

ABSTRACT BOOK

6th SETAC World Congress/SETAC Europe 22nd Annual Meeting

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This book composes the abstracts of the presentations for the platform and poster sessions of the 6th World Congress/22nd Annual Meeting of the Society of Environmental Toxicology and Chemistry (SETAC), conducted at the Estrel Hotel in Berlin, Germany from 20-24 May 2012.

The abstracts are reproduced as accepted by the scientific committee of the meeting and appear in order of abstract code, in alphabetical order per presentation type. The poster spotlight abstracts and poster corners abstracts are included in the list of poster abstracts.



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SOCIETY OF ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY

In the 1970s, no forum existed for interdisciplinary communication among environmental scientists, biologists, chemists, toxicologists, and others interested in environmental issues such as managers and engineers. The Society of Environmental Toxicology and Chemistry (SETAC) was founded in 1979 to fill the void. Based on growing membership, meetings attendance, and publications, the forum was needed.

A unique strength of SETAC is its commitment to balance the interests of academia, business, and government. The Society by-laws mandate equal representation from these three sectors for World Council Officers, Board of Directors / Council Members, and Committee members. And although there is no control mechanism, the proportion of members from each of the three sectors has remained nearly equal over the past 30 years.

Like many other professional societies, SETAC publishes esteemed scientific journals and convenes annual meetings replete with state-of-the-science poster and platform presentations. Because of its multidisciplinary approach, however, the scope of the science of SETAC is much broader in concept and application than that of most other societies.

SETAC is concerned about global environmental issues. Its members are committed to good science worldwide, to timely and effective communication of research, and to interactions among professionals so that enhanced knowledge and increased personal exchanges occur.

SETAC was founded in North America but membership was open to environmental scientists worldwide. SETAC Europe was organized in 1989; SETAC Asia / Pacific in 1997 and SETAC Latin America in 1999. Members voted overwhelmingly in 2001 to combine these “geographic units” into one global society to form the SETAC World Council. SETAC meets the professional needs of individuals at local and regional levels throughout all geographic units, throughout national branches and chapters (Argentina, Brazil, United Kingdom, Central and Eastern Europe, Africa and soon-to-be organized Japan), through regional chapters, and through national language branches (Germany). International acceptance of the SETAC model continues with widespread interest in Russia and Africa. It is now the job of SETAC World Council to oversee the myriad SETAC activities around the world and to assure the integrity of the Society.

Environmental Toxicology and Chemistry, an internationally acclaimed scientific journal, has grown from a quarterly publication of fewer than 400 pages annually in 1982 to a monthly publication of 2,915 pages in 2001. Since January 2005, SETAC publishes a second scientific journal: Integrated Environmental Assessment and Management. IEAM is devoted to bridge the gap between scientific research and the use of science in decision making, regulation and environmental management. SETAC publishes the global newsletter SETAC Globe, peer-reviewed workshop and symposia proceedings, and a variety of technical reports.

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Keynote speaker abstracts

KS01

The Biological Basis of Sustainability

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Sustainability requires the capacity to adapt to changing conditions, and the ecosystems of our planet are the result of billions of years of successfully adapting to change. Their diversity provides today's societies with a wide range of services, ranging from provision of living resources to pollination and protection against natural hazards. In recent years, many industries are learning from natural systems, finding that "biomimicry" can help to solve many challenges in efficient and sustainable product design -- everything from architecture that requires less energy to paint that washes itself. The "natural infrastructure" provided by nature continues to provide sustainable benefits to people, with no costs of construction or maintenance. The genetic diversity of plants will be essential for adapting to changing climates and meeting the increasing demands for food by a growing human population. And what does nature need in return? Being treated with respect, some sacrifices in opportunity costs, and being given full consideration as critical elements in the strategies that governments and the private sector are developing to support sustainable development. The environmental foundations of sustainability will be essential for building the economic and social dimensions of future human wellbeing.

KS02

Sustainability – an ecological, economic and social programme for future development

Jochen Flasbarth

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The concept of sustainable development was introduced to the political and scientific debate 20 years ago at the UNCED conference in Rio de Janeiro. Since then we have seen, on the one hand, rapid growth of economies worldwide, increased resource consumption and apparent evidence of climate change. On the other hand there is growing awareness of public society that planetary boundaries exist and that the limited resources should be shared with developing countries and economies in transition. Technical and political concepts have been developed for how to save energy and resources, avoid toxic emissions and tackle major problems like rising energy demand, loss of fertile soils, lack of drinking water, demographic development, etc. 20 years after 1992, governments and stakeholders will meet again in Rio. What have we achieved? What should we achieve? And how can chemistry contribute to sustainable development?

Despite all the progress already achieved we need a fundamental change in the economy and lifestyle if we want future generations to be able to meet their needs. If business continues as usual, according to estimates by UNEP, energy-related CO₂ emissions will increase continuously to more than 50 gigatonnes by 2030, although we need a reversal of emission trends if we want to comply with the 2°C target. The global demand for water will exceed supplies by 40 percent as early as 2030. According to the World Water Council currently more than 60 percent - in arid regions even 90 percent - of water withdrawals is used for irrigation.

The world economy needs to be changed in such a way that the limits of the global ecosystem are respected and irreversible damage is avoided. That means that we urgently need a greening of the economy which respects the planetary boundaries by setting environmental objectives. Key fields of action in industrialized countries include the transition to a low-carbon economy, reducing resource consumption in absolute terms and preserving biodiversity. The transformation to a green economy is a precondition for sustainable development and requires the contribution of the private sector in particular. Green economy is not an illusion of environmentalists which can only be realized in ecological niches. To the contrary, green economy offers major opportunities for business and employment and relates to the entire spectrum of economic activities. Various studies show that e.g. ambitious climate protection goals can create additional jobs, for example in developing renewable energies and in renovating buildings to enhance their energy efficiency. Looking at the chemical sector it can also be demonstrated that eco-innovations offer win-win situations for companies active in that sector.

However, a green economy will not develop on its own. In order to accelerate the transition process towards a green economy we need economic incentives for eco-innovations and sustainable consumption and production patterns. In industrialized countries, environmentally harmful subsidies need to be reduced and economic actors who behave unsustainably should bear the social costs they cause. We need a transformation from short-term to long-term responsibility as societies.

The chemical sector is one of the key sectors of Europe's economy. It is highly innovative and can therefore provide solutions to achieve the goals of sustainable development. In the past pollution by chemical industry was nearly synonymous with environmental degradation in general. Significant progress has been achieved in the meantime but problems and deficiencies still remain to be solved. One of the commitments made in Johannesburg in 2002 was that "chemicals should be used and produced in ways that lead to the minimization of significant adverse effects on human health and the environment". In Europe, REACH may provide safe chemicals but not necessarily sustainable chemicals. Complying with environmental laws is not sufficient to meet the requirements of energy and resource efficiency as well as sustainable production. Petroleum is still the main feedstock for producing chemicals. The chemical industry is not sufficiently prepared to move towards renewable alternatives or – in the long-term - to hydrogen-based production.

The addressees of a sustainable chemicals policy do not only include the chemical industry. There must also be fundamental changes in agricultural practice in order to reduce the use of pesticides, veterinary pharmaceuticals and arable soil. Producing and using increasing amounts of chemicals, pesticides and pharmaceuticals is unsustainable in ecological, economic and social terms.

KS03

Resource Efficiency and Decoupling as Drivers for Sustainability

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The remarkable economic and population growth of the 20th century was coupled to substantial increases in the extraction and consumption of natural resources, leading to increasingly-damaging negative environmental impacts. Total material extraction increased by a factor of about 8, and the price index of resources declined by about 30%. But many resources are now reaching their productive limits, as indicated by rising prices and lower grades of ores being mined.

However, economic growth globally was faster than growth of the rate of natural resources' consumption, and some negative environmental impacts have been reduced. Globally, about 25% less material input was required in 2002 compared to 1980 to produce one unit of real GDP which is a move towards a more resource efficient society. It appears that some 'dematerialization' of the world economy has occurred spontaneously.

Accelerating this process of decoupling economic activity from consumption and environmental impacts is fundamental to future human well-being. Sustainability demands that resources be used more efficiently reducing economic and environmental costs of resource depletion and negative environmental impacts. Finally, while in some parts of the world local environmental impacts have declined, global impacts on the environment are becoming ever more important and severe, requiring an international policy response. Future policies will require a better understanding of the process, so the International Resource Panel (IRP) has undertaken to define the key issues and challenges, drawing on peer-reviewed research and international experiences [1]. In seeking to find where policy interventions for decoupling will be most effective, it is essential to consider the life cycle of resources, from initial extraction to ultimate disposal (or recycling) [2]. A major conclusion drawn by the IRP [1] is that decoupling is feasible, and is already happening, but further sustainability-oriented innovations are urgently required to enable decoupling to support sustainable development more effectively. More transformative change is required to meet the size of the challenge.

References

- [1] UNEP (2011) Decoupling natural resource use and environmental impacts from economic growth, A Report of the Working Group on Decoupling to the International Resource Panel. Fischer-Kowalski, M., Swilling, M., von Weizsäcker, E.U., Ren, Y., Moriguchi, Y., Crane, W., Krausmann, F., Eisenmenger, N., Giljum, S., Hennicke, P., Romero Lankao, P., Siriban Manalang, A., Sewerin, S.
- [2] UNEP (2004) Why Take a Life Cycle Approach. Paris

Special session abstracts

SS01-1

A Statement on the Sustainability of Global Human Societies: Toward a Declaration of the 6th SETAC World Congress, May 2012

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As we develop the underpinnings for what will become the Berlin Declaration, we recognize the unique opportunity that SETAC has to contribute meaningfully to the global dialogue on sustainability. SETAC's tripartite structure of governmental, academic, and industry scientists and environmental professionals offers a model system for the trans-disciplinary development of a science of sustainability. Since its inception in 1979, SETAC's orientation has evolved from primarily environmental to include many aspects of social, economic and behavioural sciences, including decision analysis and policy analysis.

SETAC enters the worldwide sustainability discussion in an interesting time. A society's ability to reach into any corner of the planet and produce a needed material from the global resource base enables the development of megacities and in general allows our present society to live in a state far from equilibrium with our local landscapes. This reach also allows us to occupy marginal lands that could not support even the smallest of human communities long-term. Living on marginal lands and importing food, fibre, and energy places great demand from the goods side of the ecological landscape (the rate at which the system supplies desired food, water, and other materials), but also from the services side (the assimilation rate of societal wastes).

As these challenges are discussed, a new paradigm is emerging, one that is different from the widely endorsed three pillars of sustainability. The new paradigm considers a nested relationship in which economies reside within societies, which in turn are wholly dependent upon surrounding ecological systems. This new perspective relates to the Millennium Ecosystem Assessment that highlights societies' dependency on ecosystem services. SETAC's challenge is to embrace and reinforce this science-informed conceptual model of sustainable social - ecological landscapes, within this professional society and out into the greater sustainability community. The Statement is building toward a Declaration that will offer a view of how the emerging science of sustainability can guide and inform societal decision-making processes.

SS01-2

Integrated Sustainability Assessment: Providing Scientific Support for Sustainability Policy

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Sustainability is among the founding, long-term goals of the European Union and has been adopted as a guiding principle and objective for policy development by the European Commission. The EU Sustainable Development Strategy advances a vision for sustainable, prosperous communities predicated on resource efficiency, environmental protection and social cohesion. The Europe 2020 Strategy for 'Smart, Sustainable and Inclusive Growth' provides the policy agenda for sustainability.

The Sustainability Assessment Unit (Institute for Environment and Sustainability, DG Joint Research Centre) aims to advance science-based decision support for sustainability policy formulation, monitoring, and cross-cutting impact assessment. Towards this end, the Unit develops new methods and approaches for integrated sustainability assessment, including accounting frameworks and monitoring tools, reference data, and scenario modelling platforms to ultimately support policy making within the EU.

The activities of the Unit are more and more targeted towards the implementation of objectives and policies foreseen within the Europe 2020 Strategy. This includes flagship initiatives such as 'A Resource Efficient Europe', the 'Sustainable Consumption and Production and Sustainable Industrial Policy Action Plan', the Strategy for 'Innovating for Sustainable Growth: a Bioeconomy for Europe', and the 'The Raw Materials Initiative - Meeting our Critical Needs for Growth and Jobs in Europe'. Seeking to integrate and expand upon these initiatives, on-going development combines life cycle environmental, social and economic analyses within a common framework where indicator results can be assessed against sustainability targets and thresholds, or be used within a (land use) modelling platform to support the assessment of development policies via regionally-resolved sustainability scenarios.

Life cycle thinking - one of the core elements of Unit activities - is implemented via the European Platform on LCA. In recognition of the pivotal importance of quality-assured data for robust assessments, the International Reference Life Cycle Data Network (ILCD) was created as a repository for sharing quality-compliant Life Cycle Inventory (emissions and resource use) data sets. It is open to all providers including business, national LCA projects, researchers, consultants, and others. Building on the ILCD, the Life Cycle-based Indicators and the European Environmental Footprint provide the methods and framework for life cycle assessments of environmental impacts related to European production and consumption.

SS01-3

Micronutrient to the rescue! How zinc fights a global health problem

E. Van Genderen

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Due to the current dependence on metals in society, the industry has focused its research programs on many attributes that contribute to the sustainable development of basic resources - food, water and energy. For essential metals, such as zinc, the linkage between soil and human deficiency has been identified as an area that requires significant attention in order to address childhood mortality through improved agricultural productivity. For example, zinc deficiency is attributed to 800,000 deaths per year (450,000 deaths per year in children under the age of five). Further, it has been estimated that 50% of the world's agricultural soils are deficient in zinc. This high prevalence of zinc deficient soils in major agricultural zones limits crop productivity and lowers the nutritional value. In light of these critical and linked issues of zinc deficiency in soils, crops and humans, the International Zinc Association has launched programs that promote both short-term (food supplementation initiatives in conjunction with UNICEF) and long-term (improved crop production and nutrition through use of zinc containing fertilizers) strategies to address this global health problem. The presentation will provide an overview of these activities, including education outreach, policy efforts, communications and research.

SS01-4

Sustaining the Potential - making the most of nanotechnology

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Nanomaterials form an emerging and extremely promising technology, which already offers a wide range of economic and societal benefits and has the potential to unlock some of 21st Century society's biggest challenges. There are already encouraging indications that nanoscience can be an enabler to the environmental agenda, as it is in health care, electronics, construction, information technology and many other areas. However, to unlock this potential, Governments will need to bring science and policy development together, so that the benefits are maximised and the risks minimised. Developing partnerships will be key, as will the take-up of concepts such as responsible care; but all this needs to rest on a sound scientific evidence base, including life cycle analysis and risk assessment. This paper will explore these themes from the perspective of the United Kingdom and the European Union.

SS01-5

Sustainability communications: the do's and don'ts

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Organizations are seeking ways to continually describe their improved social, environmental and financial performance. Enterprise wide and product specific guidelines have been developed by a variety of groups and governments, e.g., the Global Reporting Initiative (GRI), International Organization for Standardization (ISO), DJSI, FTC environmental claims guidance, and others. These efforts ensure a level playing field by advancing harmonized 'rules' on how an organization should communicate their story/progress without 'green washing'. Additionally, with the increase in communication mechanisms, organizations are learning how to better utilize the internet and smart phones to reach broader audiences in new ways. This presentation will provide examples of good and 'not so good' sustainability communications and will conclude with general thoughts on what should be considered when developing and implementing a sustainability communications strategy.

SS02-1

Exposure assessment in ERA: from current tools to new approaches

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Environmental exposure can be assessed by means of monitoring activities and/or predictive approaches such as fate and bioaccumulation modelling. Current approaches in EU are mainly based on the use of models to estimate the fate of a chemical, as the Technical Guidance Document (TGD) dictates; however, these approaches, generally provide a steady state calculation of the fate of the chemical in a generic, standard "average" European scenario. Such static approach neglects temporal and spatial variability and can be currently adopted as a predictive tool for chemicals belonging to the classical "non polar" category, unless measured partition coefficients are provided. To overcome such issue, models should incorporate algorithm for predicting partitioning of e.g., polar and ionized chemicals, and account for more spatial and temporal variability, considering the diversity of ecosystem exposure conditions. On the monitoring side, a number of environmental data and experimental parameters are needed to improve the modelling approaches: from data on mixtures of chemical, (not commonly assessed as such), to the nanomaterials, for which a considerable lack of understanding of the fate still exists. Other knowledge gaps can be found in specific areas in which modelling approaches can be improved, from mass transfer parameters in specific environmental

scenarios to realistic environmental compartment characteristics and “validation” datasets, to name a few.

SS02-2

Environmental effect assessment and risk characterization of chemicals: what's wrong and how can do it better?

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Ecological risk assessment (RA) procedures form the basis for the development of national and international regulations aimed at protecting the environment against potential adverse effects of chemical substances. The currently used approaches, however, lack environmental realism which leads to high uncertainty and use of largely unsubstantiated ‘safety factors’. This paper will review some major drawbacks of current environmental/ecological effects assessment methods and propose new approaches based on recent scientific developments.

SS02-3

Mechanistic effect modelling for ecological risk assessment: state-of-the art, trends, and challenges

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Mechanistic effect modelling includes individual-level effect models, such as TK/TD and Dynamic Energy Budget models, and ecological models addressing populations and communities. Such models have the potential to overcome limitations of current risk assessment, in particular regarding higher tiers, complex exposure patterns, indirect ecological effects, and endpoints relevant for ecosystem vulnerability and services. The state-of-the art includes models that have been shown to be realistic enough to make robust predictions relevant for risk assessment of chemicals. However, so far such models had to be assessed on a case-by-case basis and often were not designed and communicated in a way that makes them suitable for regulatory decisions. Therefore, current initiatives are trying to establish guidelines for Good Modelling Practice, for example the TRACE documentation framework developed in the EU-funded project CREAM. Main current challenges include: establishing acceptance criteria for mechanistic effect models, which then become part of Good Modelling Practice; agreement on standard species, scenarios, models, and ecological endpoints; installation of procedures that prevent standard models from becoming static and therefore being used uncritically; and linking effect and exposure models. Mechanistic effects models are an indispensable tool for making risk assessment of chemicals more ecologically relevant. However, concerted actions, which correspond in scope and required effort to FOCUS, are needed to fully integrate such models in future regulatory risk assessments.

SS02-4

Addressing complexity in ecological systems - ignore it or embrace it?

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Despite a widespread and long-held recognition that ecological systems are complex, traditional approaches to ecological risk assessment have largely attempted to ignore complexity. Instead, standard approaches rely on simplistic tests and assumptions with complexities glossed over by applying fixed ‘safety’ or ‘uncertainty’ factors. Advances in computer science, together with the development of appropriate modeling tools and modeling practice are facilitating more sophisticated and scientifically robust ways to deal with complexity. For example, extrapolation of effects across levels of biological organization, consideration of multiple stressors, and complex exposure scenarios can all be addressed using appropriate agent-based models (ABMs). ABMs offer many benefits for ecological risk assessment: they are highly flexible, not limited by mathematical tractability, can incorporate spatial and temporal variability, can capture interactions among species and among multiple stressors, and are ideal for studying responses that cross levels of biological organization. There remain challenges for both the modeling and for model implementation so that the necessary complexities can be effectively addressed. The alternative, of course, is to continue to keep the complexities implicit and hope that by doing so will make them go away.

SS02-5

Priorities to improve ecological risk assessment for chemicals

T.C.M. Brock

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The ecosystem services concept can be applied to operationalize and harmonize the generic protection goals formulated in legislative documents. For each ecosystem service potentially affected by chemicals, important taxa/functional groups need to be identified. It is key to identify “potential vulnerable” representatives (focal species) of these taxa/functional groups on which the different risk assessment tiers should focus. From a risk manager and cost-benefit point of view it may be necessary to develop criteria for a transparent spatial differentiation in specific protection goal options. Lack of a clear conceptual basis for the interface between the exposure and the effect assessment may lead to an overall low scientific quality of the risk assessment. This interface is defined as the Ecotoxicologically Relevant Concentration (ERC). Key is that the type of ERC used to express the “C” in the exposure estimates should not be in conflict with the ERC used to express the “C” in effect estimate. In the near future many of the ERC problems might be solved by using toxicokinetic / toxicodynamic or population models and exposure scenarios specifically developed for “vulnerable” focal species. The different tiers in ERA schemes need to be calibrated. An important lesson learned from the past is that the consistency of the tiered approach needs to be re-evaluated every time new chemistries (with a novel toxic mode-of-action) come on the market. An important pitfall in ERA remains the extrapolation of results of relatively simple model ecosystem experiments and computer simulation models to the diverse reality of the field. In relatively simple model ecosystem experiments and food-web models the indirect effects of chemical-stress observed may be a caricature of reality, since not all essential feedback mechanisms that may dampen temporal chemical-stress in natural ecosystems will be captured in the simple models. In the extended abstract more detailed information is given.

SS02-6

Regulatory point of view (focused on the implementation of REACH) on the new scientific challenges for ecological risk assessment on hazardous and PBT substances

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Disclaimer: The authors are staff members of the European Chemicals Agency. The views expressed are solely the authors’ views and do not represent an official position of the Agency. The presentation will focus on the regulatory point of view (focused on implementation of REACH) on the new scientific challenges for ecological risk assessment in the case of hazardous and PBT substances which, as part of the registration process, require an environmental exposure assessment (EEA), the environmental risk characterisation, and/or the identification of operational conditions (OC) and/or risk management measures (RMM) for minimizing the emissions to the environment. The presentation will cover the following topics: - different roles and responsibilities under REACH involving EEA and risk characterisation/emission minimisation; - substances registered that require an EEA and risk characterisation/emission minimisation; and the evaluation process; - REACH-related regulatory difficulties related to EEA and risk characterisation/emission minimisation; - scientific challenges in EEA and risk characterisation/emission minimisation faced during dossier evaluation so far; - major EEA and risk characterisation/emission minimisation related deficiencies in dossiers evaluated so far; - current experience, further needs and possible solutions; - relevance of the identified challenges and proposed recommendations in the draft SCCS/SCHEER/SCENIHR opinion regarding the implementation of the REACH Regulation; - further suggestions and research needs in the regulatory scientific context.

SS02-7

The view of a regulator on the possibilities for applying more ecologically relevant effect assessments

HCI Clausen

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The presentation will address different approaches to effect assessments and risk characterisation with the view of a regulator focusing especially on the practicability. Seek simplicity, and distrust it. The simpler the system, the easier it is to handle, and it is generally more transparent. The more complex the testing and modelling is the greater the reality, and also the greater the difficulties in interpreting the results. What should the requirements be for employing the different methodologies and tests.

SS02-8

Addressing the new challenges for risk assessment: An industry perspective

P. Campbell

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This is a comprehensive overview of ecological risk assessment approaches and the high level goals of this paper, such as reduced animal testing and development and promotion of high level ecological risk assessment expertise, should be commended. Key recommendations such as: - adopting higher-tier ecological risk assessment tools; a list of requirements for new innovative approaches before they can be used in regulatory ecological risk assessments; and the need for further research on how to deal with multiple stressors are also well founded. One of more holistic challenges for this document, is the breadth of remit. Currently it encompasses both prospective and retrospective regulation as well as different types of potential chemicals contaminants (eg pesticides and general chemicals), where the issues and the available data sets are going to be very different. Therefore, one way to improve this document would be to consider restructuring the content to provide clear and specific advice to distinctly different situations eg Ecological Risk Assessment for Chemical Registration and Site Specific Ecological Risk Assessment, being 2 potential examples. One of the ecological risk assessment challenges for the pesticide industry is extrapolation of risk assessment conclusions eg between species, different environmental conditions and geographical regions. One of the tools recommended within this document to help with this issue is the expanded use of modelling, and again this is justified. However, another recommendation is for greater uncertainty analysis and application of appropriate uncertainty factor. The industry experience with this option is not so helpful eg Bird & Mammal Guidance Document,

where low toxicity herbicides are still failing the risk assessment. In addition, the current practise of application of safety factors to the results of Mesocosm/pond studies now means there is no regulatory benefit to carrying out such studies. Consequently, a real opportunity is now missed to test, observe and understand potential in-direct and community level aquatic effects, both of which are highlighted as an area where more information is required in this document. The key issue here is there will always be uncertainty within ecological risk assessment and therefore can we really reduce the need for expert judgement as recommended, without having to continue to apply large over precautionary uncertainty factors.

SS02-9

Environmental Risk Assessment - Basic principles, future trends. View from industry employed environmental scientist on the EU (DG Sanco) document 'Addressing the new challenges for risk assessment'

PD Dohmen

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The draft EU document represents the state of the art in environmental risk assessment (ERA) including new trends and combining high level environmental protection and realism. The ecosystem services concept offers a suitable approach to determine which impact may be tolerable where and when. Established ERA uses a stepwise approach starting with standardized studies combined with appropriate AF and where needed followed by higher tier assessments and respective adjusted AF. The new EU document follows this approach and presents additional refinements in order to improve the realism of risk assessment. This is certainly desirable; however, the additional data must not become standard requirement. The document describes when to ask for additional information or call for specific risk management actions; i.e. analyse data, include modelling etc. This should be verified by respective field investigations and/or monitoring. Only then specific action should be warranted. This general approach is strongly supported. The document refers to 'indirect effects'. These occur as a consequence of direct effects. If there is low risk of direct effects, then indirect impacts are of low relevance. Only when direct effects are considered acceptable, more consideration should be given to indirect effects. Site specific evaluation will improve realistic ERA. However, this may require a significant amount of additional assessments and should not be misused nationally or regionally counteracting EU harmonized approaches. "Trait-based approaches represent a promising tool capable [3DOTS] to complement taxonomically based assessments with functionally based assessment (more significant in ecological terms)". This is valid and relevant; however, this tool is only useful in the regulatory context if it is avoided that individual results from single species will override the trait-based approach. Modelling has been included in ERA allowing to extrapolate risk assessments without the need of excessive (animal) testing; it will also address uncertainties more quantitatively. This should be done in an overall realistic assessment; simply adding up various individual uncertainties has nothing to do with a realistic ERA.

SS03-1

Nanotechnology: Peeking into the Future

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There is great potential for nanotechnology to be applied in a broad range of societal sectors. New applications and product formats are likely to add to current emissions into the environment, e.g. via consumer disposal of domestic products down the drain or via direct emission to, or use in, the environment. Given the developing state of definitions and of characterisation and risk assessment methods, industry is faced with uncertainty over the likely success of new innovation involving nanomaterials and technologies. Rapid progress is needed in these assessment methodologies in order to establish guidance on data requirements and how they should be interpreted for risk-based decisions. In the meantime communication with stakeholders needs particular care to ensure that both research and safety related data can be considered in context of the risks to man and the environment. This presentation will elaborate on developments from an industry perspective.

SS03-2

The regulatory perspective on engineered nanomaterials

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As nanomaterials are used in an increasing range of applications there is growing debate regarding their safe use and possible impact on human health and the environment. There is no specific legislation in the European Union (EU) or the USA on nanotechnology or nanomaterials, but legislation on worker protection, environmental legislation, chemical legislation, specific products legislation etc. apply in principle to nanomaterials. In the EU, nanomaterials are covered by the chemicals legislation, REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals), which addresses chemical substances, in whatever size, shape or physical state, although there are no specific provisions for nanomaterials in REACH. Any introduction of provisions specific to nanomaterials requires the adoption of a definition of nanomaterial. Although in October 2011 the EU Commission adopted the Recommendation on the definition of a nanomaterial, at this stage this does not constitute as yet enforceable legislation. In the USA, nanomaterials are primarily covered by the industrial chemicals legislation TSCA (Toxic Substances Control Act), pesticides legislation FIFRA (Federal Insecticide, Fungicide, and Rodenticide Act) and for foods and drugs, the FFDCA (Federal Food, Drug, and Cosmetic Act). In the USA, before a new chemical is commercialized a manufacturer or importer must submit a new chemical notification and a determination must be made that it may not present an unreasonable risk to human health or the environment. The presentation will give an overview of the current regulatory perspectives for engineered nanomaterials including a short summary concerning available guidance on safety/risk assessment, and will briefly cover additional international activities.

SS03-3

Advances in methods for analysing nanoparticles in complex environmental media

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The analysis of engineered nanomaterials (ENMs) in the environment is still a challenging task. Advances in the study of the environmental fate, transport, and ecotoxicological effects of ENMs have been hampered by a lack of adequate techniques for the detection and quantification at environmentally relevant concentrations in complex media. The analysis of ENMs differs from traditional chemical analysis because both chemical and physical forms must be considered. Because ENMs are present as colloidal systems, their appearance and physicochemical properties are dependent on the surrounding conditions and may be of transient character. By trying to observe, isolate, and quantify ENMs their physicochemical properties may be changed, making the analysis extremely susceptible to artifacts. The most pressing research needs are the development of techniques for sample storage, extraction/separation, and cleanup that introduce minimal artifacts; to increase the speed, sensitivity, and specificity of analytical techniques, as well as the development of techniques that can differentiate between abundant, naturally occurring particles, and manufactured nanoparticles. The direct and non-invasive detection of ENPs in complex samples is hampered by the presence of sample components interacting with the NPs and/or interfering with the analytical technique. Separation and isolation of the ENPs is therefore necessary in most cases. The basic idea of most currently developed methods is to provide particle sizing capabilities by the use of high spatial resolution or chromatography-like separation techniques. These core capabilities are then combined with material, element- or mass-specific detection. Especially high resolution X-ray, Field-Flow Fractionation and single-particle ICP-MS based techniques have made considerable progress because of their particle size and compositional selectivity. The presentation will cover examples of particle/matrix combinations and discuss the pro and cons of the methodological approaches. It will also address in detail the challenges posed to analytical methods arising from the definition of Nanomaterials issued by the EU commission.

SS03-4

Fate and behaviour of engineered nanoparticles affecting exposure in natural systems

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Research over the past decade has provided a fundamental understanding of the behaviour of engineered nanomaterials in surface waters, sediments and soils. We are well aware that aggregation is an important pathway for most nanoparticles, while for some, e.g. ZnO dissolution has been shown to be an important control on exposure. These transformations will depend both on the physical and chemical properties of the nanoparticle, such as size, surface charge and the presence of coatings, and of the receiving environment, such as ionic strength, pH, and its chemical composition including both dissolved and colloidal organic and inorganic components. Because of the difficulty in working with what could be termed 'environmental concentrations' of nanoparticles, studies of their fate and toxicity have for the most part been undertaken at parts per million concentrations where both the chemical and physicochemical measurements are more reliable, and usually in controlled synthetic water, sediments and soil matrices. Interactions with organic matter have usually been studied using additions of "standard" humic acids. More recently, studies with natural waters, sediments and soils have been undertaken. Here their fate and potential toxicity can dramatically change as a result of aggregation of nanoparticles with natural mineral and organic colloids (heteroaggregation) whose concentrations are likely to exceed those of the nanoparticles, as distinct from homoaggregation with the same nanoparticles. Heteroaggregation will change the surface charge and mobility of nanomaterials and based on the low bioavailability of natural colloids, is likely to reduce toxicity to natural biota. Such reactions will also occur during wastewater treatment (as will chemical transformations, e.g. sulfidation of silver nanoparticles) and will likely contribute to the ultimate transfer of nanoparticles to sediments or to their immobilisation on the soil solid phase. Examples will be presented. The challenge will be to link the exposure concentrations determined under these more realistic environmental scenarios to bioaccumulation and potential toxicity, so that risk assessments and life cycle models can provide more reliable predictions for environmental managers.

Challenges in determining the ecotoxicological responses of engineered nanomaterialsD Handy¹, J Klaine², F Fernandes³¹University of Plymouth, PLYMOUTH, United Kingdom²Clemson University, CLEMSON, United States of America³Heriot-Watt University, EDINBURGH, United Kingdom

The ecotoxicology tool box contains a myriad of different bioassays and toxicity tests that use a variety of biological end points. These responses are from different levels of biological organisation from the molecular level to whole organisms; which in turn may be extrapolated to imply population level effects. However, while there is general agreement that many of the existing regulatory test methods may be adapted or modified to work with nanomaterials, less attention has been given to the underlying biological responses. This paper aims to critically evaluate the scientific rationale behind the selection of biological responses as end points for ecotoxicity tests with nanomaterials, and asks whether or not all the important biological responses have been considered in the testing strategy. Ideally, a mechanistic explanation should associate any given biological response with the exposure, but this understanding is lacking for many materials and organisms. Biological responses are time-dependent and for many materials the sequence of exposure, bioavailability, uptake kinetics, and the onset of the biological response has not been verified. An association between the presence of the nanomaterial in the tissue and the biological response (the target organ approach) is not the only paradigm to consider, with secondary toxicity and latent effects also possible. Evidence is emerging that some of the traditional end points in regulatory tests, such as growth or survival, may not be the most sensitive measurements for nanomaterials. However, alternative biochemical, genetic, and other molecular assays that rely on the presence of the test substance being inside the cell may be less sensitive than expected if the nanomaterial is not available to the internal compartments. It is therefore vital to understand the body distribution, target organ and sub-cellular localisation of the materials in order to identify the relevance of each response. Finally, there are many challenges for determining ecotoxicological responses in field-collected samples from real ecosystems; including linking exposure with a nano-specific biological effect(s), and differentiating the nano-effect within a mixture of chemicals.

SS05-1

Human needs, biodiversity and ecosystem services - contradiction or common grounds? Facing sustainability challenges at the third millennium' Introduction to the problem:

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The world population is growing faster than in the last century. In particular, food commerce has been increased to satisfy increasing demands of their populations, resulting in a less controlled grow of food production, particularly food commodities. This higher demand of commodities has been partially satisfied by improving the production technology but also increasing cultivated areas, which result in the use of areas previously occupied by wild biota. Modern agricultural practices make intensive use of agrochemicals to warrant both productivity and health of cultivars. On the other hand, cattle production uses veterinary drugs, accelerated grow systems (feed lot and similar), sometimes using non authorized anabolic products. Within this framework, South America (SA) is probably the area showing the highest and fastest increase in food production, which result in almost uncontrolled augment of areas dedicated to the production of soya and crops. Particularly Brazil and Argentina but also Uruguay and Chile have been strongly increased their national incomes arising from the exportation of food commodities, in addition to a growing production of bio-fuels. The increase of international prices for soya and maize have been the main argument used for small and big farmers to increase the cultivated area, affecting native forest, spreading agrochemicals in the proximity of cities, controlling pests and weeds but also affecting non target organisms. During this short introduction some figures on the growing demand on food commodities, as stated by FAO (UN) will be presented, with particular emphasis in the production of Latin American Countries (LAC). The main goal of this introductory speech is raising questions on the environmental cost that this increased production is causing in comparison with economical benefits arising from the exportation of food commodities from LAC.

SS05-2

The expansion of agricultural frontiers and its consequences on the environment. The case of Argentina during last decadesML Menone¹, KSB Miglioranza², DA Wunderlin³¹Universidad Nacional de Mar del Plata, MAR DEL PLATA, Argentina²Universidad Nacional Mar del Plata, MAR DEL PLATA, Argentina³Universidad Nacional de Córdoba, CÓRDOBA, Argentina

The agricultural expansion and intensification in South America, particularly in Argentina, Brazil and Uruguay, has triggered negative changes in the environment. The negative effect of human intervention on habitat and biodiversity would have increased and the water-use efficiency decreased. However erosion and pollution risk are today lower than those of the mid-20th century. Regarding agrochemicals, quantities and qualities of pesticides used in Argentina vary with the productive system. Pedological and climatic characteristics of the Argentinean regions determine the kind and extension of agricultural activities. Extensive soybean and wheat productions are concentrated on the Rolling Pampa region and account for the 80% of the total arable land of the country. This system is based on the direct seeding technique with the application of the herbicide glyphosate, and technical endosulfan and a-cypermethrin as insecticides. On the other hand, Patagonian agriculture is almost exclusively based on the fruit and wine production concentrated mainly on the Rio Negro watershed. The historical and current use of pesticides in the area is reflected on the occurrence of organochlorine pesticides in biotic and abiotic matrices from the Rio Negro Valley with a clear predominance of residues of DDTs followed by endosulfans. The intensive and increased agriculture developed in Argentina during the last 20 years lead to different effects on biota. Mass mortality of Swainson's hawks occurred in Argentina due to monocrotophos during 1995-96 and recent investigations in the Pampas showed that bird species richness tends to correlate negatively with annual crops. Non-target species from other ecosystems like the aquatic could suffer the negative consequences of the pesticides. Concentrations of chlorpyrifos and cypermethrin that exceeded the water quality criteria for freshwater environments have been detected in runoff and stream water of small agricultural streams in the main soybean area of Argentina; associated to 100% mortality of aquatic macroinvertebrates. Environmentally relevant concentrations of endosulfan have been demonstrated that exert oxidative stress, genotoxicity and histological damage in native fish and wetland macrophytes in laboratory experiments. Therefore, residues of this pesticide in freshwater ecosystems are expected to damage wildlife at organism or even more at population level.

SS05-3

THE SCARCE CONSOLIDER PROJECT ON WATER SCARCITY IN IBERIAN RIVER BASINSA. Navarra-Ortega¹, S. Sabater², I. Muñoz³, X. Sanchez-Vila⁴, C. Conde⁵, Y. Picó⁶, J. Blasco⁷, A. Elosegi⁸, M. Schuhmacher⁹, R. Batalla¹⁰, F. Francés¹¹, D. Barcelo¹²¹IDAEA-CSIC, Barcelona, Spain²Catalan Institute for Water Research (ICRA), Girona, Spain³UB, Barcelona, Spain⁴UPC, Spain⁵UPM, Madrid, Spain⁶UVEG, Valencia, Spain⁷Instituto de Ciencias Marinas de Andalucía, Cadiz, Spain⁸UPV/EHU, Leioa, Spain⁹Universitat Rovira i Virgili, Tarragona, Spain¹⁰UdL, Lleida, Spain¹¹UPV, Valencia, Spain¹²CSIC-IDAEA, Barcelona, Spain

Water resources in Spain are subjected to rising pressures, related to the socioeconomic activities of an increasing human population, expressed by accelerated land use changes, and the specific climate characteristic of Mediterranean countries. The main panels on climate change predict a future scenario of increasing frequency of floods and extended droughts in the Iberian Peninsula, mostly in the Mediterranean basin. This will be added to the currently existing problems, and will probably affect the available water resources, their quality, the functioning of associated ecosystems, especially rivers and their aquifers, and the ecosystem services they provide.

In such context, SCARCE is a project that aims to describe and predict the relevance of global change impacts on water availability, water quality and ecosystem services in Mediterranean river basins of the Iberian Peninsula, as well as their impacts on the human society and economy. Hence, the project has assembled a multidisciplinary team of leading scientists in the fields of hydrology, geomorphology, chemistry, ecology, ecotoxicology, economy, engineering and modelling, in an unknown effort in the CONSOLIDER framework. The project also considers the active involvement of Water Authorities and other relevant agents as stakeholders.

SCARCE has two complementary objectives. The first tackles basic research questions and will define the long-term patterns and the mechanisms that operate in the hydrology, water quality, habitat dynamics, and ecosystem structure and function of Mediterranean basins. The second objective is related to the effects of climate and human footprint (taken both as key elements of global change) that provide on the ecosystem services, rivers and streams, as well as the urgent need to implement and eventually refine the water management policies demanded by the EU Water Framework Directive (WFD). Therefore, the project emphasizes linking basic research and management practices in a single framework. The project has the external support of several Water Authorities and stakeholders. SCARCE is structured across a series of Horizontal and Thematic Work packages that coordinate the various scientific goals, as well as their interactions. These WPs deal with data collection (WP1), hydrology (WP2), sediment transport and river channel morphology (WP3), chemical and biological quality (WP4), ecosystem processes (WP5), modelling (WP6), socioeconomic scenarios (WP7), ecosystem services (WP8), river management (WP9) and coordination (WP10).

SS05-4

Emerging organic contaminants in food: a global sustainability challenge

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There are many thousands of chemical substances in food; most of them being of natural origin. A number, however, are man-made and arise from the use of agrochemicals, or due to pollution of water, soil and air, or occur during food preparation/processing. In addition food may contain biological contaminants. A range of additives may also be added for a variety of purposes (e.g. enhance the flavour, colour, improve stability etc).

Therefore, while consumers expect the food that they eat to be safe, as consequence of industrial development, pollution, and the climate change cause the variety of food contaminants to increase. Currently one of the great challenges in food safety is control the risks associated to mixtures of contaminants, which continuously are changing. Among the most prominent groups of emerging food contaminants can be considered industrial origin contaminants as perfluorinated compounds (PFCs), polybrominated biphenyls (PBBs), the new generation of pesticides, plasticisers, siloxanes, and nanomaterials can be considered. Many of them are of particular concern because can cause severe damages in human health, for example some of them are suspected to be cancer promoters. Other of selected compounds have been related to endocrine disruptor effects, or can be accumulated and biomagnified through the food chain.

In this talk, we will present a summary of current situation, as well as, the results of different research work carried out in our group investigating the presence of emerging contaminants in food. The strategies for their analysis including sample preparation, separation and detection will be presented. The results in typical food baskets of different countries will be compared. The main sources of the selected groups of contaminants will be discussed together with their toxicological data and concentrations reported during the last few years by other authors.

SS05-5

Impact of agrochemicals on the food chain. Research needs to guarantee safe and sustainable food production at the third millennium

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Consequences of the intensive use of agrochemicals have lead to detect presence of these products in edible fish and, sometimes, in fruits and vegetables. Though levels found are still bellow FAO guidelines for safe human consumption, there are many issues needing attention from research community. Particularly, the passage of single agrochemicals or their formulations from plant-soils to higher animals (cattle, etc.) and aquatic systems, impacting fish and other edible products is a topic of concern in South America. Also transport phenomena of these agrochemicals, which are affected by changes in the soil coverage (deforestation), need to be addressed in the near future. So far, Environmental Toxicology in developing countries, dealing with a rapid increase of agricultural frontiers, need to urgently address the above mentioned questions. Regional and international cooperation should be necessary to facilitate studies as well as to spread the concept of sustainable food production for future generations.

SS06-1

Historical and regulatory background of soil quality assessment

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From the 70-ties of the past century onwards concern has been raised about levels of contaminants in the environment. Main sources of contamination are industrial processes, waste disposal and intentionally applied chemicals in agriculture, such as fertilizers, pesticides and veterinary drugs. Regulations dealing with the prevention and remediation of contaminated soils are aimed at the soil as an environmental compartment (soil protection act), industries (environmental regulations), chemicals (REACH, pesticide regulation, Nitrate directive) or waste management (Waste Directive). Policies to prevent and remediate soil contamination use prognostic and diagnostic instruments. Prognostic instruments include soil quality standards and predictions of environmental concentrations of chemicals to manage (intentional) emissions of contaminants. Site specific ecological risk assessment (ERA) is a diagnostic instrument that also uses quality standards, such as intervention values, combined with other lines of evidence. Since the 90-ties species sensitivity distributions (SSD's) have been increasingly used in prognostic and diagnostic instruments, to determine e.g. safe levels and intervention values for contaminants. SSD's seem less suitable for contaminants that have a very specific mode of action, essential elements and nutrients. Some prognostic instruments, for instance for pesticides, may include higher tier testing in case predicted environmental concentrations exceed safe levels. More lines of evidence in site specific ERA may include observations from mesocosms and field tests, as well as ecological knowledge on the role and function of sensitive species in the environment. A Triad approach is often recommended, in which chemical, toxicological and ecological data for a contaminated site are assessed along converging lines of evidence. The European Soil Strategy (2006) has identified 7 additional soil threats, besides contamination. These threats refer also to, biological and physical aspects of soil. The Soil Strategy stresses the importance of soil functions by explicitly listing these functions as protection goals. The 'soil functions' largely comply with the definition of ecosystem services, a concept that gains an increasingly central role in environmental assessments. The potential of this concept for assessing soil quality will be briefly addressed.

SS06-2

Soil ecology and soil quality relationships

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Whenever dealing with environmental hazards, quality assessment of the respective compartment has been heatedly debated. Buzzwords and concepts have repeatedly changed names (e.g. critical loads, critical thresholds, tipping points), yet the key issue - what is a healthy system (here: soil)? - has never been thoroughly conceptualised. Is there an ideal species number, community structure? How do we adequately compare soils with different climates, different keystone species? How do we measure resilience? What is the role of redundancy (which appears to be specially pronounced in soils)? I will summarize theoretical approaches, scrutinize some frequently used endpoints and quality measures such as BBSQ or PICT and discuss their pro's and con's. With respect to available and desirable data I will particularly stress the relevance of various test systems and endpoints, short-term versus long-term monitoring data as well as natural stressors. Trying to structure everything in a logical way and wrapping it with my own considerations, I hope being able to give some new impulses for soil quality assessment.

SS06-3

Predicting or measuring exposure and (bio)availability assessment

M.J. McLaughlin

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Predicting or measuring exposure and bioavailability of contaminants in soils has progressed significantly in the last 10 years. For metals, robust methods to determine speciation of metals in both the solid phase and in soil porewater are now available. We also know that toxicity of metals is moderated by competitive ion effects at the receptor surface, and these interactions as well as metal partitioning between solid and solution phases can be predicted and/or modelled. "Bottom up" or mechanistic approaches to predict metal toxicity across a wide range of soils have found less success in regulatory implementation than "top down" or empirical methods, largely because of the simplicity of data requirements and ease of calibration and validation of the latter. It is important that the degree of complexity and data requirements of frameworks needed for Tier 1 risk assessments or contaminant screening values be borne in mind during development. Direct measurement of contaminant bioavailability is often regarded as a competitor to predictive models, but it should be realised these are best suited to different levels of the risk assessment process. Great improvements in measures of metal fate, exposure, and bioavailability in soils are now possible with advances in instrumentation and techniques that often now allow rapid and/or in situ measurements that will greatly enhance not only risk assessments but also could assist with risk management processes (remediation).

SS06-4

Natural stressors in ecotoxicological studies and ERA: do they matter?

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Although already some 20 years ago scientists were warning that ecological risk assessment (ERA) based on simple (eco)toxicological essays may be seriously biased, this is still the most commonly used methodology. Although the need for chronic and sub-chronic tests has been recognized to some extent in recent years, what certainly improved the confidence in ERA, these tests are also performed under standard constant laboratory conditions. Moreover, even if these conditions are usually called "optimal", this is not necessarily true. First of all, however, organisms are exposed to a whole range of suboptimal conditions in their natural environment, and these conditions are perpetually fluctuating. Organisms are, thus, exposed to a number of different stressing factors which, irrespectively of any possible pollution, affect individuals and populations. The important question from the ecotoxicological point of view is whether these non-chemical stressors can significantly bias the predictions of ERA. In recent years the number of studies showing significant and environmentally relevant interactions between chemical and non-chemical stressors have increased substantially. For soil environment, temperature, drought, food supply, and pathogens appear to affect toxicity of chemicals. On top of that, also chemical factors of natural origin, such as pH and organic matter contents, can modify the toxicity of pollutants and, most probably, the interactions between toxicants and non-chemical factors themselves, making the picture a whole lot more complicated and difficult to study. Despite these difficulties, it seems that neglecting the interactions cannot be justified any longer. This is proved not only by laboratory studies indicating on significant interactions, which always can be questioned from the point of view of their relevance to ERA, but also by field surveys which actually show substantial discrepancy between field observations in polluted environments and predictions derived from laboratory bioassays. The authors of a recent paper devoted to this problem concluded that "understanding the effects of toxic agents in a complex environmental gradient may require more research on the interaction between toxicity

and environmental factors “(Frouz et al. 2011). I subscribe to this point of view and will review the arguments towards modifying current ERA approaches to include more complicated experimental designs, at least at higher-tier assessment procedures.

SS06-5

Microbial, single species, and higher tier test for predictive assessment of chemical effects on soil quality

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Since 1984, the ecotoxicological hazard potential of chemicals in soil has to be evaluated in Europe and North America. During the 1990's and early 2000's, there was considerable research undertaken to develop soil ecotoxicological tests (e.g. SECOFASE project; Environment Canada soil methods program) which used plant species and different soil invertebrates, including earthworms, enchytraeids, nematodes, collembola, staphylinid beetles, mites, centipedes, millipedes and isopods. Semi-field test methods such as the Terrestrial Model Ecosystem (TME) and earthworm field collection have also been developed and standardized through ISO and OECD. Within the past decade, a number of these toxicity tests have been standardized by ISO, OECD and Environment Canada to allow the assessment of sublethal and/or chronic effects on organism growth, behaviour and reproduction. There has also been great methodological progress in tools for assessing the influence of chemicals and contaminant mixtures on ecological soil functions and microbial community biomass, activity and diversity. In Europe, South America and North America, these biological test methods have been further standardized for use in the area of soil quality assessment and remedial decision-making at sites where there is complex contamination in natural surface soils. However, adapting biological methods that initially were standardized for testing of individual substances has been a challenge. There is also recognition that endemic soil microbial communities will vary from site to site. To ensure the generation of high quality toxicity test data for effective application in environmental regulations or directives, standardized methodologies, laboratory quality assurance and the proper data interpretation is needed. Overall, the quality of methods, testing laboratories, and data generated ensure that decisions regarding the protection of the environment are made using the most appropriate science. This presentation will provide an overview of existing standardised test methods, focussing on invertebrates, plants and microbial community assessment methods.

SS06-6

Can we realize tangible benefits from using complex 'omics tools when assessing soil quality?

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'Omics approaches are no longer novel but rather serve as powerful tools which can substantially enrich and enhance ecotoxicological investigations. Some proteomic and metabolomic methods have exploited for approaching 20 years whilst after a decade of the exploitation of microarrays based gene measurements we may soon see this technology retired in deference to the more robust New Generation Sequencing based digital transcriptomics. The maturation of some of these technologies provides an opportunity to appraise what has been learnt so far during their application for chemical hazard assessment. An appraisal of this type considering aquatic ecosystems, and especially fish (Fent and Sumpter 2011, Aquat. Tox. 1055, 25-29), concluded that despite some notable technical achievements and scientific advances, the application of profiling techniques to aquatic ecotoxicology was not yet sufficiently proven for application in either prospective risk assessment or routine environmental monitoring. In this paper, we will discuss the current status and application of systems biology methods for assessing the biological consequences of soil contamination. The analysis will start from an assessment of the lessons learnt from traditional single biomarker analysis conducted at the Avonmouth smelter contaminated site (e.g. expression analysis for an individual gene or protein) both in terms of absolute sensitivity of gene expression measurements and also variation in biomarker responses. An understanding of biomarker sensitivity and variability can lead to hypotheses related to micro-evolutionary processes that can be tested either through exquisitely targeted analysis or genome wide screening. Such analyses can use standard methods or the emerging potential of Next Generation Sequencing. Although often forgotten in the push to move to expression analysis, the genome resources generated as precursors to expression profiling can in themselves offer a resource from which to identify potential mode and mechanisms of action coupled to the biological effects associated with chemical exposure. Linking wider gene expression analysis to physiological observations can provide support for physiological models that categorise chemicals according to their effects on relevant endpoints. Finally, the holy grail of diagnostic based monitoring can become a reality provided that the test case is clearly defined and the molecular assessment is supported by sound environmental chemistry and anchored to physiological analysis of the monitored population(s).

SS06-8

Case study 1: prognostic - risk assessment of plant protection products

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A Dutch working Group drafted a proposal for risk assessment of persistence of plant protection products in soil. The proposal was evaluated for four substances and a metabolite using public available data. A tiered approach was followed for exposure and effect assessment. In this contribution it is discussed whether sufficient knowledge (and methods) is available to ensure the basic principles of a tiered approach for in-soil risk assessment. For the effect assessment protection goals were defined. The relation between the protection goals used and soil health status is discussed.

S06-9

Site-Specific ecological risk assessment. Case-study 2

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The decision supporting and integrating assessment tool, TRIAD, is used site-specific on PAH- and heavy metal contaminated sites in Denmark. The various aspects of the TRIAD approach are used on a set of chemistry-, ecotoxicology- and ecology related data collected among others in the EU project “Development of a decision support system for sustainable management of contaminated land by linking bioavailability, ecological risk and ground water pollution of organic pollutants” or in short “LIBERATION”. The presentation includes examples on how to scale and integrate the results from various scientific disciplines.

SS07-1

The decline of the honeybee: a modelling approach

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Insect pollinators play an essential role in crop production with an estimated economic value of about 14.2 billion Euro in the European Union. Among those, honeybees belong to the most important pollinators. In Europe and North America a decline in the number of managed honeybee colonies is reported which may have a severe ecological and economic impact. There are indications that the increased colony losses are not caused by a single factor but by an interaction between several agents. To better understand these interactions we have developed a computer model, simulating the dynamics of a single honeybee colony. In order to keep computational time low but to allow for flexibility in the decision-making, we combined a cohort-based population model with an agent-based foraging module. We included the varroa mite as a bee parasite which acts as vector for two different bee viruses: Deformed Wing Virus (DWV) and Acute Paralysis Virus (APV or ABPV). Crop maps defining the availability of food sources can be used in a separate, spatially explicit landscape model to allow the application of realistic foraging scenarios with specifically defined nectar and pollen flows over time. In this talk we will describe and explain the model's design and present output for the colony dynamics under scarce and abundant forage scenarios, with and without varroa as well as for hypothetical scenarios of pesticide exposure and effects.

SS07-2

Using population models to determine the impact of herbicides on endangered species: an example with lange's metalmark butterflies at the Antioch Dunes National Wildlife Refuge, California

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The Lange's metalmark butterfly (*Apodemia mormo langei*) is in imminent danger of extinction due to the loss of its habitat caused by invasive exotic plants which are eliminating naked stem buckwheat (*Eriogonum nudum* var. *auriculatum*), its food plant. The butterfly was listed as an endangered species on June 1, 1976. Invasive plants significantly impact the few remaining acres of habitat at the Antioch Dunes National Wildlife Refuge. Plant control measures include mowing, hand pulling, fire, and herbicides. In March 2007, the Recovery Branch of the Sacramento Fish and Wildlife Office asked the Environmental Contaminants Division to design experiments to test effects of herbicide treatments on larvae of other subspecies of metalmark butterfly. Due to the lack of literature regarding butterflies and herbicide direct toxicity and the declines in native butterflies it is quite clear that a study evaluating the effects of herbicides on butterflies was necessary. We have evaluated the effects of three commonly used herbicides, triclopyr, sethoxydim, and imazapyr on a surrogate species, Behr's Metalmark and found that exposure of larvae to these products results in a reduction of the number of adults from 24-36%. The effects of these herbicides on Lange's Metalmark population dynamics were determined using stochastic matrix population models (population viability analysis) and will be discussed in this presentation.

SS07-3

The importance of absorption, elimination and feeding pattern: using toxicokinetics modelling to refine the risk assessment of pesticides to wildlife

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Current risk assessment for mammals is based on external exposure measurements, but it has long been acknowledged that effects of toxicants are better correlated with systemically available dose, which depends on many factors, e.g., bioavailability (fraction of dose reaching systemic circulation or site of physiological activity), absorption, distribution, metabolism and excretion (ADME). Toxicokinetic (TK) models are mathematical descriptions of these processes and can be used to refine risk assessments. In toxicological studies of pesticides and biocides, little internal dose data are routinely generated, and the use of toxicokinetic models in risk assessment for pesticides is relatively new. Moreover, for risk assessment of pesticides, the toxicokinetic (and toxicodynamic) should be interpreted in the context of potential exposure in the field. Therefore, it is key issue to include different timescales of exposure and behavioural factors such as feeding pattern in study. Here, we present a case study for an insecticide. A toxicokinetic model was parameterised using data from a rat study with ¹⁴C-labelled material. The number of compartments (tissues) in the model and the complexity of the processes of absorption and elimination were investigated. Feeding pattern and avoidance of contaminated food were included in the model as they influence internal concentration and hence risk in natural environments. High variability in kinetic parameters between individuals meant it was necessary to test the effects of different combinations of values for absorption k_a and elimination rate constants on concentration in the bloodstream. This was done for different scenarios, e.g. LD50 eaten as a bolus dose and more natural feeding patterns. Maximum internal concentrations (C_{MaxBody}) were compared across scenarios. Our simulations showed that, irrespective of k_a and k_e combinations, higher C_{MaxBody} were reached when the LD50 was given as a bolus dose than when realistic worse-case scenarios were used. Nevertheless, the difference between C_{MaxBody} reached after a bolus dose or feeding naturally depended on which combination of k_a and k_e was used; the lower the k_a and k_e the closer the feeding body burden came to the bolus dose body burden. Ongoing modelling is underway and the impact of avoidance mechanism on the insecticide body burden will be reported. The possible use of body burden model as a refinement option in bird and mammal risk assessments will be discussed.

SS07-4

Mechanistic modeling of the effects of perchlorate on the thyroid gland and risk to post-natal developing mammals

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Mechanistic models can be used to predict the risk to mammal population from exposure to perchlorate. We developed a physiologically-based toxicokinetic (PBTk) model with diffusion mechanisms governing the uptake and distribution of perchlorate. Compartments include blood plasma, liver, kidneys, gut wall, gut contents, and thyroid and pituitary endocrine glands. The thyroid model describes the binding, distribution, and disposal of thyroxine (T₄) and triiodothyronine (T₃) including homeostasis mechanisms. These thyroid hormones inhibit the secretion of TSH by the pituitary, which stimulates secretion of the thyroid hormones. Health risk to mammalian species depends upon the concentrations of perchlorate in consumed food and water. Prenatal and postnatal development may be impacted through perchlorate transfer by the placenta and during lactation. Non-lethal assessment endpoints, such as growth retardation and offspring deformities, define health risk. Several individuals are simulated to obtain population level effects. The model is stochastic, with random variables for residues in water and food items.

SS07-5

Why complexity matters: using ALMaSS for risk assessment of wildlife

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The primary focus of environmental risk assessment (ERA) for non-target organisms is on the direct effect of the toxicant. In some cases what are often termed large-scale sources of variation are also considered (e.g. expected crop distribution). However, these describe only part of the variation that occurs in the real world. Landscapes vary in structure, meaning that the field size and proximity to primary NTO habitats will vary. There is climatic variation driving changes in phenology and behaviour, and management changes in the proportion of crops grown, but also changes in how they are cultivated in time and space. All of these factors can affect the risk assessment. There is also another, difficult to observe, property of real systems, and that is the spatio-temporal dynamics associated with populations, climate, management, and ecology and behaviour, and the potential for feedback loops. These interactions can exacerbate or ameliorate impacts either via local feedback mechanisms e.g. multiple stressors, or by virtue of the spatial population dynamics. This uncertainty is normally considered as stochasticity in ERAs and the factors are often incorporated into a single general term, utilizing a safety factor to account for uncertainty. This, however, robs us of both understanding and predictive power, since probability distribution can only be based on statistical expectations of past events, which do not necessarily account for interactions in the future. An alternative approach capable of dealing with these system properties is agent-based modelling (ABM). ABMs are capable of integrating a range of drivers and actors in space and time and can represent detailed farming operations on a large scale, integrating these with realistic models of animal populations, and expressing each animal as an individual agent. As such they are capable of representing very complex dynamics in time and space. Another key model attribute is the ability of agents to respond to the information they gather from their local environment. This automatically integrates many of the dynamics difficult to capture in traditional models, e.g. source-sink dynamics are emergent properties and do not need to be imposed. ALMaSS is one such model system and is used here to exemplify aspects of complexity in population-level risk assessment for terrestrial mammals, birds, and arthropods exposed to pesticides. Results are used to argue for greater realism and population-level ERA.

SS07-6

Incorporating environmental complexity in assessing chemical risks for soil organisms using mechanistic effect models

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Unlike larger terrestrial vertebrates, such as birds and mammals, most soil organisms have limited mobility and may not as easily be able to seek shelter from environmental extremes or completely avoid contaminated habitats. Yet terrestrial soils are both spatially and temporally variable, and this variability may have an important influence on the susceptibility of soil organisms to toxic chemicals. The most effective way to systematically assess the influence of such environmental complexities on risk is through the use of mechanistic effect models. This talk will provide an overview of the types of mechanistic effect models that have been applied to soil organisms with an emphasis on collembolans. We will summarize the types of questions that have been addressed using models, outline the challenges in model development and implementation, and demonstrate the value added by the use of models compared to traditional methods for risk assessment. One area in need of further work is development of robust predictive relationships between soil organism population dynamics and delivery of those ecosystem services of which such species are key drivers.

SS08-1

Combined Effects of Climate Change and Contaminants on Arctic Ecosystems and Humans

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The Arctic Monitoring and Assessment Programme (AMAP) has produced several scientific assessments documenting the levels, trends and effects of specific groups of contaminants such as heavy metals, persistent organic pollutants (POPs), and radionuclides, and the status, trends and effects due to climate change on Arctic ecosystems and humans. The difficulty of attributing the effects observed to a specific problem or cause triggered an increasing need to analyze the potential combined effects of several contaminants in mixture and also together with the current observed Arctic climate change. Based on this, the project 'Combined Effects of Climate Change and Contaminants in the Arctic' was initiated in 2005. The project has focused on the combined effects of climate change, POPs, mercury and radionuclides in selected parts of the Arctic/sub-Arctic marine and terrestrial food chains and humans. The project was carried out by eight Nordic research groups. The first phase of this project has been completed and an overview of the results is presented here. The research priorities covered the following topics:

- 1) A theoretical analysis of the effects of climate change on the long-distance atmospheric transport and deposition of several selected POPs and mercury in the Arctic using a 3-D atmospheric chemistry-transport model;
- 2) Quantitative analyses of POPs in snow and air from Svalbard and Greenland to study the concentrations and mobility of these contaminants in relation to environmental conditions such as temperature and solar radiation;
- 3) A modeling study in which the effect of climate change, in terms of increased temperature and primary production, on the partitioning and bioaccumulation of organic contaminants in biota at various trophic levels in the Arctic marine environment was investigated and quantified;
- 4) An analysis of changes in the fallout of radiocesium (¹³⁷Cs) and mobility in the Arctic frozen/melt terrestrial ecosystems and its uptake in terrestrial food webs;
- 5) A review of abiotic and biological processes relevant to the concentrations and distribution of mercury in the Arctic environment and bioaccumulation in biota;

6) An analysis of the concentrations of selected POPs and mercury in the blood of several indigenous peoples populations in Arctic Russia as a prelude to a further study of their potential effects on people living mainly on local marine and terrestrial food sources.

SS08-2

Southern Ocean and the Antarctic climate system

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The planetary climatic system is sensitive to the conditions of energy exchange between the ocean and the atmosphere in the Southern Ocean. The interbasin exchange accomplished by the Antarctic Circumpolar Current (ACC) is an important link in the global overturning circulation and also admits anomalies formed in one basin be carried around the globe to influence climate in remote locations. As a result, the Southern Ocean strongly influences climate patterns and the cycling of carbon and nutrients. A reliable knowledge of the quantitative characteristics of energy, mass and gas exchange appears to be a basis for the estimation of possible climatic changes in the near decades. Recent observations suggest the Southern Ocean is indeed changing: Data from Argo floats and research vessels confirm that the Southern Ocean, particularly the southern flank of the ACC, has warmed more rapidly than the global ocean as a whole. The average anomalies for 2005-2011, i.e., water temperature deviations from the mean values in the Southern Ocean turned out to be positive in the upper 1500 m layer with a maximum of +0.2 °C in the layer between 250 and 450 m. The average for the same seven years salinity deviations from mean values indicate weak water freshening in the Southern Ocean with the largest negative deviations of about -0.04 “ achieved at 200 m depth. Strong statistically significant abyssal warming trend in the Southern Ocean between the 1990s and 2000s has been also identified. In addition, the dense bottom water formed near Antarctica has freshened in some locations. The freshening is consistent with increased melt from the Antarctic ice shelves and ice sheet. In recent decades the Antarctic Peninsula and some surrounding ocean areas have warmed faster than anywhere else in the Southern Hemisphere. The role of the Southern Ocean in global heat and freshwater balance, including the stability of the overturning circulation, the stability of the Antarctic ice sheet, the stability of the sea ice cover and the future of Southern Ocean carbon uptake, demands the sustained, multi-disciplinary observations system - the Southern Ocean Observing System (SOOS). There is an urgent and compelling need to make progress in the SOOS development to inform decision-makers confronted with the challenges of climate change, sea-level rise, ocean acidification, and the sustainable management of marine resources.

SS08-3

Persistent Organic Pollutants in Antarctica; System Input from Distant and Local Contaminant Sources

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Persistent Organic Pollutants (POPs) are ubiquitous toxic compounds that are incorporated into food chains with high efficiency. Polar Regions have long been established as receiving environments for POPs. In order to manage environmental contamination by POPs in Antarctica, information regarding system input to this remote region is required.

Here we present the first results of atmospheric input of POPs to the Australian Antarctic Territory in over a decade. Further, we present the first audit of an Australian research base as a local emitter of newly listed POPs and explain biota accumulation of PFCs in terms of species foraging ecology and the dynamics of the Antarctic circumpolar current.

It has recently been shown that climate change is beginning to mobilise global POP reservoirs. Our findings therefore also provide a baseline for temporal monitoring of how input to this remote region stands to be impacted as global secondary sources are perturbed.

SS08-4

Impacts of black carbon on Arctic climate

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Black carbon (BC) particles are products of incomplete combustion. Major emission sources globally are transport and residential heating and cooking as well as wildfires. BC is the most efficient aerosol species absorbing visible light and it exerts a warming effect in the atmosphere. The significance of BC is higher in the Arctic than elsewhere. Its atmospheric light absorption is enhanced by the high albedo of snow and ice surfaces in snow covered areas, and the deposited BC particles in snow and ice reduce the surface albedo and accelerate the melt rate. BC has a relatively short lifetime in the atmosphere, in the order of days, which implies that emission reductions of BC rich sources would lead to fast responses in its climate impacts. However, since it is emitted along with other particulate and gaseous species that have multiple cooling and warming effects, the net-effect of all relevant species should be taken into account when designing emission mitigation strategies. Recent assessments indicate that a set of measures targeting BC could reduce the projected Arctic climate impacts significantly. The reductions would also bring about co-benefits for public health.

SS08-5

Climate change and the effects on Arctic animals

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The Arctic is one of the regions that the Intergovernmental Panel on Climate Change (IPCC) believes will be most affected by climate change. Some of the expected and already documented alterations are: thinning of the ice sheet, less multi-year ice, increased river discharge into the Arctic Basin, accelerating melting of glaciers, warmer surface temperatures and greater inflow of Atlantic water masses. These changes may potentially alter species distributions, food web structures and carbon cycling, and subsequently tropho-dynamics and transport and uptake of POPs into and within the Arctic.

The Arctic environment is also characterised by high seasonality in light intensity, primary production, food availability, lipid concentration in organisms, migration of organisms, and ice cover. These 'naturally occurring' factors influence the availability of POPs and their uptake in the organisms and in food webs.

In the international polar year project 'Contaminants in Polar Regions (COPOL)' we were able to study the links between climate and contaminants. The COPOL project has shown that accumulation of POPs in marine organisms varies with year and season. However, the seasonal pattern in accumulation and magnification differs depending on the chemical and group of organisms involved.

In all species of zooplankton studied, POP concentrations decrease from May to October. This coincides with decreasing POP concentrations in seawater and increasing lipid stores in the plankton during the same period. Investigation of the accumulation and magnification from zooplankton to fish and birds generally identified July as the month when magnification was greatest, since POP concentrations in the fish species and black-legged kittiwakes were highest in July. The fact that POP concentration in zooplankton was observed to decrease from May to October further underscored the magnitude of increased accumulation through the food web. These results indicate that estimates of the uptake and accumulation of POPs are dependent on the time of sampling (i.e. season).

The level of POPs in marine birds varies between years. In kittiwakes (pelagic feeder) the levels of PCBs and DDE were 50% higher in 2008 compared to 2007. In common eider ducks (benthic feeder) concentration levels were reduced by 50-60% from 2007 to 2008. This shows that diet can exert a great influence on contaminant load. Concentrations of POPs continue to be found at high levels in top-level predators such as glaucous gulls, ivory gull and great skua. Studies investigating effects show that these species are influenced by the current contaminant loads.

Knowledge about the significance of seasonality is essential to be able to differentiate between seasonal variation in accumulation and magnification of POPs versus alterations caused by climate change. Identifying possible alterations caused by climate change in a seasonal environment is a complex and difficult task. Clearly, climate change has the potential to alter POP concentrations in the Arctic. However, the degree to which these concentrations will change, and whether a warmer climate will bring a net increase or decrease of POPs in the Arctic, is still uncertain.

SS09-1

Designing an ecosystem approach for ecological risk assessment of radiation: a path forward for radioecology

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The current approach to ecological risk assessment of radiation based upon "reference organisms" evolved from the radio-toxicological methodology designed to support the development of the system of human radioprotection (concept of "reference man"). Exploiting knowledge on the mechanistic aspects of radiation effects on living matter, it integrates therefore classical ecotoxicological data for some test species along different effect endpoints gathered for individual organisms. As such, it relies exclusively on

the dose-response relationships at the organism level. The approach is limited in light of the extent of both, biodiversity and interaction among species in the environment. Founded essentially on organism-related data, such a method cannot fully meet the actual environment protection objectives that have been set, in the vast majority of situations, at the population- and ecosystem-levels. Furthermore, perturbations induced by stressors within ecosystems cannot be entirely grasped from an exclusive toxicological understanding of the stressor's interaction at the organism level. Such effects only act as triggers of perturbation, which propagate within ecosystems, with ultimate responses that may differ radically from those observed at the organism-level (due to non-linear behaviour of complex inter-population relationships). Also, inter-population relationships, such as predator-prey, are capable of mediating indirect effects by means of which the population actually exposed to the stressor may not be the most affected. This is particularly relevant when considering the long-term ecological effect of chronic exposure to toxicants, like radiation, where damage may not be most due to the direct radiotoxicological effect of radiation per se (upon individual organisms), but rather to the build up of imbalances between interacting populations within ecosystems as a result of differences in their sensitivity to radiation. This communication, therefore, will discuss the wide array of justifications supporting to widen the scope towards an "ecosystem approach" for ecological risk assessment of ionizing radiation, as already adopted in other areas of environment protection (biodiversity, halieutics, etc.).

SS09-2

Are we discriminating against ionising radiation? A comparison of environmental risk assessment for radiation and chemical pollutants

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SS09-3

Are adverse effects of uranium mainly due to its chemotoxicity or its radiotoxicity?

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The ecotoxic profile of uranium (U) has not been studied extensively for non-human biota, particularly for aquatic invertebrates. The toxic action of U in organisms potentially originates from both its chemical and radiological properties, the latter depending on the specific activity of U isotopes and their associated energy radiation. However, information about distinguishing the hazardous effects of its chemotoxicity and radiotoxicity on aquatic organisms is scarce. The main aim of this study was to identify the contribution of the chemotoxicity and the radiotoxicity to U effects on mitochondria and the oxidative balance using transcriptional responses (mt, sod(Mn), cox1, atp6, 12S) and enzymatic activity as endpoints (SOD, CAT, GPx, GST). The opportunity was also taken to evaluate the sensitivity of the used biomarkers by comparing the impacts on the different biological levels of organization after a low contamination level. Therefore, groups of crayfish *Procambarus clarkii* were exposed for 4 and 10 days to either 30 µg/L of depleted uranium (DU) or 233U which only differ from each other in their specific activity (DU = 1.7[GREEKX]104 Bq.g⁻¹, 233U = 3.57[GREEKX]108 Bq.g⁻¹) and hence in their radiotoxicity. The chosen U concentration is close to some environmental values measured near U mining sites and is twice higher than the world health organization recommendation value for drinking-water. U accumulation levels were measured in different organs (gills, hepatopancreas (HP), stomach, intestine, green gland, muscles, and carapace) whereas biological effects of the different types of U were evaluated only in the gills and the HP, because they provided sufficient amount of tissues for conducting various analyses on the same organ. In order to evaluate the radiotoxicity of both DU and 233U, internal dose rates were calculated with EDEN-2.2 software in the HP for the two sets of experiments. Results showed a significant U accumulation in organs of *P. clarkii* and some effects on the studied biological parameters (mitochondrial damage and antioxidant response). But despite the huge difference of the specific activities (21000x) between DU and 233U, few significant differences in biological responses were noticed for these two pollutants, indicating that the radiotoxicity was low compared to the chemotoxicity in our experimental conditions. Finally, endpoints measured by genes expression levels gave more sensitive responses than those expressed by enzymatic activities.

SS09-4

Importance of the biological role of elements and the trophic level of organisms for transfer of radionuclides to biota

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New requirements in environmental radiation protection imply an increased need for estimations of exposure to biota from radionuclides by risk assessors. A large number of radionuclides that will require assessment in combination with knowledge gaps and complex relations for many radionuclides concerning uptake in biota increases the need to make generalizing assumptions in risk assessments. If an element is biologically essential for an organism this may increase the likelihood of it being taken up, e.g., from food or soil. By that principle, a radioactive isotope of an essential element or a radionuclide with similar characteristics as an essential element might be more likely to be taken up by and accumulated in an organism than a non-essential element. Furthermore, the position an organism has in the food web, i.e., its trophic level, can affect its uptake of radionuclides. For example, uptake pathways for primary producers and consumers vary greatly. This study explores the importance of the biological role of elements and trophic level of organisms for the transfer of radionuclides to biota. The concentration ratios (CRs) for radionuclides and reference organisms in the ERICA tool (July 2007) that originate from empirical data and the new CRs compiled by IAEA and ICRP in the Wildlife Transfer Database (June 2011) for terrestrial, freshwater, marine and brackish ecosystems were used to study patterns in the transfer of radionuclides to biota. The CRs were divided into three element groups on the basis of their biological requirement (major essential, trace, and non-essential). Further, the reference organisms were divided into groups of trophic level (primary producers, consumers etc). The results show that CR values are different for the ecosystems. Moreover, results suggest that for terrestrial biota radioisotopes of essential elements and radionuclides with similar characteristics as an essential element are more likely to be taken up than radioisotopes of non-essential elements. In aquatic ecosystems trace elements have highest CR values. Furthermore, primary producers in all ecosystems and primary consumers in aquatic ecosystems have higher CR values than higher trophic levels. Recognizing the large knowledge gaps for many radionuclides and organisms concerning transfer to biota this type of grouping of elements and organisms can save resources in future risk assessments.

SS09-5

Effects of chronic radiation exposure on plant populations

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One of the major difficulties in the implementation of an ecological risk assessment is a lack of knowledge about the effects from chronic low-level exposures to radioactive contaminants. To understand effects of real-world contaminant exposure properly we must pay attention to what is actually going on in the field. However, for many wildlife groups and endpoints, there are no, or very few, studies that link accumulation, chronic exposure and biological effects in natural settings. The results of long-term field observations in the 30-km Chernobyl NPP zone, in the vicinity of the radioactive wastes storage facility (Leningrad Region), at radium production industry storage cell territory (the Komi Republic), in the Bryansk Region affected by the Chernobyl accident, and in Semipalatinsk Test Site, Kazakhstan that have been carried out on different species of wild and agricultural plants are discussed. Although radionuclides cause primary damage at the molecular level, there are emergent effects at the level of populations, non-predictable from the knowledge of elementary mechanisms of the pollutants' influence. Plant populations growing in areas with relatively low levels of pollution are characterized by the increased level of both cytogenetic alterations and genetic diversity. Accumulation of cellular alterations may afterward influence biological parameters important for populations such as health and reproduction. Presented data provide evidence that in plant populations inhabiting heavily contaminated territories cytogenetic damage were accompanied by decrease in reproductive ability. In less contaminated sites, because of the scarcity of data available, it is impossible to establish exactly the relationship between cytogenetic effects and reproductive ability. Radioactive contamination of the plants environment activates genetic mechanisms, changing a population's resistance to exposure. However, there are ecological situations in which enhanced resistance has not evolved or has not persisted. Consequently, there are good theoretical and practical reasons for more attention being paid to the mechanisms by which populations become more radioresistant and to those situations where radio-adaptation appears not to be taking place. Since radio-adaptation plays an important role in response of populations on radiation exposure, this process needs to be incorporated into management programmes.

SS09-6

Analysis of bacterial diversity in a Chernobyl contaminated soil by pyrosequencing

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Chernobyl and recent Fukushima Daiichi nuclear disaster revives the importance of understanding the transfer of radioactive contamination in the environment and its ecological consequences. While some studies have been performed on higher organisms, only a few focused on bacterial communities. It is however well known that bacteria play an essential role in contaminant mobility in soils by lowering or enhancing their transfer to other compartments (e.g. water, plant, animals). Radionuclides (RN) contaminants

might also exert toxic effects on bacteria hence inhibiting their role in the transfer. Thus, the objective of this study was to evaluate the impact of RNs contamination at the bacterial community level by the determination of its phylogenetic diversity. Following the Chernobyl nuclear accident, contaminated soils, vegetation and other radioactive debris were buried in situ in trenches. In this area, the trench n° 22 has been a pilot site for the study of RNs migration in soil for many years. In a previous study, the bacterial diversity was estimated on a set of contaminated and control soil samples on this pilot site using a genetic fingerprint method (DGGE). This analysis revealed the presence of complex communities in the soils but did not give access to the taxonomic diversity. To refine the results, an in depth analysis of the same samples has been conducted by a new high throughput sequencing method, pyrosequencing, leading to 19,000 sequences per sample in average. This molecular technique gave access to unprecedented results and evidenced a huge diversity in the soils with 963 genera and 39 phyla represented. The 4 most predominant phyla, detected in all samples, were Chloroflexi, Proteobacteria, Acidobacteria, and Verrucomicrobia. These data demonstrated definitively that a long term exposure to RNs did not lead to the decrease of bacterial diversity as concluded from the DGGE analysis. However, statistical analysis of the pyrosequencing data evidenced a distinction of bacterial community between contaminated and control samples, suggesting the presence of RN adapted species in the contaminated samples. The pyrosequencing data will guide us for the selection of a model bacteria among a collection of 250 culturable isolates retrieved from these contaminated and non contaminated areas. This model strain will be further used for laboratory experiments to study interactions with representative RNs of the trench (137Cs, U, 90Sr).

SS09-7

Bioaccumulation of Fukushima-derived radionuclides by local marine biota

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The earthquake and tsunami related failure of the nuclear reactors at the Dai-ichi power plant in Fukushima caused radiocesium release amounting to the magnitude of petabecquerels. Two thirds of this release were absorbed by the ocean, raising concerns of radiocesium (i.e. 134Cs and 137Cs) bioaccumulation by resident marine organisms and subsequent trophic transfer in the marine food chain. During an international cruise in June 2011, samples of water, zooplankton, and micronektonic fish were collected and later analyzed for gamma-emitting radionuclides. To obtain sufficient biological material for radioanalyses, bulk samples of zooplankton (>0.3mm) and micro nektonic communities (>4mm) were obtained by oblique tows using Bongo and Methot nets. The naturally occurring 40K dominated the total gamma radioactivity in the biota. Generally, radioactivity levels in biota were higher nearer the coast, with concentrations reaching 46 Bq kg⁻¹ dry wt for 134Cs and 56 Bq kg⁻¹ for 137Cs. Crustacean zooplankton displayed higher radioactivity levels than gelatinous zooplankton. We calculated the concentration factors for mixed zooplankton at all stations, which ranged from 9 to 352 with an average of 40, by using Cs concentrations in biota and seawater. Taxonomic analyses revealed that while all zooplankton samples were copepod dominated those that also included copepod consuming chaetognaths had higher concentration factors suggesting Cs biomagnification in the food chain. The ratios of 134Cs:137Cs in both seawater and biota samples were ~1, consistent with reports for their release into the coastal ocean. While no 110mAg was detected in seawater, zooplankton but not fish contained elevated concentrations ranging from 0.5 to 21.5 Bq kg⁻¹ dry wt.

SS09-8

The effect of radionuclide contamination of the Yenisei River on cytogenetic characteristics of aquatic plants

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The Yenisei River, one of the world's largest rivers, is contaminated with artificial radionuclides released by one of the Russian facilities producing weapons-grade plutonium (the Mining-and-Chemical Combine, MCC), which has been in operation for many years. Aquatic plants are an important component of water ecosystems, which can accumulate high levels of radionuclides and, thus, can be used in biomonitoring and bioremediation. Internal dose rates to aquatic organisms of the Yenisei River were previously estimated for the area near the MCC. The water moss (*Fontinalis antipyretica*) accumulated the largest artificial exposure dose among the study aquatic organisms. Preliminary results showed that at the MCC discharge site the occurrence of chromosomal aberrations in cells of *Elodea* was considerably higher than in the control areas. However, plants growing in other parts of the Yenisei, including those with elevated uranium levels, have not been either analyzed for radionuclides or examined cytogenetically. The purpose of the study was to assess levels of radionuclides and to evaluate the frequency of chromosomal aberrations in samples of submerged plants, collected in different parts of the Yenisei River. The following species were studied: *Fontinalis antipyretica*, *Batrachium kauffmannii*, *Myriophyllum spicatum*, *Elodea canadensis*, *Ceratophyllum demersum* and various Potamogeton species. Detailed analysis of radioactive contamination of aquatic plants of the Yenisei River revealed large-scale contamination of aquatic plants as far as 250 km downstream of the MCC. About 30 radionuclides, including uranium and transuranium elements, were detected in the biomass of aquatic plants. The highest concentration factors of the major radionuclides were obtained for *Fontinalis antipyretica* and *Potamogeton lucens*. Results of cytogenetic investigations of aquatic plants suggest that at the MCC discharge site and downstream the occurrence of chromosomal aberrations in anaphase and metaphase cells of the plants was considerably higher (up to 30%) than in the control areas (6%). Cytogenetic studies of *Elodea canadensis* samples collected at positions with elevated uranium levels (and decreased levels of artificial radionuclides) showed that the overall frequency of chromosomal aberrations reached 18%. Thus, not only artificial radionuclides but also uranium concentrated in the biomass of aquatic plants can be responsible for cytogenetic aberrations observed in them.

SS09-9

Speciation, bioavailability and toxicity of uranium in different *Lemna minor* growth media

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Anthropogenic activities have led to a local increase of U concentration to levels that pose potential ecological risks. Uranium toxicity is known to depend on its redox state, speciation and physiological form. The speciation of U strongly varies with parameters such as pH, dissolved organic matter, carbonate and phosphate concentrations and water hardness. The objective of this work was to investigate the bioavailability and toxicity of U to the freshwater macrophyte *Lemna minor* L. using the standard growth inhibition test [1]. However, as the bioavailable fraction of U strongly depends on the medium composition the total U concentration, speciation and growth of *Lemna* in different growth media was evaluated. Three different growth media were selected based on their previous use in *Lemna* growth inhibition tests: (i) the OECD medium [1], (ii) K-medium [2], and (iii) a synthetic freshwater [3]. For each of the media the pH, CO₃ and phosphate concentrations were varied. As expected the OECD medium with normal phosphate concentrations (13.4 mg/L) sustained growth (figure 1A). Changing the pH (figure 1A) or omitting CO₃ from the medium did not affect any of the tested growth parameters. On the other hand lowering the phosphate levels in the OECD medium clearly negatively influenced growth rate. In contrast for the K-medium it was shown that lowering the phosphate concentration did not adversely influence the growth rate (figure 1B). Only when no phosphate was added the growth rate was below 0.250 average and as such also below OECD guidelines. Finally none of the tested Synthetic Freshwater compositions could sufficiently sustain growth of the *Lemna* plants (average growth rate 0.15/day). Towards the bioavailability of U only in the K-medium with phosphate concentrations of 0.5mg/L or below U in solution could be retrieved. A dose-response curve for U was set up (0.05µM up to 500µM). In the K-medium with low phosphate concentrations U concentrations from 50µM or higher induced more than 50% growth inhibition in the plants. In contrast in all other tested media similar concentration did not induce growth inhibiting effects probably due to a changed U speciation. The speciation of U in the different media is currently under investigation. [1] OECD, 2006 *Lemna* sp. growth inhibition tests. Guideline 221 [2] Cedergreen, N, et al., 2007 *Env. Tox. Chem.* 26(1):149-156. [3] Charles, AL, SJ Markich, and P Ralph, 2006 *Chemosphere*. 62(8):1224-1233.

SS09-10

Change of radiocesium concentration in tree leaves before and after abscission

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Due to the Fukushima Daiichi Nuclear Power Plant (FDNPP) accident, several large releases of radionuclides occurred in March, 2011, and in early April, radionuclide releases had dropped compared to the amounts released in March. National Institute of Radiological Sciences (NIRS) located in Chiba, where 220 km away from FDNPP, also had radioactive deposition from FDNPP in March. In newly emerged tree leaves collected at NIRS in late April 2011, radiocesium were found although they had not contaminated directly from the deposition. According to the previous reports and our observation 1-3), we concluded that radiocesium was absorbed from tree surface, e.g. leaves (old leaves of evergreen trees) and stems; radiocesium uptake through roots would be negligible.

In autumn, deciduous trees shed leaves, and a part of leaves fall from evergreen trees. It is known that some nutrients in grass leaves withdrawn before abscission 4), therefore, we thought it would be possible that leaves return radiocesium to tree stems and trunks before leaf-fall. If so, radiocesium remained in trees for a long time period.

Tree leaf samples both living and dead were collected from 13 trees (9 species) in November, 2011 at NIRS. Radiocesium concentrations as well as stable elements, e.g., Mg, Al, Fe, Cu, Zn, Sr, Cs, etc., were measured. Radiocesium and stable cesium concentrations decreased in dead leaves than that in living leaves for deciduous trees, while evergreen tree leaves tended to show no change before and after fall. Potassium in dead decreased for all the trees except a ginkgo tree. Thus, for most trees, Cs in leaves could be withdrawn to the tree body before fall, and which possibly could be depended on tree types and nutritional condition of the tree. More details will be discussed at the presentation.

This work has been partially supported by the Agency for Natural Resources and Energy, the Ministry of Economy, Trade and Industry (METI), Japan.

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SS09-11

Dose-dependent effects induced by uranium at pH 4.5 in *Arabidopsis thaliana*

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To evaluate the environmental impact of uranium (U)-contamination, it is important to unravel the mechanisms by which plants respond to U-stress. It was already shown that U-exposure at pH 5.5 can disrupt the cellular redox balance and induce oxidative stress related responses in *Arabidopsis thaliana* plants (Vanhoudt et al., 2008). However, U-speciation and as such its toxicity strongly depend on environmental parameters such as pH. In a previous experiment, it was shown that U-toxicity at pH 4.5 was higher than at pH 7.5. Therefore, we want to investigate dose-dependent effects at low pH. *Arabidopsis thaliana* plants were exposed to U-concentrations ranging from 0 to 100 µM at pH 4.5 during 3 days. U-concentration, fresh weight, lipid peroxidation and photosynthetic efficiency were analysed. On protein level, the enzymes of the antioxidative defence system were analysed to evaluate the importance of the cellular redox balance in *Arabidopsis thaliana* plants exposed to U. Results indicate that the U-content in roots and shoots increased with increasing U-concentration added to the nutrient solution. However, there was a low root-to-shoot transfer. Fresh weight of roots and leaves decreased after exposure to 50, 75 and 100 µM U. In contrast, plants exposed to 6.25 and 12.5 µM U had an increased fresh weight as compared to the control plants, which alludes to a hormesis effect as was observed before (Vanhoudt et al., 2008, Straczek et al., 2009). One of the most important physiological processes in plants is photosynthesis. Apparently, the photosynthetic efficiency of plants exposed to 25-100 µM U was increased. This indicates that the photosynthetic system is not damaged but in contrast plants try to optimize their photosynthesis under U-stress. In addition to the reduced biomass production, plants exposed to 25-100 µM U showed an increased lipid peroxidation. This increase indicates an affected membrane integrity and functionality. Assessing antioxidative enzyme capacity of the plants indicated an increased defence against ROS in the U-exposed plants as evidenced by increased activities of ROS scavenging enzymes. The increased activity of guaiacol peroxidase could indicate an increased cell wall lignification as a defence reaction that limits the entry of toxic metals. In conclusion, this study indicates that elevated U-concentrations at low pH can cause important morphological, physiological and biochemical effects in *Arabidopsis thaliana* seedlings.

SS09-12

Environmental sensitivity as a tool for the risk assessment of the use of nuclear energy

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Approaches to the management of the risk in radioecology have to take into account geographic, climatic, living and dietary habit differences and in more detail ecosystem differences. The understanding of the factors of sensitivity of different environments, populations or geographic areas is important for scientists and policy makers, to set priorities for the allocation of limited resources. Furthermore the identification of vulnerable environments will be valuable in planning the locations of new nuclear facilities. A Task Group on Radioecological sensitivity was organized by the International Union of Radioecology (IUR) in 2007 on the basis of studies of the Radioecological Sensitivity Forum, 1998-2001. The objective was to discuss a standardization to represent the radiological state of the environment following accidental pollution and a scale of radioecological sensitivity of areas, useful in emergency planning and preparedness. The work of the Group continued under the International Atomic Energy Agency (IAEA) EM-RAS II Programme, from 2009 to 2011, as Working Group 8 on Environmental Sensitivity. The WG8 focused its studies on sensitive non-urban environments. The aim was to investigate which environments, which components of each environment, and which seasons of the year would be most sensitive to a major release of radionuclides. The overall aim is to aid in the planning and implementation of emergency as well as long-term countermeasures following a nuclear accident. Sensitivity analysis was performed for different environments: temperate agricultural and alpine (Europe and Canada), coastal marine (Nordic seas, North-East Aegean Sea, Thermaikos Gulf Mediterranean Sea), temperate forest (Northern Saskatchewan and Canadian), freshwater aquatic (Norway, Italy, Northern Saskatchewan) and Arctic (Northern Canada). Each environment was to receive the same deposition of ¹³⁷Cs, ¹³¹I and ⁹⁰Sr. The concentrations of these radionuclides in key environment compartments as functions of time after the event were calculated, and also the doses to human populations who receive most or all of their food intake from the respective environments. Models participating to the work were CHERPAC, ECOSYS, FDMT-RODOS, HealthCanada model, IMPACT, MOIRA-PLUS, NRPA box model, NTUA 3D model. Results are presented, showing as the interaction of environmental but also of social and economic factors plays a role in defining the radiological sensitivity of geographic areas.

SS09-13

Agricultural land management options following large-scale environmental contamination

HA Vandenhove

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The accident at the Fukushima Dai-ichi Nuclear Power Plant has raised questions about the accumulation of radionuclides in soils and the transfer in the food chain. Following a large-scale nuclear accident, the application of countermeasures is a key issue. Numerous countermeasures were developed since the Chernobyl accident and applied on large scale. This presentation discusses countermeasure strategies and their effectiveness and feasibility against the background of the Fukushima Dai-ichi nuclear accident and the agricultural areas affected. Land management options should be selected based on a criteria such as effectiveness; constraints on implementation; wastes generated and waste management options; doses received during implementation; side-effects; cost/benefit considerations; acceptance (stakeholder opinion). Mechanical (soil removal, ploughing) and agrochemical (e.g. application of fertilizers or caesium sorbents) management options will be discussed in terms of their potential effectiveness in reducing the soil-plant transfer but also potential side effects will be highlighted. Specific attention will be attributed to andosols, a prevailing soil group in Japan. In addition phytomanagement options such as food crop selection and cultivation of technical crops (e.g. bioenergy crops, fibre crops) will be briefly discussed. Phytoremediation will be critically examined. Land management options will be evaluated based on available information on soil contamination levels, soil characteristics, food and non-food crop production and conversion systems in Japan and following interaction with institutes belonging to NARO (Japanese National Agricultural and food Research Organisation), and considering the evaluation by international teams of the remediation activities in Japan. For optimizing agricultural management options, a good knowledge on agricultural practice and soil characteristics is required. Alternative land uses for areas where contamination levels are considered too high, need careful evaluation. Planning for agricultural management options requires a holistic approach, considering radioecological, radiological, environmental, economic and socio-cultural and political aspects.

SS09P-1

The concept of radiocapacity: theory and application for estimations of ecological risks in ecosystems

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Russian Federation

After the accident in Chernobyl, the most part of Ukraine has been contaminated with cesium-137. Meanwhile, cesium is the closest analogue of potassium that is known as the macro element of great importance. Therefore, ¹³⁷Cs has turned out to become the inescapable tracer in biological objects of all ecosystems in Ukraine, almost without exceptions. Thus, there is every reason to believe that the monitoring of this radionuclide can be employed for quantitative estimations of robustness of biota in the ecosystems. We suggest the concept of radio-capacity, as the novel approach, for estimation and prognosis of the states of ecosystems biota exposed to unfavorable physical and chemical factors. The radio-capacity is defined as the threshold (limit) amount of ¹³⁷Cs, i.e. - the amount at which there are still no visible violations in the basic functions of biota, such as the capability to retain the biota biomass and the environmental conditions. Besides, basing on this concept and the theory of reliability, we have developed the mathematical model of the radio-capacity. Comparison analysis of the data for different kinds of ecosystems, including terrestrial, aqueous, forest, grasslands and urban landscapes, demonstrates that the parameters of our reliability-theory model are adequately sensitive to the impacts of γ -irradiation and heavy metals on the biota. We show that both, distribution and redistribution, of the cesium-tracer in aqueous and terrestrial ecosystems neatly follow all essential external factors, like the changes in climate, floods, contra-measures, and so on, as well as they respond to different kinds of contaminations, like the ionizing radiation, heat fluxes from atomic power stations and chemical pollutants. The higher is the radio-capacity factor, the greater is the biota robustness and the less is the ecological risk for the biota. Moreover, we have demonstrated that the ecological risk for the biota inevitably and essentially increases when the radio-capacity of the biota decreases under the influence of the pollutants. Furthermore, we have demonstrated the key role of the reparation and restore processes on exposure of the biota simultaneously to radiation and chemical pollutants. Therewith, the interference of the different factors may change from synergism to antagonism. Thus, it may be concluded that the radiocapacity holds much promise as the unified equidimensional parameter for quantitative estimations of impacts of different factors on the ecosystems biota. In addition, basing on the radio-capacity concept, we suggest the method of ecological standardization that is prospect to determine the threshold levels of pollutants acceptable for the ecosystems biota, as well as to assess the levels of ecological risks.

SS09P-2

Radiocesium distribution in a bamboo forest

Due to the accident of Fukushima Daiichi Nuclear Power Plants (FDNPP) occurred on 11 March, 2011, some areas were contaminated with radionuclides. Since one year has been almost passed, we can find largely released, long-lived radionuclides in the terrestrial environment, that is, Cs-134 and Cs-137. The behavior in forests is of interest because large areas are covered by forest in Japan. In this study, we focused on bamboo forest, which is commonly found in Asian countries. Since Japanese use young bamboo shoot as a food materials and bamboo stems as various craftwork products, radiocesium behaviors in bamboo forests are of interest.

We collected stems, leaves, shoots, litter, and soil samples of a bamboo forest (Ibaraki Prefecture) on 8 May, 2012 to see the distribution about 2 months after the large radionuclide releases from FDNPP. The results showed that most radiocesium was on bamboo body and litter layers; the concentrations of Cs-137 in twigs, leaves and litter samples were 0.9, 6.4 and 9.6 kBq/kg-wet, respectively. However, the concentration in soil was 0.5 kg/kg-dry, possibly because of the interception effect by the litter layer. The young bamboo shoots contained some radiocesium due to translocation from old bamboo trees. About 30% of radiocesium was removed from the young bamboo shoot by boiling. In October, we collected samples from the same forest again. The Cs-137 concentrations in the leaves decreased by ca. 100-fold, and that in the litter layer was ca. 6-fold. Bamboo trees in Japan shed their leaves in late spring, and thus Cs concentration decreased dramatically in leaves. On the other hand, Cs-137 concentration in the soil samples increased by three times; Cs leached from the dead leaves to the soil. Since radiocesium concentration in the bamboo tree decreased, we expect that radiocesium concentration in young shoot will be much smaller in this year than that we observed in the last year.

This work has been partially supported by the Agency for Natural Resources and Energy, the Ministry of Economy, Trade and Industry (METI), Japan.

SS09P-3

The activity of uranium, thorium and some decay products in chickens and eggs

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The ingestion of radionuclides during food consumption can represent major source of radiation exposure to humans. Uranium, thorium and their decay products, are naturally present in the environment and can be transferred to animals and animal products. Even if the background levels in animal products don't represent a significant risk due to the low activities of radionuclides, their evaluation is important. Indeed, the assessment of the magnitude of a contamination due to accidental or chronic releases of radionuclides depend on the knowledge of the natural activities. The present study aims in the first instance to quantify the activities of uranium, thorium and their decay products in chicken meat and eggs in five regions of the French territory. The highest activities (mBq.kg⁻¹ fresh weight) are measured for radium isotopes in eggs and range between 136 and 190 for ²²⁶Ra. In comparison, uranium activities in eggs are much lower and range between 0.51 and 1.30 for ²³⁸U. In chicken meat, ²³⁸U activity concentrations show higher values than in eggs and range between 1.7 and 9.7. Concerning ²³²Th, its activities are lower than those of ²³⁸U and range between 0.5 and 4.9. Secondly the contribution of the potential sources of the radionuclide for chicken meat and eggs (grain mixture, soil particles and drinking water) are studied in order to calculate the concentration ratios and the transfer factors. These values could enrich the data given by the technical report series N472 of IAEA (2010). In this report the mean transfer values for uranium in chicken meat and eggs (0.75 d.kg⁻¹ and 1.1 d.kg⁻¹ respectively) are only based on two results from the study of Prister (1967). Moreover no concentration ratios are given up to now in the literature for uranium, radium and thorium in eggs and chicken meat. It is important to complete this database, to provide reference transfer coefficient values which could be used to predict the behaviours of radionuclides in the environment.

SS09P-4

Annual intakes of ²²⁶Ra, ²²⁸Ra and ⁴⁰K in staple foodstuffs from a high background radiation area in the southwest region of Cameroon

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Annual intakes of ²²⁶Ra, ²²⁸Ra and ⁴⁰K in staple foodstuffs from a high background radiation area in the southwest region of Cameroon

Concentrations of naturally occurring radionuclides ²²⁶Ra, ²²⁸Ra and ⁴⁰K were determined in five most consumed vegetables in a high-level background radiation area (HLBRA) in the southwest region of Cameroon. A total of 25 foodstuff samples collected from Akongo, Ngombas, Awanda, Bikoué and Lolodorf rural districts were analyzed by gamma spectrometry. The average activity concentration values of ²²⁶Ra, ²²⁸Ra and ⁴⁰K were respectively 2.30, 1.50 and 140.40 Bq kg⁻¹ fresh-weights. The effective dose for individual consumption of the investigated foodstuff types was calculated on an estimated annual intake of such diets for Adults, Children and Infants in the study area. The estimated total daily effective doses from the ingestion of the investigated foodstuffs for each studied long-life natural radionuclide were respectively 0.41μSv for ²²⁶Ra, 0.84μSv for ²²⁸Ra and 0.71μSv for ⁴⁰K. The total annual effective dose was estimated at 0.70 mSv y⁻¹. ²²⁸Ra (44%) and ⁴⁰K (36%) were found to be the main sources for internal irradiation which is very likely due to the specific uptake of these radionuclides by the studied plants.

SS09P-5

Incorporating plant physiological pathways to mechanistic modelling soil-to-plant transfer of radionuclides

T. Sauras, J. Casadesus, R. Vallejo

BIORUR is a mechanistic model developed to assess the soil-to-plant transfer of radionuclides. BIORUR algorithms assume that the movement of radionuclides in an ecosystem and inside the plants is through the same network of pathways than nutrient cycling. The fluxes of radionuclides between compartments inside an ecosystem or a plant are estimated from the flux of a nutrient analogue between these compartments, the ratio of concentration radionuclide to nutrient and a selectivity coefficient which compares the affinity of the pathway for radionuclide to that for its nutrient analogue. One advantage of BIORUR algorithms is the flexibility to adapt the model to a wide range of scenarios where the only requirements are that nutrient cycling have been quantified and that each radionuclide must be associated to a nutrient analogue. However the scope of radionuclides is restricted to those which are associated to a nutrient analogue, such as Cs-K, Sr-Ca. BIORUR model incorporates some of the biological processes relevant to radionuclide availability such as plant physiology, mycorrhizal transfer, organic matter mineralisation and micro-organism. In order to study the plant physiological mechanisms involved, we have developed a hydroponics plant growing system in which we manipulate the plant-environmental processes affecting the uptake of radionuclides (i.e. transpiration, plant growth rate, nutrient demand, nutrient supply). The experimental system permits to verify the mechanisms of Cs and Sr uptake already included in BIORUR.

SS10-1

REACH: what can be learned from the first registration deadline?

W. De Wolf

European Chemicals Agency - ECHA, Helsinki, Finland

The first REACH registration deadline for substances produced at 1000 tonnes or more per year, carcinogenic/mutagenic/reprotoxic substances (CMRs) and substances produced at 100 tonnes or more which are classified as R50/53 has passed 30 November 2010. ECHA has started testing proposal evaluations and compliance checks on registration dossiers. In 2011 for instance, ECHA has started 472 testing proposal evaluations and completed 146 compliance checks. This presentation will give feedback from ECHA on the first REACH registration deadline, with an emphasis on environmental hazard and exposure data. Main topics are: 1) amount of data submitted, 2) type of data submitted, 3) quality of the data, 4) feedback from evaluation, 5) lessons learnt and recommendations for the next registration deadline of 31 May 2013, 6) opportunities for scientific developments from data mining from public registration data. Substance evaluation and authorisation will be briefly discussed as well..

SS10-2

REACH and Science

D. Merckel

Environment Agency, Oxfordshire, United Kingdom

This talk will explore scientific work the Chemicals Assessment Unit (CAU) of the Environment Agency has undertaken that is directly relevant for chemical hazard and risk assessment under REACH. Aspects of our work as they relate to the existing legislation, and to research and development in the field of bioaccumulation will be covered. In the last two years there have been a number of significant revisions for the regulation and testing of PBT (persistent, bioaccumulative and toxic) and vPvB (very persistent, very bioaccumulative) chemicals in the EU. Annex XIII of REACH, which lists the PBT/vPvB criteria, has been revised introducing additional ways to capture substances as being 'PBT-like', or of equivalent concern to PBT substances, even though they do not meet the numerical criteria of degradation half-life, aquatic bioconcentration factor and long-term aquatic toxicity. Some of these additions to the annex will be discussed and illustrated with practical examples that CAU have been working on. The OECD Test Guideline (TG) for measuring aquatic bioconcentration (TG 305), a very important guideline for both risk assessment and PBT assessment, has been the subject of a revision over the last four years. CAU, on behalf of the UK and in partnership with the Netherlands and Germany, led on this work. The revisions mean that fewer animals can be used in the existing aqueous test in certain cases and, for highly hydrophobic substances, a dietary exposure test has been added. The revised test guideline has more detail for interpreting test results, as our understanding of important factors in laboratory aquatic bioaccumulation testing has grown. The major changes concerning data treatment and interpretation relevant for both exposure methods (e.g. fish growth, kinetics, metabolism, etc) and specific for each method (e.g. kinetic BCF derivation, BCF estimation from dietary study data) will be presented with reference to chapter R.7.10.1 of the REACH technical guidance and desk-based research and development work published by CAU. Finally, the relevance of the revised test guideline for B testing under REACH and the apparent changing emphasis of B assessment will be briefly explored.

SS10-3

CSR Preparation Experiences - Science Behind the CSR

L.I. Sweet

Lubrizol, United States of America

Important lessons can be learned from the CSR preparation work already conducted for the first REACH registration deadline. While the Chemical Safety Assessment (CSA) and Chemical Safety Report (CSR) documents the conditions under which the risks associated with the use of a substance during its life cycle are managed to a level that is considered safe to humans and the environment, it can also be a reflection of the state of the regulatory science, tools, and resources used to understand chemical hazard, exposure, and risk. This presentation highlights a number of key areas in the CSR where use of the state of the science, weight of evidence, and read across is especially important; including PBT assessments, PNEC and DNEL calculations, environmental modeling, release factors, and others; and provides insights to help industry put the best science forward in complying with this complex legislation.

SS10-4

Updates of dossiers in light of new scientific data: expectations on what is regarded as new in a practical way. What are the problems and challenges for the future?

P. Douben
REACHWise, Nederland

REACH places a duty on registrants to keep their dossiers up to date. Amongst those possible update reasons is new scientific data. This begs the question what the boundaries of this concept are, and what it means in a practical way. Does the discovery in a lab trigger updating obligations or not? In other words what are possible criteria, time line, etc. The issue cannot be seen in isolation but must be placed in the entire framework of operations in which companies operate to meet their legal obligations.

SS10-5

What can be learned from the first registration deadline? Expectations for the next registration deadline

N Nimpuno
ChemSec, GÖTEBORG, Sweden

NGOs had very high expectations on the first registration deadline. At last, large amounts of information on individual substances would be published by ECHA, giving the general public, authorities, scientists, as well as NGOs, access to high quality environmental information for all high tonnage substances. However, the reality has instead caused concern due to delays in dissemination and because important information has not been published, in particular each registrant's name and tonnage, thus making it impossible for non-authorities to connect the submitted information to a responsible company. Further, many registration dossiers have proven to be of poor quality, and there are data gaps for key parameters from higher-tier studies such as endocrine disrupting properties, persistence and environmental fate. The lack of correct and sufficient data in the registration dossiers can adversely affect enforcement, with enforcing activities already strained. For the coming registration deadline in 2013, an emphasis must therefore be placed on correctly compiled registration dossiers from companies and a rapid dissemination of the submitted information by ECHA. NGOs call for the dissemination portal to flag the status of assessing confidentiality claims on data that has been claimed as confidential by the registrants. NGOs also call for other information such as the presence and amount of SVHCs in imported articles should also be disseminated, ideally by function/ product group and volumes. NGOs want to have such information to have input into the processes for dealing with substances of concern, i.e. restrictions, candidate list prioritisation and of uses under the authorisation procedure.

SS10-6

ITS Schemes for Human and Environmental Toxicology in REACH

G. Schüürmann
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Through the implementation of the European chemical legislation REACH, alternative methods have gained an increased importance as non-animal tools for the toxicological assessment of chemical substances. In this context, the 3R principle of reducing, refining and replacing animal testing has resulted in a paradigm shift: While the original idea was to replace a given animal test by a single in vitro alternative (1:1 replacement), the integrated testing strategy (ITS) approach aims at exploiting the combined information generated from several (n) non-animal methods in order to - partly or fully - replace the respective animal experiment (1:n replacement). In the presentation, the ITS concept and its major components covering non-test (in silico) and test (in chemico, in vitro, omics) methods as well as information theory are outlined. For illustrating their way of application, both human and environmental toxicology endpoints are taken, building on respective results as developed within the EU-funded OSIRIS project.¹ Particular emphasis is put on the mode of action as guiding principle for extrapolating, and on consensus evaluation as a practical tool to characterize and improve the level of confidence in ITS scheme applications.

[1] OSIRIS. Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Test Information. EU Project, contract no. GOCE-CT-2007-037017, 2007-2011. OSIRIS project website: <http://www.osiris-reach.eu/>. OSIRIS webtool website: <http://osiris.simppl.com/OSIRIS-ITS/welcome.do>.

SS10-7

From stress genes to toxic effects and vice versa. A risk assessment perspective

B. Piña
IDAEA-CSIC, Barcelona, Spain

Toxicant-induced changes in gene expression often constitute the very first level of a cascade of events that result in toxic effects at the whole-animal level. Their analysis provides valuable information about the mode of action of the toxicant and, in many cases, sets molecular concentration thresholds below which no effect is predicted. However, and since the analyses of single regulatory events are often perceived as not representative for systemic effects, regulatory toxicology is still reluctant to fully incorporate these technologies. Recent major advances may provide a bridge between molecular responses and whole-organism effects. The ability to characterize effects at the whole genome level allows the identification of targets for each toxicant or for different physiological modes of action. In addition, our ever-increasing knowledge of cell regulatory networks facilitates the systemic interpretation of these coordinated transcriptome changes. Finally, the development and validation of relevant non-animal models for vertebrates and humans, like cell lines, fish and amphibian embryos, or stem cells, diminishes or even eliminates the need for animal testing and allows high throughput screening schemes. In this regard, the combination of gene expression-based methodologies with chemical structure-oriented analyses (as QSAR) may constitute a solid and reliable strategy for the assessment of health risks associated to the thousands of new chemical products released into the market every year.

SS10-8

Chemical Risk Assessment under Chemical Substances Control Law in Japan and comparison with REACH

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The purpose of Chemical Substances Control Law (CSCL) is to evaluate, before manufacture or import, whether or not new chemical substances have properties such as persistence, and to implement necessary regulations, in order to prevent environmental pollution caused by chemical substances that pose a risk of impairing human health or interfering with the inhabitation and/or growth of flora and fauna.

To minimize the significant adverse effect of chemical substances on human health and environment by 2020 (Agreement in the Environmental Summit in 2002), CSCL has been amended in 2009 and implemented in 2011. Under amended CSCL, risk assessment will be conducted in step-wise manner for all chemicals including existing chemicals. In this presentation, chemical risk assessment approach under amended CSCL and the results of comparison between CSCL and REACH will be introduced.

SS11-1

Non-standard methods for environmental endpoints under REACH: scientific challenges & opportunities

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The overall purpose of both the REACH (EC No 1907/2006) and the CLP (EC No 1272/2008) Regulations is to ensure a high level of protection of human health and the environment. Industry has to ensure that chemical substances are used safely. A key motivation for developing REACH was to fill information gaps for the large number of substances already in use in the EU, as for many there was inadequate information on their hazards and risks they pose. REACH registrants have to provide information on the intrinsic properties and hazards of the substance in the registration dossier. The standard information required depends on the tonnage manufactured or imported; the higher the tonnage, the more information needed. In addition for substances at 10 tonnes per annum (tpa) or above, the registration dossier must include a Chemical Safety Report (CSR). New studies using vertebrate animals for REACH registration should only be conducted as a last resort. For information specified in Annexes IX and X (more than 100 and 1000 tpa, respectively), a testing proposal should be approved first before testing on vertebrate animals is done. In addition there are data sharing obligations for registrants of the same substance to avoid duplicate testing using experimental animals. Registrants must first collect and assess all existing data. Then they have to identify data gaps and consider whether they can be filled by using non-standard data before any new tests are conducted. This means that all available information is collected: in vivo and in vitro studies, information from human exposure, information from structurally-related substances (i.e. 'read-across' and 'chemical categories') and predictions from valid (Q)SARs. The non-standard information has to be equivalent to the information obtained from the standard test data. The key point is that the non-standard data must be suitable for an adequate risk assessment to ensure the substance can be used safely and also for adequate classification for hazard communication. This presentation summarises the current experience, discusses the scientific challenges and opportunities, and presents some recommendations for improving the use of alternative methods in the environmental hazard assessment under REACH/CLP. Disclaimer: The authors are staff members of the European Chemicals Agency. The views expressed are solely the authors' views and do not represent an official position of the Agency.

SS11-2

Opportunities for Animal Alternatives Implementation in the Evolving OECD Fish Testing Framework

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The OECD Fish Testing Framework (FTF) was proposed by the USEPA to OECD in 2009. The goals were to review existing fish testing guidelines in the context of regulatory needs and data requirements, address animal welfare concerns and to define a possible testing strategy for fish tests. A workshop in 2010 and subsequent follow-up meeting in 2011 developed a document that focused on assessments of 13 existing/proposed fish Test Guidelines and subjects relevant to fish testing. Chapters were reviewed in detail and future recommendations for action were proposed to address various needs. Flow diagrams exemplify how tests could be integrated into an overall strategy for assessing toxicity of chemicals to fish.

This presentation reviews opportunities for animal alternative approaches in the FTF. These will be placed in the context of in vivo tests required in many regulatory situations for the registration of industrial chemicals, pharmaceuticals, and agrochemicals. The Framework integrates current thinking regarding screening assessments, acute toxicity, chronic toxicity, bioaccumulation, and endocrine disruption determinations. Ample room exists for improved use of in vitro, embryo tests, threshold approaches, and implementation of robust experimental designs. The overall goal is to minimize animal use while maximizing the collection of information useful in hazard and risk assessment, and classification and labelling. Method development needs and future opportunities will be identified throughout.

SS11-3

The validation journey - What is validation and how does it work?

M. Halder

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It is common understanding and a regulatory need that alternative methods to animal tests are reliable and relevant, i.e. validated. According to OECD Guidance Document 34 on the validation and international acceptance of new or updated test methods for hazard assessment, the process of validation establishes the reliability and relevance of a method for the given purpose. Reliability refers to the reproducibility of the method within and between laboratories over time, relevance to its scientific value and practical usefulness, and purpose to its intended application. The presentation will outline the various steps in the validation process: a) the definition of a test method and how to assess its readiness to enter validation; b) the assessment of its transferability, within- and between-laboratory reproducibility; c) assessment of its relevance; and d) the peer review by independent experts (e.g. the ECVAM Scientific Advisory Committee, ESAC). Crucial aspects in the design of a validation study (e.g. number of laboratories, selection of chemicals, number of chemicals to be tested, etc) will be discussed and examples will be given. The role of the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) is to promote the validation and use of alternative approaches and their international regulatory acceptance. It has been formally established by Council Directive 2010/63/EU on the protection of animals used for scientific purposes. In this context, two key bodies have been created over the past year: the PARERE (Preliminary Assessment of Regulatory relevance) network to advise EURL ECVAM on the regulatory relevance of alternative test methods submitted for validation and ESTAF (ECVAM stakeholder forum) to maintain the dialogue with the stakeholders. In addition, the EURL ECVAM is setting up NETVAL, a network of laboratories, which have been nominated by European Union Member States and will participate in EURL ECVAM coordinated validation studies.

SS11-4

Can cell-based systems help predict adverse outcomes in fish?

K Schirmer

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Fish cell-based systems hold great potential for deciphering the molecular mode of action of chemicals and, provided the right choice of in vitro model and exposure conditions, may supplement or even substitute fish toxicity tests. Conceptually, if the cell-based responses reflect the initial stage for an adverse outcome seen at the organism level, it must be possible to develop an array of cellular systems suitable to identify mechanisms of action and adverse outcomes. In support of this concept, we have recently demonstrated that a gill cell line from rainbow trout (*Onchorynchus mykiss*), RTgill-W1, responds with a loss of cell viability at comparable effect concentrations as fish respond with death in acute exposure scenarios for a wide range of organic industrial chemicals with different modes of actions. The gill epithelia is the primary uptake site of water-borne contaminants into fish; thus, damage to gill epithelia can be seen as the link to an impairment of the whole organism and eventually death. Transferring this concept to adverse outcomes other than acute lethality certainly requires more sophistication. For example, cell-based models could be used to elucidate the role of epithelial barriers (such as gills, intestine) or tissues (such as liver) in transforming and excreting chemicals. Different cell cultures maybe put together, e.g. on microfluidic devices, to better resemble the interaction of different cell types and transport of biotransformation products and signaling molecules from one compartment to another. Moreover, molecular mechanisms of toxicant action can be identified as part of a tiered testing approach but as well, cellular parameters, such as ATP production or proliferation should be explored as potential indicators for, e.g. reduced growth in fish. If these systems are combined in an intelligent way and systematically linked to chemical concentrations and response pathways and outcomes in fish via computational approaches, they provide a foundation for the development of a "virtual fish".

SS11-5

In-vitro methods for bioaccumulation: a modular approach including passive uptake and metabolism assessment

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Predictive tools for the assessment of bioaccumulation are critical components in an overall strategy for assessing bioaccumulation potential. This presentation reviews the potential of in-vitro test systems for improved prioritization and assessment of potentially bioaccumulative chemicals. The toxicokinetic processes of adsorption, distribution, metabolism and elimination (ADME) determine the bioaccumulation of hydrophobic organic compounds in fish and mammals. Mechanistic models of bioaccumulation explicitly consider these ADME processes but there is a lack of appropriate data as model input parameters, particularly for compounds that do not partition simply to tissue lipid. There are a variety of in-vitro systems available for estimating ADME properties. Here we explore the applicability of these in-vitro assays for estimating ADME input parameters for bioaccumulation models. Caco2 cell lines, the parallel artificial membrane permeability assay and related assays can only be applied for rather hydrophilic chemicals and need adaptation for hydrophobic chemicals. Passive absorption and elimination through fish gills can be described by a modified parallel artificial membrane permeability assay that is tuned to hydrophobic chemicals, for which resistance of overall permeability is aqueous boundary layer controlled. In-vivo metabolism rate can be estimated using a variety of in-vitro systems including hepatocytes and microsomal or S9 fractions of fish liver in combination with a physiologically based prediction model. The reviewed in-vitro approaches are promising tools for identification and prioritization of chemicals in bioaccumulation assessment. Their application in regulatory assessment schemes is relatively new and will require continued validation to fully elucidate their potential advantages and limitations.

SS11-6

The EUROECOTOX EU-7th-framework project - identification of gaps and limiting steps for reduction, replacement and/or refinement of animal experiments used in environmental risk assessment

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Animal experiments play an integral role in current environmental risk assessment for the registration of chemicals, pesticides, biocides, pharmaceuticals and testing of whole effluents. As with risk assessment in human toxicology, there is a strong societal demand to replace, reduce or refine the animal experiments performed in order to protect the environment. However, efforts to develop and validate alternatives for human risk assessment are relatively more advanced: international OECD guidelines based on alternative methods are already available for some endpoints (e.g. skin corrosion, eye irritation, phototoxicity, genotoxicity). EUROECOTOX (European Network for Alternative Testing Strategies in Ecotoxicology) is an European Union coordinated action project which aims at identifying the gaps and limiting steps for reduction, replacement and/or refinement of animal experiments used in environmental risk assessment. EUROECOTOX is performing an analysis of regulatory requirements for ecotoxicity testing, novel

strategies and approaches to reduce vertebrate testing (fish, birds, amphibians), potential bottlenecks for validating new methods and measures to accelerate development and validation of alternatives. Recently, EUROECOTOX has identified the lack of OECD guidelines, the need to consider criteria of validation (reliability and relevance) early in development, (financial) support for validation of new approaches and limited access to existing animal test data for the scientific community as one of the major limiting issues. A detailed analysis and statistics of currently available alternative approaches and limitations will be given, supplemented by experimental findings from inside the consortium and published studies. The audience is invited to subscribe to the EUROECOTOX network and to discuss measures for advancing the development and acceptance of alternative methods in environmental risk assessment (<http://www.euroecotox.eu>).

SS12-1 Introduction and resume of approaches and criteria to establish critical loads and related concepts for selected environmental impact categories and their applicability to a global chemical footprint

TV Rydberg

IVL Swedish Environmental Research Institute, GÖTEBORG, Sweden

The presentation will give an introduction to how the critical load concept, and related concepts to characterise allowable pollution loads, have been developed, and what considerations have been necessary to arrive at agreed levels. The applicability of such concepts to the global chemical footprint will be briefly discussed.

SS12-10 Taking action on high-concern chemicals - The NGO perspective

N Nimpuno

ChemSec, GÖTEBORG, Sweden

The ever-increasing use of chemicals in virtually every product used in modern life has enabled improvements in society globally. However, the widespread use of chemicals has introduced actual and potential problems to man and the environment on a scale that is virtually impossible to estimate. The rise of different forms of cancer, decrease in reproductivity, increased obesity, emergence of behavioral disorders among children etc. are only some examples of the problems increasingly associated with exposure to high concern chemicals. In parallel with improving the tools to measure these effects, and reaching agreement on how to classify the chemicals causing these effects, one must, from an environmental health perspective focus on what to do to prevent damage from occurring in the first place. Our focus is not so much the cut-off values that can potentially be reached before irreversible damage is achieved. Rather, the question also has to be asked: What negative effect of chemicals exposure is accepted? On an individual as well as societal level. Not only how far can we go? But how far are we, as a society willing to go?

SS12-2 Planetary boundaries and chemical pollution

A. De Wit

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The planetary boundaries concept includes 9 boundaries for different global environmental variables within which humanity can operate safely: climate change, ocean acidification, stratospheric ozone depletion, nitrogen and phosphorus biogeochemical cycles, global freshwater use, land use, biodiversity loss, atmospheric aerosol loading and chemical pollution [1]. Seven of these boundaries could be quantified. However, boundaries for aerosol loading and chemical pollution were not possible to quantify. Possible criteria for quantifying the global chemical pollution boundary are emissions, concentrations or effects on ecosystems of persistent organic pollutants (POPs), endocrine disruptors, plastics, heavy metals and nuclear waste. Due to the large number of chemicals in commerce, it is impossible to measure all possible chemicals in the environment. Another major stumbling block is lack of understanding of the effects of chemical mixtures on organisms, ecosystems and Earth system functions. Two complementary approaches were identified as possible ways to define a planetary boundary for chemical pollution: 1) focus on POPs with global distributions and 2) identify unacceptable, long-term, large-scale effects of chemical pollution on living organisms [1]. Data for only a few chemicals with POPs characteristics are available, for example, mercury, DDT and PCBs. In the second case, boundaries focussing on effects could be based on impacts on reproduction, immune systems and neurobehavior, particularly in sensitive species at sensitive life stages. However, it is currently difficult to link many chemicals to specific effects due to lack of toxicity data. One example of how this approach might work was the observed increase in neurodevelopmental disorders (autism, ADHD etc.) seen in children. A large number of chemicals are known to be neurotoxic in experimental animals and in humans, and five (methyl mercury, arsenic, lead, PCBs, toluene) are known to be toxic to human neurodevelopment. Thus, widespread exposure to low concentrations of many chemicals with known or suspected neurotoxic effects may have created a silent pandemic of neurodevelopmental disorders in children on a global scale [2]. [1] Rockström J et al. 2009. A safe operating space for humanity. *Nature* 461:472-475. [2] Grandjean P, Landrigan PJ. 2006. Developmental neurotoxicity of industrial chemicals. *Lancet* 366:2167-2178.

SS12-3 Emissions of chemicals - important or misleading for a planetary boundary of chemical pollution?

9 Molander

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The concept of planetary boundaries is designed to indicate possible serious anthropogenic disturbances of the function of the Earth system, and chemical pollution might be an important contributor to the overall burden of humans on the planet. The concept as such (Rockström, Steffen et al. 2009) want to define a "safe operating space for humanity" in order to maintain the planetary conditions within ranges that we know are safe. The primary example is global warming where several studies point to the possibility of defining a range of radiative forcing within which climate will still be without unexpected serious changes related to nonlinear responses of the system. Is this approach applicable to all forms of chemical pollution? Chemicals may impact through specific mechanisms at sensitive points of the earth system, such as e.g. CFCs on the ozone layer, or due to other effects, more or less specific, on biota. In that case, also possible combined effects of many small contributions must be considered. In the case of impacts on biota, there is also links to another - already defined - planetary boundary for biodiversity that need consideration. However, before chemicals can have any effect they need to be emitted, but traditionally the scientific focus has been on fate (environmental chemistry) or effects (environmental toxicology) possibly due to the simple fact that persistence early was found to be a key property of substances identified as problematic - the silent spring paradigm regarded highly persistent, biomagnifying and very toxic substances - the PBT-approach to chemical regulation has since then dominated the scene. Present chemical regulation in Europe (REACH) focus on single substances and chemical products and does consequently only touch lightly upon the range of chemicals occurring in the societal stocks of products with longevities spanning from months to decades. These products include ordinary consumer goods like textiles, kitchenware and food packaging and products made to last for long time like furniture, carpets, flooring, wallpapers and other building materials. The presentation will attempt at a discussion of the inclusion or omission of emission as part of a planetary boundary for chemical pollution in the light of what is known regarding other kinds of emissions of importance for the concept of planetary boundaries and on the possibility to use models to estimate emissions from materials.

SS12-4 Do we have the concepts and tools to estimate the global exposure to anthropogenic chemicals?

M. Scheringer

ETH Zürich, Zürich, Switzerland

Chemical pollution is not only a combination of many local or regional cases of environmental contamination, but has also truly global aspects. These include that there are chemicals with the potential to be distributed around the globe, in particular Persistent Organic Pollutants (POPs), and that there is a global trade in chemicals and chemical wastes that leads to widespread distribution even of chemicals without POP properties. This global nature of the chemical pollution problem is illustrated by the fact that the new UNEP Global Environmental Outlook (GEO-5 [1]) will, for the first time, include a chapter on chemicals and wastes. The Planetary Boundaries concept is important because it makes it possible to explicitly address these global aspects of chemical pollution. As an essential element of an assessment of chemical pollution at the global level, we need to quantify the total global emissions of a wide range of chemicals and the resulting global exposure to these chemicals. In this talk, it will be demonstrated that the combination of educated guesses of emission rates and multi-media mass balance models provides a means to reliably estimate the global exposure to anthropogenic chemicals. Global exposure includes levels of chemicals in various environmental media such as air, water, soil, and vegetation and in various regions of the globe, reaching from urban to pristine. For several types of chemicals, model results based on estimates of global emissions show good agreement with field data. It is concluded that today the tools that are needed to estimate global exposure to anthropogenic chemicals are available. Because of large data gaps in emissions and chemical property data, these estimates of global exposure to chemicals are still surrounded by uncertainties, but nevertheless a relatively clear picture of the global burden of anthropogenic chemicals can be established. Even on the global scale with maximum dilution by large volumes of water and air, average concentrations in ocean water in the pg/L range are observed for single POP-type chemicals when current emission rates are used. The combination of all relevant chemicals in commerce makes the total concentration of chemicals available for uptake by humans and wildlife even higher. [1] Global Environmental Outlook, to be published by the United Nations Environment Programme in May 2012: <http://www.unep.org/geol/>.

SS12-5 Planetary boundaries: a suitable concept for chemical pollution?

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The concept of planetary boundaries has been introduced by Rockström et al. in a publication in *Nature* in 2009. They are defined as 'scientifically informed values of the control variable established by societies at a 'safe' distance from dangerous thresholds'. That is, planetary boundaries indicate critical thresholds that must not be exceeded, in order to avoid catastrophic environmental impacts. Rockström and his colleagues suggested specific planetary boundaries for climate change, ocean acidification, stratospheric

ozone depletion, land and freshwater use, biodiversity loss and interferences with phosphorus and nitrogen cycling. They also suggest setting a planetary boundary for chemical pollution, which has not yet been quantified. This presentation will therefore explore whether it is indeed possible and sensible to apply the concept of planetary boundaries to toxic chemicals in the environment. Limitations of and challenges for the planetary boundary concept are not only related to emission, fate and exposure assessment (which were discussed in the previous talks). To a large extent they are also connected to the ecotoxicology of chemicals and include (a) the need to account for the ecological effects of complex multi-component mixtures, (b) the ignorance of local conditions and local effects, and (c) the enormous differences in the ecotoxicological profiles of the myriads of chemicals used in society, which, together with the ever-changing chemical use patterns, make any boundary a constantly moving target. We will explore those issues and their consequences for the concept of planetary boundaries for chemical pollution. To this aim we will use two groups of environmentally important chemical groups as cases in point: (1) a particular group of pesticides, so-called photosystem-II inhibiting herbicides, (2) unspecifically acting industrial chemicals (so-called baseline toxicants).

SS12-6

Fate-based categorization of chemicals and GIS Fate Model for the planetary boundaries concept

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It may be difficult to set up single criteria for the Planetary Boundary of chemical pollution, because chemicals have different mechanisms of effects on organisms including humans and different modes of emissions to the environment. First of all, it is necessary to consider simultaneously both the categorization of chemicals based on characteristics and the prioritization of the categorized groups. Categorization of chemicals might be decided as matrix of their characteristics such as environmental fate, toxicities, and other relevant information. As for environmental fate, though POPs (Persistent Organic Pollutants) and POPs-like chemicals should be important, chemicals which are not POPs-like but having temporal-spatial local peak should also be considered in terms of the Planetary Boundaries discussion. For example, if some chemicals locally affect some microorganisms on their reproduction and this impact affect other plants or animals, this issue is no longer a local problem. This means we should consider not only environmental fate of chemicals but also these ultimate impact to the planet. Data gaps and amount of available information, for especially new chemicals, also should be important when we select an appropriate method of the chemical risk assessment. Each category of chemicals should have each appropriate method. Although the simplified models like SimpleBox, CalTox, and ChemRange are useful for wide range of chemicals in faster and with less resource, the models are also indispensable that aims to reproduce the chemical fate processes in more details. In this presentation, we introduce our research about the detailed multimedia fate models developed for pesticides (PeCHREM/G-CIEMS) and the global model developed for POPs (FATE). As for the PeCHREM/G-CIEMS, in order to accurately predict the temporal-spatial variations of many pesticides in the environment, we constructed the prediction methods using general statistics of pesticide use, geographical, meteorological, hydrological database. As for the FATE, two issues were important that are to solve the temporally-spatially resolved emission inventory and to solve the general circulation of the atmosphere and the ocean. We also show the possible way to contribute these models to the Planetary Boundaries concept.

SS12-7

A planetary boundary for chemical pollution

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Johan Rockström and co-authors (2009) wrote a persuasive argument that human activity is exceeding the planet's biophysical boundaries. Rockström et al. defined 10 boundaries that we need to live within, or alternatively, face a radically different future. Chemical pollution was one of the 10 boundaries discussed, but the authors were unable to define the actual boundary. Unlike the other 9 boundaries, defining a boundary for chemical pollution will be challenging because the boundary for chemical pollution does not have a single variable, metric or indicator. We argue that a planetary boundary for chemical pollution is necessary to address the local to global nature of chemical release and transport, and the myriad and often subtle adverse effects that are arising from single and multiple chemical exposures. Furthermore, populations are increasingly vulnerable to adverse effects from chemical pollution as a result of experiencing multiple stresses, i.e., the global system abutting against other planetary boundaries. We suggest starting the planetary boundary discussion by exploring the similarity with the "critical load" concept. A critical load is defined as the highest load that can be added to a system without causing a specified adverse effect in a sensitive population or ecosystem. A critical load is estimated by first choosing a sensitive toxicity endpoint in a sensitive organism, population or ecosystem, from which the corresponding emission rate is back-calculated. For the planetary boundary, the calculation should be done on a spatially resolved global scale with attention paid to ecologically vulnerable systems. Into the calculation we need to specify chemical emissions as tied to annual chemical production and inventory. Environmental chemical persistence, in addition to chemical persistence brought on by human stockpiling, must also be addressed. Perhaps most challenging, we need to consider multiple chemical emissions rather than take a chemical-by-chemical approach. As a first step, we suggest calculating single-chemical critical loads or planetary boundaries for the few, relatively well understood high production volume chemicals for which we can gather sufficient information on physical-chemical properties, emission rates, inventory, environmental fate, exposure and toxicity. Considering lower production volume chemicals may be unnecessary.

SS12-8

Assessing the principle of a planetary boundary for chemicals used in personal care products

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As the per capita GDP of the world's population continues to grow, particularly in developing countries, such as Brazil, Russia, India, and China, which are expected to be the four biggest economies by 2050, it is likely that the use of chemicals in commerce that is associated with modern societies will significantly increase over the next several decades. It is thus important that governance approaches are sufficient to inform risk management discussions for reducing environmental impacts related to economic growth, and are based on rigorous scientific assessment of risks. Johan Rockström and colleagues (2009) proposed nine planetary boundaries of safe operating space for humanity. The authors suggest that these boundaries might stimulate a discussion for novel and adaptive governance approaches at the global, regional, and local levels. Included in the nine planetary boundaries is a boundary proposed for chemical pollution, which Rockström and colleagues do not quantitatively define. A number of questions thus follow from the proposal to define a safe operating space for humanity with respect to quantifying a planetary boundary for chemical pollution. For instance, what is meant by safe operating space and what is the relationship with other concepts, such as current approaches to environmental risk assessment, life-cycle assessment, ecosystem services or source to outcome pathways? In an effort to ensure that the use of chemicals in society associated with economic growth do not negatively impact the environment, we consider here the opportunities that the principle of a planetary boundary for chemical pollution offers, by exploring the utility of existing science-based assessment methods to inform decision making, assessing the role of technological innovation in relation to economic growth as well as issues of spatial and temporal scales, and how these parameters influence our ability to quantify a planetary boundary for chemical pollution.

SS12-9

Sound Chemicals Management - The policy perspective

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The aim of the Strategic Approach to International Chemicals Management is to achieve, by 2020, that throughout their life cycle chemicals are used and produced in ways that lead to the minimization of significant adverse effects on human health and the environment. While significant progress has been made in some parts of the world, progress is sometimes uneven and that not enough is being done to reach the 2020 WSSD chemicals goal. The unsound management of chemicals can lead to the contamination of air, water and soil, resulting in increased human exposure and associated health risks. Sound chemicals management through the life cycle should be one of the key factors underpinning efforts to achieve poverty eradication and a green economy. The intention of this paper is to give state of art and to draw the attention to some areas where further progress needs to be made.

EC01A-1

PAH, PCB and OCP concentrations in the sediments, local mussels, transplanted mussels and passive samplers in the Istanbul Strait and Marmara Sea, Turkey

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Lipophilic organic pollutants such as polycyclic aromatic hydrocarbons (PAHs) and persistent organic pollutants (POPs) are accumulated in sediments and in organisms. The accumulated pollutants especially in fish and mussels may lead to serious human health hazards. In this study, the surface sediments and mussels collected from five sites of the Istanbul Strait and Marmara Sea as well as the transplanted mussels and deployed passive samplers were analyzed by using a high resolution mass spectrometer coupled with an gas chromatography to determine the concentrations of PAHs, polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCP). Istanbul Strait is a part of the Turkish Strait system connecting the Black Sea to the Mediterranean and one of the busiest waterways in the world. Maximum sediment T-PAH concentration (6431 ng/g) was measured at the sampling site representing the shipyard area, whereas the highest mussel T-PAH concentration (201 ng/g) was determined at a site situated at the middle part of the Strait at the mouth of a creek. The lower amounts of T-PAH concentrations in mussels were detected compared to passive samplers. The maximum sediment (25602 ng/g) and local mussel (881 ng/g) T-PCB concentrations were measured at the middle part of the Strait. In general, the differences in the T-PCB concentrations of the measured matrices were not significant. This shows that the PCB compounds could not be metabolized as the PAH compounds by the mussels. The accumulation of PCBs by the BR sorbents were not efficient as in case of PAHs. When SPMDs and BR sorbents were compared, the accumulation of PCB congeners in SPMDs were found much higher compared to the BR sorbents. OCP concentrations were only measured in sediments and mussels. The highest concentrations were measured at shipyard area. The most dominant OCP compounds were HCH and DDT derivatives. DDT concentrations in sediments were higher compared to the concentrations in mussels.

EC01A-2

Equilibrium sampling of environmental pollutants in Baltic Sea sediment along a transect in the Stockholm Archipelago

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The determination of the fugacity, freely dissolved concentration (C_{free}) or chemical activity of environmental pollutants in sediment is important since sediment is the dominant exposure medium for bottom-dwelling organisms and therefore is particularly relevant in a bioaccumulation context. Further, sediment represents the major reservoir for numerous hydrophobic organic chemicals (HOCs) in the aquatic environment, and the chemical activity in the sediment can govern the chemical activity in the water column.

To measure C_{free} of HOCs such as polychlorinated biphenyls (PCBs), a polymer is brought into contact with the sediment and analysed after equilibrium between the two phases is established. Here we used a coated glass jar with a very thin coating of polydimethylsiloxane (PDMS) on the vertical walls. The method makes use of three different coating thicknesses (2 μ m, 4 μ m and 8 μ m), which allows equilibrium between sample and PDMS to be confirmed for each sample and analyte (QA/QC), while at the same time verifying the absence of sampling artefacts.

In this study, we investigated C_{free} of PCBs in sediment samples from the Baltic Sea, collected along a gradient from central Stockholm towards the middle of the Stockholm archipelago. We confirmed the applicability of the coated glass jar method in the open Baltic Sea. The results showed a decrease in C_{free} with increasing distance from the urban center. This indicates that the Stockholm waters continue to be a source of PCBs to the Baltic Sea close to four decades after the PCB ban in the 1970ies. The gradient could be caused by either ongoing releases to water or PCB residues in sediment from past emissions.

Further, we calculated PCB concentrations in biota lipids (C_{lipid}) and K_d values that express the sorption strength of PCBs to the different sediments. These data were compared to values from the literature and the spatial distribution of C_{free} and K_d was examined. In summary, passive equilibrium sampling using coated glass jars was shown to be a convenient, accurate and sensitive technique to determine C_{free} of PCBs in Baltic Sea sediments.

EC01A-3

Effects of flow velocity and calibration conditions on a passive sampler for perfluorinated alkyl carboxylates and sulfonates in water

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Perfluorinated chemicals (PFCs) are emerging environmental contaminants with a global distribution. Due to the moderate water solubility of some PFCs, the majority of the environmental burden is in the water phase. Passive sampling provides a low cost and time integrative sampling approach that has already proven useful for a broad range of environmental contaminants. A newly developed and validated Polar Organic Chemical Integrative Sampler (POCIS) with a weak anion exchange sorbent has shown potential as a passive sampler for PFCs in water. However more work was required to further validate the sampler. The aim of this work was to evaluate the influence of water flow rate and calibration conditions on the uptake of PFCs into POCIS sampler. Uptake kinetics and sampling rates for PFCs did not vary significantly with flow velocity. Sampling rates derived (0.08 - 0.28 L day⁻¹) are comparable to sampling rates determined in a previous study under different conditions. A passive sampler for PFC and similar compounds could help elucidate potential aquatic exposure routes to PFCs.

EC01A-4

Occurrence and fate of brominated and organophosphorus flame retardants in river water

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The aim of this study was to evaluate the occurrence and fate of a wide range of flame retardants (FR) in river waters using a novel passive sampler and active sampling. Target compounds included PBDEs (BDE 28, 47, 99, 100, 153, 154, 183 and 209), new brominated flame retardants (hexabromobenzene - HBB, pentabromoethyl benzene - PBEB, pentabromotoluene - PBT, 2,3-dibromopropyl 2,4,6 tribromophenyl ether - DPTE, 2-ethylhexyl 2,3,4,5 tetrabromobenzoate - EHTBB, Bis(2-ethyl-1-hexyl)tetrabromophthalate - BEHTBP, 1,2 bis (2,4,6 tribromophenoxy)ethane - BTBPE and decabromodiphenyl ethane - DBDPE) and organophosphorus flame retardants (tris(2-chloroethyl) phosphate - TCEP, tris(2-chloro-1-methylethyl) phosphate - TCPP, tris[2-chloro-1-(chloromethyl)ethyl] phosphate - TDCP and triphenylphosphate - TPhP). Analysis was performed by gas chromatography coupled to mass spectrometry (GC-MS). As a first step, a passive sampler design based on a ceramic dosimeter and HLB as receive phase was tested and calibrated in laboratory conditions to determine uptake kinetics of a set of six flame retardants. The sampling rate obtained for TCPP, TCEP, TDCP and TPhP were 2.4, 2.9, 2.3 and 2.1 mL day⁻¹, respectively, while lower values were found for the rest of FR, such as for HBB and PBEB (0.4 and 0.6 mL day⁻¹, respectively). In a second step, a monitoring study was performed along the River Aire, in Yorkshire, Northeastern England using grab samples and the passive sampler. In this latter case, unfiltered waters were extracted by means of solid phase extraction (SPE), through OASIS HLB cartridges. In grab samples, TCPP was the most abundant compound, probably due to its increased local usage and punctual sources, being detected in all samples, ranging from 0.11 to 26 μ g L⁻¹ (average 8.3 μ g L⁻¹). TCEP, TDCP and TPhP were detected in most of the sampling points ranging from 0.12 to 0.32 μ g L⁻¹, 0.06 to 0.15 μ g L⁻¹ and 0.006 to 0.02 μ g L⁻¹, respectively. From the brominated flame retardants, the BDE 209 was frequently detected, ranging from 0.02 to 0.29 μ g L⁻¹. When comparing the performance of active and passive sampling, a good correlation was observed for organophosphorus flame retardants after a 3-week deployment period, while BDE 209 was only detected in the grab samples. Overall, the developed passive sampler proved to be efficient for the monitoring of organophosphorus flame retardants, a family of compounds which is characterized for their widespread occurrence and high toxicity.

EC01A-5

Porewater profiles of As, Se, Fe, Mn, V and P in spiked marine sediment measured using DGT and DET techniques

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Arsenic and selenium are anionic contaminants, released into aquatic environments from the mineralisation of naturally-rich ores and industrial discharges. A diffusive gradients in thin films (DGT) technique to measure pore water profiles of As, Se, Fe, Mn, V and P in spiked marine sediment is described. The study evaluates the performance of DET and two DGTs (precipitated ferrihydrite and titanium dioxide (Metsorb[TRADEMARK]) binding layers) in sediments that were sieved (<1 mm), aged for 8 months. The mangrove sediments were then spiked at 60 mm depth with either As(III) and Se(IV), or As(V) and Se(VI) 48-h prior to probe deployment. DET profiles identified a Fe/Mn redox boundary at 40 mm below the sediment water interface, which correlated with the DET-P maxima. Both ferrihydrite and Metsorb[TRADEMARK] DGTs successfully bound As(III) and As(V) (as total As), Se(IV), and natural levels of V(V) and PO₄³⁻ from marine sediment porewaters (Se(VI) was not DGT labile). There was more

DGT-labile Se, As and V in the sub-oxic and oxic regions of the sediment and overlying waters. Differences in DGT and DET profiles are attributed to DET measuring total dissolved solutes whereas only the oxyanions were DGT labile, demonstrating the benefits of dual DET and DGT deployments

EC01A-6

Polar (un)charged micropollutants on SPE materials - which factors control the adsorption?

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Adsorption of organic solutes from the water phase onto solid matter is a crucial process in the environment, water purification and in analytical science. The ability to adsorb to and absorb into a material dictates the fate of solutes in the environment. Many of these solutes are polar or even charged. Adsorption of these compounds to SPE materials is a promising way to help detecting them, as concentrations are often still too low for MS and preconcentration can overcome this problem. Many materials have been employed for this purpose, but little is known about the exact sorption mechanism of these polar compounds. The role of functional groups of these solutes and the SPE materials is still ambiguous. How do they interact with each other and what is the part of water in the adsorption process? Apart from that, the effects of other compounds, such as inorganic salts, on the sorption behaviour is not yet well-understood. This knowledge can also be used for the removal of these compounds from drinking water. Often they pass through the treatment plant, as the current treatment methods cannot remove them effectively.

Aim of the research is to get a better insight into the influence of various functional groups of selected chemicals and SPE materials on the sorption to be able to take a grounded decision which SPE material should be used. In case of charged organic compounds, additionally the impact of competing inorganic electrolytes was monitored. We decided to use OASIS polymers as SPE material. These polymers carry polar moieties, hydrophilic parts as well as charged groups, which should allow the adsorption of the target compounds.

The results of this research indicate that especially apolar functionalities have a great impact on the sorption, whether the compound is charged or not. The more pronounced the apolar moiety is, the better the compound can adsorb. However, the results show that there is no direct correlation between solubility and adsorption behaviour.

Furthermore, it emerged that the conditions of the aqueous phase, such as salt concentration, influence the sorption behaviour of charged molecules dramatically. The higher the salt concentration is, the lower the adsorption of the charged compounds. Apart from concentration also the type of ion is important.

EC01B-1

Stimulation and Inhibition of bacterial growth by caffeine dependent on antibiotics and silver nanoparticles - a ternary toxicity study using a microfluid segment technique

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A droplet-based micro segmented flow technique for the fast generation of three dimensional concentration spaces for the screening of toxic effects of selected antibiotic substance and silver nanoparticles on the toxicity and activation of bacterial growth by caffeine at the nanoliter scale was introduced. Up to about 1200 well separated fluidic compartments containing 216 different combinations of concentrations were realized in a single experimental run. To evaluate the toxicity of the ternary mixtures a time resolved miniaturized optical double endpoint detection unit using a microflow-through fluorimeter and a three channel microflow-through photometer were used for the simultaneous analysis of changes on the endogenous cellular fluorescence signal and on the cell density of *E. coli* cultivated inside 500 nL microfluid segments. As a result, a complex response pattern was discovered including synergistic and compensatory effects as well as strong non-linear combination effects, concentration dependent stimulation and the formation of activity summits on isobolographic maps. The results reflect a complex response of growing bacterial cultures depending on the combined effectors. A strong caffeine induced enhancement of bacterial growth was found at sublethal chloramphenicol and sublethal silver nanoparticle concentrations. The reliability of the method was proven through the high redundancy of fluidic experiments. The results indicate the importance of multi-parameter investigations for toxicological studies and prove the potential of the microsegmented flow technique for analyzing combined effects.

EC01B-2

Applying novel passive dosing systems for the control of chemical and drought stress to a terrestrial invertebrate

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Two passive dosing systems were used in this study to control (1) chemical stress and (2) combined chemical and drought stress in toxicity tests with the terrestrial springtail *Folsomia candida*. *F. candida* lives in the interstitial air in the top soil and is very sensitive to low humidity. The test compounds were the PAHs naphthalene, phenanthrene and pyrene. The aims of the studies were (1) to link lethality of naphthalene, phenanthrene, pyrene and their mixtures to (sum) chemical activity and (2) to determine springtail lethality in a two-factor experiment with both chemical and drought stress. Chemical activity is a multimedia parameter that expresses the energetic state of the contaminant. Passive dosing from PAH loaded silicone elastomer was used to control defined chemical activities of the test compounds in glass vials. To start the 7-days test, 10 springtails were transferred to each loaded vial. *F. candida* could move freely on the loaded silicone, resulting in exposure through direct contact and headspace. In the test with combined chemical and drought stress, the chemical exposure was controlled by passive dosing, as described above. Passive dosing vials were covered with a net and placed inside a closed glass jar with aqueous saline solution in the bottom which controlled the humidity within the entire jar. In this way, *F. candida* was exposed to controlled chemical stress from below, while the drought stress was controlled from above. The PAH exposure parameter was in all cases chemical activity (unitless, [0-1]), and the humidity was expressed as relative humidity, that is also known as water activity. The two passive dosing systems were simple and practical to use, and the systems worked well with high survival in controls and low variation in lethality within treatments. Springtail lethality caused by naphthalene, phenanthrene and pyrene was successfully linked to chemical activity. Springtail lethality caused by four PAH mixtures (each in three dilutions) was plotted as a function of sum chemical activities, and the toxicity data of all tested mixtures was successfully fitted to one chemical activity response relationship. The lethal chemical activities (La-50) of the individual PAHs and the mixtures were all well within the expected range of 0.01-0.1 for baseline toxicity. The results from the combined stressors experiment showed stress synergy in springtail lethality between the chemical and drought stress.

EC01B-3

Passive dosing under the microscope reveals that microorganisms enhance the mass transfer of hydrophobic organic chemicals

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The transport of hydrophobic organic chemicals (HOCs) is often rate limited by their diffusive mass transfer through stagnant boundary layers (SBLs). This then results in gradients on the microscale, which are crucial for the diffusive transport of HOCs and for organisms living in such boundary layers. To study how microorganisms react to such gradients, and whether they can alter the mass transfer of HOCs through SBLs, a new experimental setup was developed. In this setup, microgradients of HOCs could be precisely controlled and quantified. At the same time microscopic observation of organisms exposed to the gradients could be performed. Passive dosing was employed on microscope slides to produce gradients of polyaromatic hydrocarbons (PAHs) by integrating silicone O-rings into the Dunn Chemotaxis chamber. An outer clean ring thereby served as a sink and an inner PAH-loaded ring as a source. The mass transfer of the PAHs from source to sink by diffusion through protozoa medium was then quantified, and compared to that in the presence of the ciliate *Tetrahymena pyriformis*. In the presence of *T. pyriformis* the mass transfer was enhanced, increasing with the hydrophobicity of the PAHs. This enhancement was nearly hundred-fold for benzo[a]pyrene, the most hydrophobic compound tested. Fluorescence video imaging microscopy showed that this enhancement was based on a mechanism where protozoa acted as a transport vector of PAHs via their diffusive uptake and release. Such a transport mechanisms has, to our knowledge, not yet been described.

EC01B-4

Comparison of two monitoring strategies for organic pollutants in seawater: passive sampler devices vs transplanted clams

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The concentration of organic pollutants in seawater shows a great spatial and temporal variability as a result of a combination of natural and anthropogenic effects. In this study we have characterized simultaneously the bioaccumulation in transplanted clams and two passive sampling devices (semipermeable membrane device, SPMD, and continuous flow integrative sampler, CFIS). The combination of these monitoring techniques such as water, biota and passive samplers with the analysis of water concentrations allows a comprehensive knowledge of the environmental status and to assess the representativity of these integrative sampling methods. The efficiency of these two passive samplers and the determination of the bioaccumulation of organic pollutants in transplanted clams were tested in the marine environment in spring and autumn comparing the obtained integrative concentration with the real water one. The study was performed in four selected sampling points in Mar Menor Lagoon (SE of Spain), which is subjected to direct and indirect discharges of organic pollutants. El Albujón Wadi is the main surface watercourse that flows into this lagoon from Cartagena Field, which is one of the most relevant horticulture areas in Europe. The organic pollutants determined in clam were PAHs, PCBs and organochlorinated pesticides. In surface and marine waters PAHs, PCBs, triazines, organophosphorus and organochlorinated pesticides were analysed by stir bar sorptive extraction and thermal desorption coupled to capillary gas chromatography-mass spectrometry (SBSE-TD/GC/MS). Significant daily, weekly and seasonally differences were observed in the input of organic pollutants. Chlorpyrifos ranged from 0 to 12 ng/L in spring and from 3 to 6000 ng/L in autumn. Duplicate and triplicate of CFIS and SPMD were studied in several sampling points in

order to study the repeatability (RSD<20%) of passive samplers. Chlorpyrifos was detected in spring in all cases, both in SPMD (4.8-130 ng/L), showing a great variability, and CFIS (4.68-17.25 ng/L) this insecticide only detected with CFIS (2-18 ng/L) in autumn. An increase of concentrations in clams for pesticides and PCBs were observed after a month of exposition in the mouth of El Albuñón watercourse. In general both in Spring and Autumn the intermediate exposure time was too short to reach similar pollutants levels found in CFIS or SPMD.

EC01B-5

A passive sampling method for the estimation of concentrations in pore water and accessible concentrations of polycyclic aromatic hydrocarbons in contaminated sediments

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For the environmental risk assessment of contaminated sediments it is important to know both the equilibrium concentrations in the pore water (C_w) and the accessible concentration in the sediment phase (C_{as}). C_w is highly relevant for uptake by organisms and transport, and the accessible concentrations in sediment are important for distinguishing between the contaminant fractions that may be subject to bioaccumulation and bioremediation on one hand, and fractions that are strongly bound to resistant sediment constituents, such as soot and coal, on the other. Traditional methods for determining accessible concentrations are extraction of sediment suspensions for a fixed period of time using gas purge or TENAX. Concentrations in the pore water traditionally are measured in sediment suspensions by passive sampling (e.g. SPME, polyoxymethylene, low density polyethylene or silicone rubber), assuming that uptake by the passive sampler does not substantially lower the initial C_w . We developed a silicone passive sampler based method for the simultaneous determination of C_w and C_{as} , by incubating sediment suspensions at increasing silicone /sediment phase ratios. This method allows for determining sediment-water sorption isotherms, because higher silicone masses cause a lowering of the concentrations in both the sediment and the pore water. Sediment samples from 3 different areas in the Netherlands were equilibrated with silicone samplers by shaking at 150 rpm for 28 days with 4-5 different sampler sediment ratios ranging from 0.03 to 1 at a suspension density of 0.1 g/mL. The results show that the accessible contaminant fraction follows linear sorption isotherms, and that only 10-25% of the total concentrations can be released from the sediment to the aqueous phase on the timescale of the experiments. In additional experiments, a strong increase in passive sampler uptake rates was observed at higher suspension densities. The results are highly relevant for the risk assessment of contaminated sediments.

EC01B-6

Evaluation of an in-situ equilibrium sampling device for persistent organic pollutants in sediment pore water systems on the basis of solid phase microextraction (SPME)

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In this study, equilibrium passive sampling device is introduced that makes POP bioavailability in terms of freely dissolved aqueous concentrations assessable on site, i.e. in the sediments. The developed in-situ sampling device has several advantages compared to in-vitro or solvent-based sample extractions that make it an ideal monitoring tool: (1) The ecologically relevant parameter 'in-situ bioavailability' is addressed instead of total sediment or pore water concentrations. (2) Due to short/medium equilibrium times, the temporal resolution of the measurements is suitable for analysis of both long-term trends and seasonal effects. (3) The device is of very solid construction and can be reused practically ad infinitum; only replacement of the disposable sampling materials contributes to its operational costs. (4) Sample treatment is reduced to a minimum which in turn reduces possible sources of sample manipulations, measurement errors and analysis costs. Both sampling and analysis procedures are thus simple, robust and cost-effective.

The device is already applicable in a multitude of aquatic environments, especially where currents are low and sediments are muddy and well-mixed e.g. by bioturbation. Examples for such environments are mud flats, harbor basins, river banks and lakes.

EC01C-1

Use of plants as passive samplers for volatile organic compounds (VOCs) in indoor environments

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Concerns about the potential build up of volatile organic compounds (VOCs) in indoor air have increased as energy conservation methods minimized the introduction of outdoor air. VOCs, including many with documented short- and long-term adverse health effects, can enter indoor environments through internal (i.e. paints, paint strippers, fuels, cleaning supplies, pesticides, building materials, adhesives) and external sources (i.e. vapor intrusion from contaminated groundwater). Indoor air concentrations of VOCs vary widely, but concentrations of most VOCs are consistently higher indoors than outdoors. Typical approaches used to sample indoor air include evacuated canisters and sorbent tubes. The use of ornamental plants has been suggested as a simple, unobtrusive, aesthetically pleasing, and cost effective method for sampling and purifying indoor air. The waxy surface of the leaves has the potential to provide a good surface for the passive capture of VOCs. However, the efficiency and kinetics of capture has not been well characterized. To investigate the potential use of plants as indoor air VOC samplers, three types of studies were performed. The first consisted of monitoring air and plant concentrations over time after a controlled release of several VOCs into a residential building containing several plant species. The second study used a flow-through glass and stainless plant growth chamber to evaluate the relationship between air and plant leaf VOC concentrations. The third study used a headspace approach to measure equilibrium leaf-air partition coefficients. Good correlations between the leaf and air concentrations observed in the three different studies suggest that plant leaves can be used to monitor indoor air concentrations of VOCs.

EC01C-2

Calibration and field evaluation of Polar organic chemical integrative samplers (POCIS) for monitoring pharmaceuticals in hospital sewage water

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Pharmaceuticals have become an important issue in the last few years due to their continuous release in the environment. In this context, we made the MEDIFLUX study to estimate the contribution of hospital to the urban sewage pollution by pharmaceuticals. To go on, we decided to test Polar Organic Chemical Integrative Samplers (POCIS). Passive samplers allow the measurement of Time Weighted Average (TWA) concentrations, overcoming many shortcomings of the spot sampling techniques. The application of POCIS was studied using six compounds already selected as representative of the great families of pharmaceuticals used at the hospital (Atenolol, Prednisolone, Methylprednisolone, Sulfamethoxazole, Ofloxacin, Ketoprofen).

In a first step, POCIS were calibrated in tap water under laboratory conditions for the analytes of interest taking into account various relevant environmental conditions (temperature, flow rate...). POCIS were exposed under a constant agitation to tap water spiked with the selected pharmaceuticals, renewed every two days. Sampling rates were determined and compared. It appears that Rs increase significantly when flow rate increase between 10 to 25 cm.s⁻¹ for all the compounds (except sulfamethoxazole). A slight difference of Rs between 15 and 25°C was observed for sulfamethoxazole, prednisolone, ketoprofen.

In a second step, the flow rate and temperature of waste water were recorded during a week on the selected hospital effluent. For the calibration, 20 L of wastewater were taken from hospital effluents and brought to the laboratory to carry out Rs measurement as for the previous measurement in tap water. Sewage water was changed every two days. The Rs values obtained were close to Rs values in tap water.

As the laboratory calibration step shows the feasibility of POCIS to sample sewage water, we plan to deploy POCIS in situ.

The step of calibration was the main part of the work and its completion was needed before the application of POCIS in situ. The follow-up of accumulation made it possible to estimate the field of linearity in order to choose the optimal length of POCIS implantation in situ.

The use of POCIS for the follow-up of contamination by organic pollutants is especially described for surface waters such as rivers, but very little studies report its use in wastewaters. This work gives encouraging results for the deployment of POCIS in sewage that could be a useful tool for pharmaceutical pollution management.

EC01C-3

Accumulation kinetics and sampling rates for 56 polar organic compounds, identification and validation of 5 PRCs

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POCIS (Polar Organic Chemical Integrative Sampler) is a new emerging tool for sampling polar organic micropollutants in water. There is still a need of research concerning its domain of validity (e.g., molecules sampled, type of water studied, optimal exposure duration) and its performances (molecules sampling rates, repeatability, accuracy of the evaluation of time-weighted average or TWA concentrations).

In order to obtain in situ TWA concentrations, POCIS needs to be calibrated in laboratory for each molecule of interest. We performed 3 different laboratory calibration experiments using a flow-through calibration system in order to evaluate the optimal exposure duration and to calculate the sampling rates for 56 polar organic contaminants, and to identify and validate performance reference compounds (PRCs). The calibration system was composed of 2 aquaria (50 L) containing i) spiked tap water (circa 3 µg/L) and non-spiked POCIS for the determination of accumulation kinetics, ii) non-spiked tap water and spiked POCIS for the determination of desorption kinetics and iii) spiked tap water and spiked POCIS for the validation of the potential PRCs. In all experiments, water temperature, pH, conductivity and dissolved organic carbon were controlled. Agitation in the aquaria was ensured via a submerged pump. The resulting flow velocity was 10±5 cm/s and was directed perpendicularly towards POCIS surface. Triplicate POCIS were analyzed at 0, 1, 3, 6 and 12 hours and at 1, 3, 7, 11, 14, 21 and 28 days.

We present results for the 56 studied molecules, including the duration of linear accumulation phase, sampling rates and possible performance reference compounds (PRCs). The accumulation phase was curvilinear for almost all molecules; so, we obtained the duration of optimal linear accumulation phase using $t_{1/2}$ criteria. Concerning the desorption experiments, 5 compounds showed a good potential as PRC; they enable to decrease the effect of variable environmental conditions, so they can be used to obtain more reliable in situ TWA concentrations. We also checked isotropic exchange for these molecules comparing the exchange constant k_e in accumulation and desorption experiments. At last, we validated the PRC strategy by calculating TWA concentrations from the third laboratory experiment mentioned above.

EC01C-4

Polar organic chemical integrative sampler (POCIS) calibration for steroid hormones and pharmaceuticals, and flow modelling in the calibration system

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Water pollution from emerging contaminants such as steroid hormones and pharmaceuticals is one of the important stakes of current environmental research. Pharmaceuticals that are most often encountered in surface water are antibiotics, non-steroidal anti-inflammatory drugs, anti-epileptic drugs and hypolipidemic agents with a proven toxicity on living organisms. Steroid hormones, either natural or synthetic, are also of major concern. These compounds are typically encountered in the aqueous environment at very low, but toxicologically relevant, concentrations (ng/L).

One of the main drawbacks of conventional grab sampling is the poor representativeness of the actual contamination. Passive sampling can be an alternative approach that provides a more accurate image of the contamination, while being easier to handle than large volume water samples. Polar Organic Chemical Integrative Sampler (POCIS) in its "Pharm" configuration contains Oasis HLB material enclosed within two polyethersulfone membranes. This design enables the POCIS to trap polar organic compounds. Another configuration with Nylon membranes has also been tested, allowing less polar compounds to reach the sequestration medium.

This work presents the results of a calibration with these two different POCIS designs, as well as desorption of target compounds from samplers. Various steroid hormones (Estrone, α -Estradiol, β -Estradiol, Ethinylestradiol, Estriol, Mestranol, Progesterone, Testosterone, Norethindrone, Levonorgestrel) and some widely used pharmaceuticals (Sulfamethoxazole, Erythromycin-H2O, Bezafibrate, Diclofenac, Carbamazepine) were studied.

In the literature, various calibration systems are used. In the present work, calibration of POCIS was carried out in a flow-through exposure system with stirring action provided by rotating blades. Computational Fluid Dynamics (CFD) modelling with Fluent software confirmed the homogeneity offered by this design and gave an estimation of the flow at the surface of the samplers.

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EC01C-5

The use of passive samplers to constrain distributed models for pesticide runoff simulations

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Passive samplers yield data on average concentration during their exposure period. Yet their application is most useful to cover periods where concentrations are strongly fluctuating and the information hidden in the average value should be decrypted. Pesticides are emitted by two main episodically active pathways: emissions from Wastewater Treatment Plants (WWTP), which are the result of the spilling of leftovers and the cleaning of spraying equipment during application periods, and surface-runoff from agricultural fields during precipitation events. Passive samplers are easy to install in greater numbers and can therefore be used to track critical source areas for pesticides.

The main issue relates to the quantitative nature of passive samplers. Sampling rates (R_s) can deviate rather largely under field conditions depending on the flow environment and the compound properties. Moreover, for the evaluation of peak concentrations the elimination rate (k_e) for the substances during non-polluted periods following the peak is equally important. Hence a robust determination of k_u (uptake rate) and k_e is the basis for the interpretation of plumes that have been monitored by a passive sampler.

This contribution shows uptake and elimination rates that have been determined in the laboratory and the field and how these parameters can be used to estimate peak concentrations during exposure periods of passive samplers. In combination with autosampler data from one site located near the basin outlet, contributions from low-flow and precipitation events can be calculated with simple balancing of pesticide uptake by passive samplers during floodwaves for each site. The data have been used to refine sub-catchment emissions in a SWAT simulation.

EC01C-6

Calibration and exposure correction methods for the polar organic chemical integrative sampler (POCIS)

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The measurement of polar compounds of interest in complex environmental matrices and at trace concentrations represents a significant analytical challenge. In this regard, passive sampling devices may have much to offer the analytical process, for example providing a time-integrative sample, with low detection limits and in-situ extraction of analytes. The polar organic chemical integrative sampler (POCIS) has been shown to accumulate a wide range of such chemicals. However in contrast to hydrophobic passive samplers, results remain semi-quantitative. This is due to the lack of an uptake model and an exposure correction method which may adjust sampling rates for differences in environmental conditions during deployment. Thus the purpose of this study was to examine the variation due to environmental factors on uptake in POCIS and the possibility of correcting differences using performance reference compounds from hydrophobic passive samplers. Sampling rates for several hundred compounds from over 20 calibration studies published in the literature were critically examined. In addition, several flow through calibration experiments were conducted at a local sewage treatment works. Most published calibration studies describe static type experiments with either batch renewal or modelled depletion of exposure water. Whilst such experiments may be reasonably reproducible they are likely to be poorly replicated in the environment. Whilst the effect of environmental factors of POCIS sampling rates is generally somewhat smaller than that reported for hydrophobic samplers, water flow rates, temperature, fouling, are all reported to affect uptake. These effects were not uniform for all compounds examined. Additionally polar compounds may be more greatly affected by pH and salinity than hydrophobic ones. Calculated in situ sampling rates were in general lower than those for laboratory studies of similar compounds. Changes in sampling rates in co-deployed hydrophobic samplers were not easily related to those of POCIS, where both samplers were subjected to variable water flows. There remain gaps in our knowledge concerning polar samplers and the processes involved in chemical accumulation. In summary we need to address some of these basic questions before we can hope to use polar passive samplers in a truly quantitative way.

EC02 - Contaminant pathways, trends and biological effects in a warmer Arctic

EC02A-1

Influence of climate change on contaminant distribution and effects in Arctic marine food webs - Summary of the IPY project COPOL

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An overall aim of COPOL was to evaluate and quantify the seasonal and annual variation in bioaccumulation and effects of contaminants (organic contaminants and trace elements) in benthic and pelagic Arctic marine food webs. This was important in order to be able to separate the effect of a climate signal from other expected system variability. Two fjords on Svalbard with different climate conditions were selected as study sites: Kongsfjorden (Atlantic signal) and Liefdefjorden (Arctic signal). In summary, the seasonal variation in the pelagic food web was greater than both between year and between fjord variations. This was found both for absolute concentrations from zooplankton to fish and birds, and for the average concentration increase per trophic level (food web biomagnification estimated by trophic magnification factors). The high within year variability underlines the importance of coherent studies of specific time periods rather than pooling samples across a year. It also means that if studies should repeat annually, it is important to select one representative time period.

We assumed that the selected fjords in the site for time study would represent different climate scenario. However, there are several indicators that even the fjord on Svalbard's northern coastline was influenced by Atlantic waters. This was reflected in the pelagic food web that contained both Atlantic zooplankton and Atlantic cod. The benthic food web on the other hand, was a better reflection an Arctic site, and is like to better reflect the average conditions of the site.

The present study has documented large changes in bioaccumulation during the year, at a larger scale than between year or between fjords. It is therefore important to standardize future studies to identify the true source of the observed variation.

EC02A-2

Snow, contaminants and climate change

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The impact of a changing climate on contaminant fate in the physical environment of cold regions is likely to be amplified by the sensitivity of the cryosphere to relatively small temperature changes. It is not so much the change in temperature that will modify a contaminant's environmental behaviour, but the changes in the extent and duration of a seasonal snow and sea ice cover and in the nature of the snow melt. In order to anticipate the potential influence of climate change, we seek to improve the general understanding of contaminant-cryosphere interactions through a combination of laboratory experiments, field studies and computer simulations. Snow is subjected to controlled melting in a cold room laboratory and the fractionated melt water is filtered and the sorbed and dissolved fractions analysed with appropriate trace analytical techniques. Elution curves for different contaminants of variable partitioning properties, for different types of snow and for different melting conditions are recorded. Field studies in a highly urbanised watershed involve the repeated sampling of river water throughout the snow melt season. Sorbed and dissolved fractions are analysed by trace analytical techniques. Elution curves of contaminants from a melting snow pack are predicted with a simple mass balance model that simulates the sequential melting of several horizontal snow layers and the resulting downward percolation of melt water. The model assumes equilibrium partitioning between the various snow pack phases. Laboratory experiments revealed at least five types of elution curves for organic contaminants from a melting snow pack. All types could be reproduced with, and thereby mechanistically explained by, the snow pack melt model. Elution curves in river water do not resemble those eluting from laboratory snow packs, except that water soluble contaminants tend to appear early during the melt period. Particle-bound contaminant concentrations in the river tend to peak sharply during snow melt, and correlate with river run-off rates. Concentration time profiles of particle bound contaminants in rivers during snow melt are less controlled by processes occurring within the snow pack, and depend more on factors that determine run-off rate and the mode of melt water ablation from the snow pack to the stream. Many of these factors are influenced by a changing climate.

EC02A-3

Climate change and Arctic marine mercury biogeochemistry - conclusions and research needs from the AMAP 2011 Arctic Mercury Science Assessment

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Recent studies have shown that climate change is already having significant impacts on many aspects of transport pathways, speciation and cycling of mercury (Hg) within Arctic ecosystems and ultimately biological exposure, including humans. For example, the extensive loss of sea-ice in the Arctic Ocean and the concurrent shift from greater proportions of perennial to annual ice have numerous effects including promoting increases in primary productivity, shifting foodweb structures, possibly altering methylation and demethylation rates, and influencing Hg distribution and transport across the ocean-sea-ice-atmosphere interface (i.e., bottom-up processes). In addition, changes in animal social behavior, such as habitat selection in association with changing sea-ice regimes by top predators such as beluga, can also affect dietary exposure to Hg (i.e., top-down processes). In this presentation, adapted from one chapter of the recent AMAP Mercury Science Assessment, we address these and other possible ramifications of climate variability on marine Hg cycling, processes and exposure in the Arctic.

EC02A-4

Local contaminant sources in the Arctic: volatile and non-volatile residues from combustion engines in surface soils from snow mobile tracks in the vicinity of Longyearbyen (Svalbard Norway)

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A summer field campaign was conducted in 2010 for investigating potential long-term effects on surface soil from fossil fuel burning. A total of 18 soil samples and two surface snow samples were collected for the analysis of polycyclic aromatic hydrocarbons (PAHs) along frequently used snowmobile tracks in/out of Longyearbyen (78° 13' 0 N, 15° 37' 60 E). In addition, Benzene-Toluene-Xylene (BTX) components were measured in ambient air at the University Centre in Svalbard Laboratory facilities during spring and in autumn 2010. Polycyclic aromatic hydrocarbons (PAH) was determined in all 18 soil samples in the concentration range 21 to 1883 ng/g dw (dry weight, SUM 15 PAH). All samples were characterized with pyrogenic PAH patterns. The highest concentrations were found in a sample from the former winter airfield close to the old Northern Light observatory. Only 30% of the 2007 concentrations were detected for Benzene in 2010 Longyearbyen air. This significant concentration reduction is assumed to be caused by the higher proportion of 4-stroke engine driven snowmobiles during the 2010 season.

EC02A-5

The impact of thawing permafrost on lakes of the Mackenzie Delta uplands, NT, Canada

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Total permafrost in the Northern Hemisphere currently occupies an area of 26 million km², and by 2100, this area is expected to decrease by 19-35%. In the Mackenzie Delta, NWT, temperatures are projected to rise by 4 to 5°C in the next 50 years. Over the past 20 years, mercury and PCBs have been steadily rising in burbot from the Mackenzie River, which has prompted speculation on how the changing physical environment, such as thawing permafrost, might be affecting contaminant cycles in these thermokarst environments. We tested the hypothesis that the presence of retrogressive thaw slumps in the Mackenzie Delta Uplands (north of Inuvik, NT, Canada) is affecting nutrients (total and dissolved N and P), persistent organic pollutants, metal concentrations, and phytoplankton community assemblages in small tundra lakes. Dissolved organic carbon, total phosphorus, soluble reactive phosphorus, and total and methyl mercury were significantly lower in lakes with retrogressive thaw slumps than reference lakes, likely due to deeper water infiltration through clay-rich tundra soils. These results provide compelling evidence that thawing permafrost near lakes of the Mackenzie Delta uplands are not responsible for the rising trend in mercury concentrations of fish in the Mackenzie River.

EC02A-6

Burdens and inputs of perfluorinated compounds in the Lomonosovfonna Ice Core, Svalbard (2009)

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In 2009, we drilled a 40-meter ice core representing ~1953 - 2008+ from Lomonosovfonna (78°49' 24.4' N; 17°25' 59.2' E), the highest elevation glacier on Svalbard (1250 m.a.s.l.). We sub-sampled the upper 21 meters of the core to identify inputs of 12 perfluorinated compounds (PFCs) delivered to the site by long-range atmospheric transport. Each sample had liquid volumes of 291 mL (average), and dating resolution averaged 2.4 years from 1976 - 2008. Five compounds were not detected in any segment of the core, including PFBA, PFPeA, PFHpA, PFBS, 6:2FTS. PFNA dominated the accumulated burden in the core, showing 29.4 pg cm⁻², 81% more than the second highest, PFOA, 16.2 pg cm⁻². It is thought that the PFNA and PFOA may be an atmospheric oxidation products of 8:2 FTOH which has been found to be the dominant gas-phase PFC in Europe. PFOA however, is considered to be the dominant PFC in the atmospheric particle phase in Europe; these results suggest that PFCs in both phases are reaching high-elevation ice on Svalbard. Other burdens include PFHxA and PFDA which were approximately equal (9.91 and 9.85 pg cm⁻²) and about one-third PFNA. No other PFC had a burden greater than 5.8 pg cm⁻² (PFOS), including PFUnA, PFHpA and PFHxS. Net flux trends greater than 10 years duration appeared for PFNA, PFDA, and PFOS. The trend for PFNA begins in 1982 (0.388 pg cm⁻² yr⁻¹), increasing regularly until 1995 when it doubled to 1.25 pg cm⁻² yr⁻¹ by 1997, and reaching 5.5 pg cm⁻² yr⁻¹ by 2008. PFDA has the longest flux record beginning with 0.10 pg cm⁻² yr⁻¹ in 1976, increasing and decreasing in no predictable pattern, leveling off at ~ 0.4 pg cm⁻² yr⁻¹ after 2000 before increasing rapidly to 1.31 pg cm⁻² yr⁻¹ in 2008. PFOS has an irregular flux beginning with 0.24 pg cm⁻² yr⁻¹ in 1982, never exceeding 0.29 pg cm⁻² yr⁻¹ by 1995 before increasing to 1.23 pg cm⁻² yr⁻¹ in 2008. Although PFOA has the second-highest burden, its short record begins with 0.77 pg cm⁻² yr⁻¹ in 1990, doubling to 1.69 pg cm⁻² yr⁻¹ by 1993, but not observed again until 2002 when it dropped by nearly 4 times (0.46 pg cm⁻² yr⁻¹). It nearly doubled by 2006, and increased by > 4x by 2008 (3.93 pg cm⁻² yr⁻¹). The dominance in burden by PFNA and PFOA is consistent with high concentrations of these PFCs observed in polar bear liver from Greenland.

EC02B-1

The deposition and fate of perfluorinated alkyl substances (PFAS) in the Norwegian Arctic snowpack

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Poly- and perfluorinated alkyl substances (PFAS) are man-made chemicals that are ubiquitous in the environment and are present in humans and wildlife. These chemicals occur in the Arctic through long-range transport processes with the perfluorinated acids (PFAs) [e.g. carboxylates (PFCAs) and sulfonates (PFASs)], present in sentinel

organisms such as the Polar bear (*Ursus maritimus*) and the ringed seal (*Phoca hispida*). PFAs are present in arctic media through a number of processes, including transport with ocean currents, oxidative degradation of 'neutral' precursors (e.g. fluorotelomer alcohols) in the atmosphere and possibly transport with airborne particles and aerosols. A combination of these processes results in their deposition with snowfall, although only a few studies to date have examined accumulation of these chemicals in the Arctic snowpack. Questions arise regarding the sources of these chemicals in atmospheric deposition in remote locations and the role of snow in delivering PFAs to catchment systems during periods of melt. In this study, a detailed examination of PFAS was undertaken in the seasonal snowpack at remote terrestrial sites in Northern Norway. The purpose was to investigate, in some detail, their accumulation in different snow layers and to relate their profile and concentrations to physical and chemical characteristics of the separate snow layers as well as their accumulation history. PFAS measurements in air (gas phase and particle-bound) and snow (dissolved and particulate matter) were conducted. Air mass trajectory analysis was used to examine air mass origins for specific air samples and snowfall events. In the snowpack, the vertical evolution of snow density, snow layer hardness, crystal morphology (macrophotography) was examined alongside temperature, pH, conductivity, organic matter content, major anions and cations, as well as particle characteristics, to help understand sources of PFAS to fresh snowfall and their fate during snow ageing. Initial results show large variations of pH and particle matter content between snow layers, with pH measurements as low as pH 4.68 in some layers, indicating the potential for a fraction of the PFAs to exist in their neutral acid form and hence be 'remobilised' during thaw periods.

EC02B-2

Secondary emissions of legacy pollutants and their pathways to the Arctic under a changing climate

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After the phase-out of a persistent chemical it can be expected to endure in the environment for some time, up to several decades. How long and where it will persist depends on its emission history and transport, partitioning between environmental compartments, and degradation. With respect to the accumulation of legacy pollutants the Arctic is a particularly interesting region. It is also in the Arctic, where future climate change is expected to affect most the presence of persistent pollutants.

The mechanisms leading to a particular global distribution of the contaminant are addressed by multimedia chemical fate models. These tools provide not only quantitative estimates of the global distribution of a chemical, but also an understanding of the pathways involved, and allow forecasts under different emission and climatic scenarios.

In this work we model concentrations of some representative persistent organic pollutants in the Arctic after their phase out. In particular we indicate the timescales that can be expected for those chemicals to persist in the Arctic, and analyze the relationship between Arctic receptor sites and global secondary sources. Finally, we investigate the effect that the projected climate change may have on levels in the Arctic and the global distribution of secondary sources.

We show that beta-HCH and PCB153 will persist in the Arctic Environment for several decades after cessation of primary emissions with apparent half-lives of about 8 and 28 years, respectively. These results are only slightly sensitive to forecasted climate change. We further demonstrate that the location of secondary sources important for the Arctic is strongly dependent on the chemical properties of each pollutant.

EC02B-3

Spatial and temporal trends of persistent organic pollutants and mercury in ringed seals from the Canadian Arctic

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The goal of this ongoing study is to determine temporal trends of legacy POPs, new/emerging POPs, as well as mercury (Hg) in Canadian Arctic ringed seals using annual collections at Arviat, Resolute, and Sachs Harbour as well as less frequently at nearby communities. The study builds on results for legacy POPs and Hg going back to the 1980s, and earlier in some cases. Results for PBDEs, HBCD, perfluorinated chemicals (PFCs), and endosulfan, along with $\delta^{13}C$ and $\delta^{15}N$ data, have been added to samples collected since 2001 and on selected archived samples from the 1970s and 1990s. Sample collections (10 to 25 adult ringed seals; blubber, liver, muscle, tooth/lower jaw for aging) are carried out by local hunters each year (June-October). For neutral POPs, only blubber of females and juveniles are analysed to limit the influence of age. For Hg, muscle and liver samples are analysed, while PFCs are also determined in liver. Highest Hg concentrations in seal muscle and liver (age ≥ 5 yrs) were found in western and Central Arctic. No significant increase or decline of Hg in seal muscle was found over a 7 to 9 year period at Arviat, Resolute and Sachs Harbour. $\delta^{13}C$ and $\delta^{15}N$ in seal muscle varied only over a narrow range (± 0.5 ‰) indicating little change in diet over the same period. Hg concentrations in liver were more variable over the same period. This was not related to diet or to age but could reflect more recent diets of individual animals compared to measurements of muscle N. To assess time trends of legacy and new POPs we combined data for nearby communities. Overall, there are declining trends in all regions with the relative magnitude of $\Sigma DDT > \alpha HCH > \Sigma 10 PCB > \Sigma CHL$. Largest declines of all legacy POPs were in Hudson Bay possibly reflecting proximity to source regions in North America. PBDEs, PFOS, and PFCAs show increasing concentrations in the 1990s to early 2000s and then recent declines. Endosulfan and HBCD were present at low concentrations in seal blubber (0.01-2.0 ng/g) and appear to be increasing in concentration over the period 2005-2010. Temporal trends of legacy POPs in the Canadian Arctic generally show declining trends. However trends for new POPs differ from those in Greenland particularly for PFCs. Continued annual sampling is improving the statistical power of the study and enabling testing of factors influencing trends of POPs and Hg including climate, diet and changes in global emissions.

EC02B-4

An observation-based mass balance assessment of PCBs in the Arctic Ocean

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The predictability of future distribution and exposure of PCBs in the Arctic hinges centrally on the quality of our understanding of the present-day distribution and processing of PCBs in the Arctic system. Real observations of PCBs in abiotic compartments in the Arctic Ocean are scarce, increasing the importance of model simulations for understanding the fate of PCBs in this extreme but sensitive environment. The objectives of the current study were to a) present an observation-based inventory of PCBs in the Arctic Ocean (AO), b) to relate observations to total global emissions and modeled simulations of transport to the Arctic, offering an assessment of how well we understand transport and fate of PCBs in the Arctic today, and c) to present a mass balance for the entire AO, identifying the major inventories, import and export fluxes of PCBs. Water samples were collected covering all the water masses of the central AO (CAO) and surface water from the polar mixed layer in all seven shelf seas. Observed concentrations in combination with annual global emissions for the years 1930-2000 were used to estimate concentrations back to 1930. The total $\Sigma PCB7$ inventory for all Arctic Ocean water masses and ice was 64 t. The largest reservoir was the Arctic deep water, containing 77% of the total inventory. All water masses were dominated by tri- and tetra-chlorinated PCBs (40-70%). The Arctic inventory of PCB 52 corresponds to only 0.5% of the global total emission, contrasting to numbers of more than 5 % suggested by model simulations. Hence, a redistribution of PCBs to the atmosphere from the AO should not have a major impact on human and environmental exposure globally.

River discharge was a major input pathway (26-56%) to the AO during 1930-2000. The inventory for PCB 52 (including sediments) of 31 t fits well to the mass balance of 34 t imported and 16 t exported to the Arctic Ocean during 1930-2000, considering the uncertainties in these calculations. The average residence time of the Atlantic water in the CAO of approximately 25 years allowed this water to accumulate PCBs. Hence, water from the Atlantic layer exported from the AO contains higher concentrations of PCBs than the water that entered the AO, potentially offering a partial explanation for the discrepancy between model simulations and observation-based calculations presented here.

EC02B-5

Exposure of persistent organic pollutants in avian top predators in a changing northern climate

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Top predators in the Arctic ecosystem are the final destination of bioaccumulative POPs and mercury, and in such organisms the negative impacts are most likely to occur. However, little is known about how climate change will affect the accumulation of POPs in top predators. Changes in POPs in arctic top predators may occur by two pathways: 1) directly through increased POP transport or 2) indirectly through changes of POP uptake in the food chain and changes in diets of top predators. The importance of these pathways is little understood, and the aim of this presentation is to discuss how changing climate and feeding ecology may affect the uptake of POPs by using different examples on avian top predators in the arctic and upper temperate regions. Relationships are shown to factors both related to POP transport and to indirect mechanisms such as local climate and feeding conditions.

EC02B-6

Persistent organic pollutants (POPs) in Svalbard water food webs: is the Arctic far enough?

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Persistent organic pollutants (POPs) are semi volatile and have high environmental half-lives. These characteristics produce a long-range atmospheric transport and global planetary distribution, reaching locations far away from the pollution source. The overall process results in an enrichment of concentrations of some POPs in polar ecosystems, which became a global sink due to their environmental conditions. As a consequence of their biomagnification properties, high trophic food web organisms

provide a great opportunity for the analysis of background concentrations of POPs accumulated in Arctic biota. Two water ecosystems from Svalbard archipelago (Arctic) were selected encompassing freshwater and marine fish as targets (summer 2010). 18 Arctic char (*Salvelinus alpinus*) and two local marine fish species (17 Cod (*Gadus morhua*) and 9 Haddock (*Melanogrammus aeglefinus*)) were sampled. Traditional persistent organic pollutants (PeCB, HCB, HCHs, DDTs, PCBs) were analysed in all samples and recently introduced compounds such as the polybrominated diphenyl ethers (PBDEs) have also been assessed. Arctic char showed higher heterogeneity in the abundance of groups of POPs (83-95% for PeCB, HCB, HCHs and DDTs and 50% for PCBs) while in marine species these compound groups were found in 100% of the samples. Mean concentrations of these first 5 groups ranged between 0.4 and 17.8 ng/g. Higher values were found for HCHs in all species. This could be explained by the high solubility and volatility of these compounds in relation to other compounds. For the PBDEs, at least one congener in each sample was quantified in 39% of Arctic chars and in 35% of marine samples. Concentrations of total PBDEs ranged between 0.004-0.306 ng/g; 0.010-0.177 ng/g and 0.005-0.538 ng/g in Arctic char, Cod and Haddock, respectively. The most abundant congeners were PBDE-47, PBDE-99 and PBDE-209. The results from our work confirm a background level of organochlorine compounds in Arctic regions. Further studies should also include other emerging organic compounds of concern in freshwater and marine biota.

EC04 - Novel approaches to addressing metal and metal nanomaterial bioavailability in soils

EC04-1

Validation and first deployment of the DGT technique in artificial human gastrointestinal fluids after ingestion of metal-containing soil particles

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The accumulation of metals in soils due to human activities constitutes a potential health risk if directly ingested, especially by children via hand-to-mouth behaviour. Contaminants can be partially or totally released from soil by ingestion, depending on their speciation under gastrointestinal conditions. In vitro tests provide estimates of bioaccessibility, defined as the proportion of contaminant that is dissolved in the artificial gastrointestinal fluids and is potentially available for absorption. The flux toward the intestinal membrane corresponds to both the free metal ion and labile metal species. In contrast, inert species cannot dissociate and thus do not contribute to transport across the intestinal membrane. Estimation of the labile fraction can be assessed with the Diffusive Gradient in Thin films (DGT) technique. Whereas the effectiveness of DGT has been demonstrated for various metals (Cd, Zn, Cu, Ni, Pb) in different exposure media (natural waters, soils and sediments), no data is available in artificial human gastrointestinal fluids. The objectives of this study were firstly to validate the performance of the DGT technique for Cd, Pb and Zn in controlled digestive solutions for different times of exposure and different metal concentrations, and secondly to use the technique in the gastrointestinal solutions obtained after carrying out the in vitro Unified Barge Method (UBM) test on highly contaminated soils. The results demonstrated the suitability of the DGT technique for Cd, Pb and Zn measurements in the gastrointestinal media with linear response and stable accumulation up to 6 hours at 25°C and then at 37°C. Combining the in vitro test with the DGT technique provided an approach to the labile metal species available for transport across the intestinal epithelium. Thus, the gastrointestinal absorption of ingested metals ranged from 8 to 30% for Cd, 0.6 to 11% for Pb, and 0.8 to 7% for Zn and was influenced by metal speciation. In this original approach, the DGT technique was found to be simple and reliable in the investigation of labile metal species in digestive fluids. Extrapolation to the in vivo situation should be undertaken very cautiously and requires further investigation.

EC04-2

Comparison of the determination of free Zn(II) concentration in soils using AGNES and DMT

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Key paradigms in environmental sciences, such as the Free Ion Activity Model (FIAM) or the Biotic Ligand Model (BLM), attribute a central role to the free metal ion concentration (rather than the total metal concentration) in the toxic or nutritional effects of a particular element. Consequently, there is a significant interest for analytical techniques that can act as selective probes for the free Zn(II) concentration in a large variety of environmental systems, and, specifically, in soils. The measurement of free Zn(II) concentration is particularly challenging, because there is no commercial Ion Selective Electrode for this element. Only a very few techniques have a direct access to the free metal ion concentration, while many other popular techniques (such as DGT, Diffusion Gradients in Thin Films) measure operationally defined fractions (e.g. a certain labile fraction). AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) and DMT (Donnan Membrane Technique) provide robust and direct measurements of the free metal ion concentrations. In the literature, AGNES has been applied for the determination of free Zn in seawater, freshwater, humic acid solutions, ZnO nanoparticles dispersions, etc. DMT has been applied to soils, natural waters, etc.

In this work, the application of both techniques to the same synthetic and natural systems allows a cross-validation. AGNES and DMT are validated in synthetic solutions of Zn+NTA, yielding results in agreement with each other and with the theoretical code VMINTEQ. A further validation came from analysis of Rhine river water. A critical comparison of the characteristics of both techniques can be performed in terms of time of analysis, limit of detection, required instrumentation, etc. This work is the first application of AGNES to different type of soils extracts. The analyses of free Zn in 4 soil extracts (river clay, cover sand, loam and reclaimed peat; all from the Netherlands) also gave similar concentrations with both techniques, and consistent with ECOSAT theoretical predictions. The impact of the small pH drift in DMT donor solutions is negligible. The percentages of free Zn (with respect to the total zinc concentration) range between 40% and 80%. This indicates that, in the four considered soils, Zn is much loosely bound than Cu, which, in principle, should imply a larger bioavailability of Zn (II) to plants and animals.

EC04-3

Using radioactive and stable metal isotopes to study metal and metalloid availability and ecotoxicity in soils

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Methods to study the speciation of metals in the environment has progressed rapidly over the last few decades, with the principal advances being in aqueous phase methodologies. In soils (and sediments), speciation of metals in the solid phase is problematic, with classical sequential fractionation schemes being operationally defined and having several drawbacks. Synchrotron x-ray spectroscopy has opened up new avenues to examine solid phase speciation of metals in soils, but suffers from the drawback that it cannot quantitate metal availability and behaviour, which must be inferred from knowledge of solid-phase forms identified. Isotopic methods can be used to either trace metal/metalloids in particular forms added to soils, or isotopic dilution can be used to examine the fate and behaviour of materials that cannot easily be isotopically labelled (e.g. manufactured materials or wastes).

Isotopic methods provide an extremely valuable tool to probe the fate, behaviour and biological availability of metals and metalloids in soils. The information provided by isotopic methods has already been used in regulatory frameworks for metals risk assessment [and will continue to be a vital tool in probing metal and metalloid behaviour in soils. Looking to the future, new methods examining the differences in natural abundance of metal/metalloid isotope abundance will open up our understanding of metal/metalloid availability in soils

EC04-4

Stable isotopes for micronutrient metal bioavailability to earthworms

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In urban environments two of the most common metal contaminants are copper and zinc. In addition to being contaminants, these metals are regulated micronutrients in most organisms. Both have stable isotopes (⁶⁵Cu and ⁶⁸Zn) that are readily quantified and differentiated from the more abundant isotopes (⁶³Cu and ⁶⁶Zn) by modern instrumental techniques (ICP-MS). There are two parts to our previous studies, first we hypothesized that by modifying the metal isotopic ratio in a soil, we could elevate the ratio of ⁶⁵Cu/⁶³Cu or ⁶⁸Zn/⁶⁶Zn in an earthworm (either *Eisenia fetida* or *Lumbricus terrestris*) living in that soil in a short period of time. Second, after increasing the isotopic ratio, the change in that ratio after placing the worm into a soil with a normal isotopic ratio could be used as a metric of bioavailability. The change in ratio would be a result of either an exchange of stable isotope for the more abundant isotope or the dilution of the ratio due to accumulation of the abundant isotope; in either case the ratio would decrease proportional to the metal available from the soil to the organism. Our research using stable isotopes has shown usefulness in determining micronutrient metal bioavailability in soil systems. With both Cu and Zn, isotopic ratios were increased 6-8 fold over background ratios by exposing worms to labeled soils. When placed back into soils with natural isotopic abundances ratios returned to near background levels. Understanding differences in how organisms store and utilize these metals is important if these methods are to be further developed. Zinc isotopes show promise due to its longer term storage in the anterior organ tissues of *L. terrestris*, while the extremely fast turnover rate of copper in *E. fetida* suggests that stable isotopes may not be as useful as first hypothesized.

EC04-5

Weathering of silver nanoparticles could increase their bioavailability

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The major challenge in tracing engineered nanoparticles (ENPs) in complex media, such as soils, is to detect their presence, transfer to organisms and their interactions with

the surrounding environment. One possibility to overcome this issue is to use neutron activation where ENPs are subjected to a strong neutron flux that induces a more or less transient radioactive property in the ENPs without changing other chemical or physical properties that influence their behaviour during experimentation. Radioactive ENPs are subsequently detected and quantified by counting of gamma rays emitted by the isotopes that are formed.

To date, an important amount of toxicity data on ENPs is available, but data on exposure are still needed to be able to conduct risk assessment. In a first experiment, we followed the uptake and excretion of Ag from either AgNPs or AgNO₃ in the earthworm *Eisenia fetida*. In a second experiment, we studied the partitioning of Ag from either AgNPs or AgNO₃ in two natural soils of contrasting organic matter content, and over time.

At the end of the exposure period (day 28), earthworms had body concentrations corresponding to 5.1 ± 0.5 % and 11.0 ± 0.3 % of the concentration in the food for AgNPs and Ag ions, respectively. These values decreased by 80 % and 93 % within 48h depuration in clean soil, for AgNO₃ and Ag NPs, respectively. After two months depuration, 97 % and 99 % of the accumulated Ag from Ag ions and AgNPs, respectively, were excreted. Bioaccessible Ag was defined as the sum of Ag extracted by water and ammonium acetate. A rapid reduction of Ag ions occurred when they get in contact with soil organic matter, turning Ag ions into NPs and colloids. Soil properties had a limited impact on Ag speciation. Interestingly, the bioaccessible fraction increased over time in case of AgNPs, contrary to what happened with AgNO₃. We showed that the studied AgNPs were more bioaccessible than Ag ions over time, since they could act as a constant source of relatively stable and bioaccessible Ag. This increase in bioaccessible Ag in soil spiked with AgNPs, being between 8-9 times greater than the bioaccessible fraction of AgNO₃ after 70 days contact time, calls for caution in ENP risk assessment, and the low bioavailability of AgNPs to earthworms should be re-evaluated in light of the increase of silver bioaccessibility as particles weather in soil.

EC04-6

Evaluating the impact of soil ageing on the toxicity of Ag nanoparticles to *Eisenia fetida*

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The increased production and use of engineered nanoparticles in a variety applications increases the potential for exposure to these particles in the environment. Currently, very little is known about how metal nanoparticles behave once released into the environment and even less is known about the effect of these particles on terrestrial organisms. The purpose of this investigation was to characterize the difference in toxicity and the impact of ageing between silver nanoparticles and silver nitrate in OECD soil on the earthworm *Eisenia fetida*. The potential to modify the toxicity of a silver nanoparticle contaminated soil over time is an important temporal consideration when conducting a terrestrial risk assessment.

A set of Earthworms were exposed to the AgNO₃ and AgNP spiked soils 24 hours after initial preparation and then a second set of earthworms were exposed to the soils aged 28 days. Both exposures lasted 14 days with endpoints of mortality and body burden. OECD soil treatments were prepared with dissolved AgNO₃ or suspensions of 30-50 nm Ag nanoparticles (NP). Soil treatments were then split and one batch was allowed to age for 28 days in an incubator and kept at constant moisture. The aged soils were sampled every 7 days and subjected to a sequential extraction procedure to evaluate changes in Ag speciation.

Results from the first exposure showed high mortality for worms exposed to silver nitrate and almost no mortality for those individuals exposed to Ag nanoparticles. The exposure to soils aged 28 days showed a decrease in mortality in individuals exposed to the AgNO₃ treatments and an increase in mortality in those exposed to the aged AgNP soils. The Ag body burden for both AgNP exposures were indistinguishable while there was a decrease in the Ag body burden for earthworms in the AgNO₃ exposures aged 28 days as compared to freshly amended soils. There were changes in the speciation of Ag over the 28 day ageing period in both soil treatments that point toward an increase in the potentially bioavailable pool of metal in AgNP amended soils as compared to the AgNO₃ amended soils. Based on these results, the potential exists that morphological changes to the AgNPs in the soil matrix occurred during ageing and that these changes in conjunction with more bioavailable Ag made these treatments more toxic to exposed individuals.

EC05 - Occurrence, fate and impact of atmospheric pollutants on environmental and human health

EC05A-1

Application of flow-through air samplers and passive air samplers to assess the vertical variability of semi-volatile organic contaminants in the atmosphere of Hawaii

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Many semi-volatile organic chemicals (SVOCs) such as organochlorine pesticides and polybrominated diphenyl ethers (PBDEs) have been ubiquitously detected in the global atmosphere, even in remote regions where such chemicals have never been produced or used. While many studies have focused on SVOCs transported to remote polar and highland regions, few studies have investigated SVOCs undergoing long range transport to remote tropical islands far from emission sources. The global long range atmospheric transport of SVOCs occurs mainly in the free troposphere, where little interaction with the earth surface boundary layers occurs. Investigating the occurrence of SVOCs in the free troposphere is important to understand their long range atmospheric transport. In this study, air from the free troposphere and from the marine boundary layer of the Big Island of Hawaii were sampled at Mauna Loa (19°43'53"N, 155°2'52"W, 3398 m above sea level) and at the east coast of the island (19°32'9"N, 155°34'31"W, sea level, 50 m horizontal distance from the ocean) using flow-through air samplers (FASs). FASs were operated simultaneously at the two sites from April to September, 2011. Polyurethane foam (PUF) plugs used as the sampling medium were replaced every month. Three PUF plugs were installed in each FAS and analyzed separately to assess and correct for break-through. Each month, 3100-7700 m³ and 300-800 m³ of air was sampled at the Mauna Loa site and at the coastal site, respectively. Among the PBDEs analyzed in the samples using an Agilent 7000A triple quadrupole GC/MS/MS system, only BDE-47 and -99 were detected frequently over the five months. The concentrations in the marine boundary layer (4.4-24 pg/m³ BDE-47 and 2.4-88 pg/m³ BDE-99) were higher than in the free troposphere (0.1-0.4 pg/m³ BDE-47 and 0.1-0.3 pg/m³ BDE-99). PBDE concentrations measured at the mountain site were lower than what has been reported for other global background sites in the Arctic and on the Tibetan Plateau, suggesting that Mauna Loa may truly represent global background levels for SVOCs. Between the two FAS sampling sites, within a horizontal distance of 50 km, the elevation changes from 0 m to 4000 m above sea levels. Along this large gradient, we also deployed XAD-based passive air samplers to assess the vertical variability of SVOCs.

EC05A-2

Atmospheric deposition of polychlorinated naphthalenes in Dongjiang River Basin of Guangdong province, South China

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Polychlorinated naphthalenes (PCNs) are a group of chemicals consisting of naphthalene substituted with 1-8 chlorine atoms, which were historically used for their thermal stability in dielectric fluids and insulators. PCNs are no longer commercially produced ever since 1977, but they are still routinely observed in the environment, and have been identified as persistent, toxic, and bioaccumulative substances. Atmospheric deposition of PCNs was investigated at 10 sites in Dongjiang River basin (The eastern Pearl River Delta) of Guangdong Province South China, during the winter and summer of 2010. The average daily deposition flux of total PCNs was 828 pg/(m² d), and the corresponding TEQ was 0.14 pg/(m² d). Based on these values, the presumed average annual deposition of total PCNs was 8.5 kg for Dongjiang River basin within Guangdong province, and the corresponding TEQ was 1.3 g. Tri-CNs dominated the deposition fluxes in all samples and contributed to more than 50% of total PCNs. In addition, high contents of more chlorinated PCNs (penta-CNs to octa-CN) implying the source areas were discovered in Guangzhou and Dongguan instead of Huizhou. Spatially, the average daily deposition fluxes of PCNs in Guangzhou city and Dongguan city were much higher than those in less developed Huizhou city. Seasonally, the fluxes were generally higher in summer than in winter, while the corresponding TEQ fluxes were the reverse. The results indicated the PCN emissions in the Dongjiang River basin could be ascribed to both the combustions and other sources, while to contemporary PCN the combustion sources was becoming important. Few studies have focused on the atmospheric deposition of PCNs, so the results can be useful for evaluating the potential effects of PCNs to the Dongjiang River.

EC05A-3

Environmental monitoring networks are important tools to assess chemical regulations

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A recent expert meeting organized by the RECETOX Centre in Brno, Czech Republic, has identified gaps, challenges, and research needs associated with the global assessment of hazardous chemicals. One of the important areas of concern recognized by this workshop was a need in developing a range of techniques addressing an improved assessment of spatial and temporal trends of the chemicals of global concern. Establishment of carefully designed environmental monitoring networks can be an important tool in the assessment of trends of toxic compounds in the environment and development of policy regulations on production and use of these chemicals, as well as testing the effectiveness of already existing regulations. The goal of this paper is to present an example of a North American environmental monitoring network, emphasizing methodological and technical details, as well as the results of these monitoring efforts. The Integrated Atmospheric Deposition Network (IADN) is a joint monitoring and research program between the United States Environmental Protection Agency and Environment Canada. IADN was begun in 1990 through mandates of the Clean Air Act and the Great Lakes Water Quality Agreement to monitor the atmospheric deposition of persistent organic pollutants into the Great Lakes. IADN flame retardant data for 2005-2009 time period will be presented here as an example of how environmental monitoring data can be used to assess temporal and spatial trends of persistent chemicals. Overall, Chicago and Cleveland have the highest concentrations of PBDEs, BTBPE, and DBDPE in all three phases, suggesting a strong urban atmospheric source of these pollutants. The two remote sites, Sleeping Bear Dunes and Eagle Harbor, have the lowest concentrations of these contaminants. The remote site at Eagle Harbor had particularly high levels of PBEB in all three phases, and the rural site at Sturgeon Point had the highest HBB concentrations in the vapor phase. To investigate temporal trends

of these compounds in the period of 2005-2009, a multiple linear regression was applied to the concentrations of these chemicals in all three phases combined together. PBDE concentrations showed decreasing trends over time, with halving times of ~6 years. The concentrations of HBB and BTBPE are decreasing with halving times of ~10 years. PBEB and DBDPE concentrations did not show any change between 2005 and 2009, suggesting continuing source for these compounds.

EC05A-4

Assessment of persistent organic pollutant in the atmosphere of Latin America

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The widespread use and distribution of pesticides, industrial and urban chemicals and the consequent release into the environment, is of great worldwide relevance. Atmospheric transport is responsible for pollutant dispersal over long distances. As part of an atmospheric regional network, a monitoring program involving the use of pine needles, epiphytes and passive samplers is conducted. Pine needles (*Pinus* sp.) can accumulate hydrophobic compounds such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), from air. Moreover, they integrate contaminants loads over a long time. All these compounds, included in the Stockholm Convention, are of concern due to their distribution, global transport and toxicity. Pine needles were used to evaluate latitudinal and longitudinal transport of contaminants in central and Patagonian regions from Argentina, with a 20 stations network. The epiphyte *Tillandsia bergerii* was used to evaluate local sources at small scale, setting 7 transplanting points including urban, periurban and rural land uses. Passive samplers (XAD-2) constitute the aim of our Latin American Atmospheric Passive Sampling Network (LAPAN) where 46 sites are actually covered while 80 sites are at least our endeavor to reach. The network involves regions of Antarctica and 12 countries including urban, agricultural and industrial areas. Analyses are performed by GC-ECD and GC-MS. Pine needles analyses revealed that among OCPs, the currently used endosulfans, are the main pesticides found, particularly associated with agricultural areas, however DDE, the DDT metabolite, is also found in all sites independently of particular sources, as a consequence of their intensive past use in the region. Regarding PCBs, a predominance of #110, 118, 153, 138 is found, related with punctual sources. Considering the latitudinal gradient, until the southeast region of Argentina, an increasing in DDT, PCBs and PBDEs (BDE-28 and 47) is observed inferring an atmospheric transport of these compounds. PBDEs and PCBs levels in *T. bergerii* showed a concentric distribution around the urban settlement with a clear hot spot near a waste disposition site, that is accomplished by the presence of PBDEs 28, 47, 99 and 100 and PCBs 153, 138, 110 and 118. In all industrial or urban sites the relation PBDEs/PCBs >1 reflect the general trend of diminishing PCBs levels and increasing the emergent PBDEs

EC05A-5

Occurrence of currently use pesticides and selected degradation products in agricultural regions of Western Canada

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Initial studies on occurrence of currently used pesticides in agricultural regions of Western Canada focused on the Lower Fraser Valley where berries and fruits are the dominant crops, and the Canadian prairies where grains and oil seed crops dominate. These two agricultural regions were selected due to their differences in crop types, climate, and expected usage of pesticides. Western Canadian agricultural regions have the highest historical usage of pesticides in Canada. In the initial studies we focused on key pesticides that we suspected to be of concern and was prior to the availability of a 2003 usage inventory. These pesticides had little information about their atmospheric occurrence. In the Lower Fraser Valley (LFV) we examined trihalomethyl thiofungicides and were able to detect captan and folpet, while in the prairies triallate, trifluralin, and ethalfluralin were determined to be key herbicides that were still dominant in the atmosphere. From these studies the gas/particle partitioning of captan and folpet was also examined. Due to its lower vapour pressure, captan was found to be present in both the particle and gas phase, while folpet was only detected in the gas phase. Seasonal atmospheric concentrations in the LFV were dominated by the particle phase concentrations of captan and showed the importance of examining atmospheric transport processes on particles. In the prairies we also provided the first detection of azole fungicides in the atmosphere in the gas phase and related these to precipitation during a wetter than normal year (2010). In addition we also examined the differences between the two distinct agricultural regions, and provided the first detection and seasonal trends of organophosphorus oxon degradation products along with their active OP ingredient in North America. Ratio of degradation product/active ingredient were also used to identify the age of the source and to provide insight into future studies on the relative importance of local, regional, and long-range atmospheric transport sources. In the next phase of the research we expanded the number of sampling locations in each of these agricultural regions starting in 2011 in the LFV and to an adjacent agricultural region, Okanagan Valley, which is more heavily dominated by orchards and vineyards. A summary of the currently used pesticides that are part of this 5-year study and some preliminary results will be presented.

EC05A-6

Semi-volatile organic pollutants and trace metals associated with Saharan dust air masses: estimated inhalation exposures at source and downwind sites

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Saharan dust air masses transport eroded mineral dust, chemical contaminants, and microorganisms in the atmosphere thousands of kilometers from the Sahara/Sahel of Africa to the Americas, Europe and Asia. Global ocean-atmosphere interactions, regional meteorology, surface material composition, and human activities are the primary factors driving the composition and quantities of transported dust particles and associated contaminants. Semi-volatile organic compounds (SOCs) such as banned and current use pesticides, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) have been detected in dust air masses over source (Mali) and downwind locations (Cape Verde, Trinidad and Tobago, and U.S. Virgin Islands). All are known to persist in the environment, bioaccumulate and be hazardous to humans and other organisms. Some banned pesticides such as DDT are currently used in the source region against agricultural pests (locust plagues) and disease vectors (e.g., malarial mosquitos). Primary sources of PAHs include small, garbage-and-biomass-burning low temperature fires and vehicle exhaust (primarily diesel and two-stroke gasoline engines). Concentration data from source and downwind sites during Saharan dust conditions were used to estimate inhalation exposure to total and individual (14) pesticides, total PAHs, total PCBs, and biologically active trace metal(loid)s. Inhalation exposures ranged from 17-1085 nanograms per day (ng/d) for detected SOCs in the source region and <1-6 ng/d at downwind sites during dust conditions. Bioaccessible metal(loid) exposure ranged from 0-2519 ng/d Fe, and < 1-11 ng/d As in the source region and 55-2006 ng/d Fe and < 1-4 ng/d As at Caribbean sites. Because biological exposure to metal(loid)s is dependent on species accessibility, preliminary investigations of bioaccessibility of inhaled or ingested particle-associated trace metals were conducted using simulated lung and gastric fluids. Bioaccessibility varied (0-100%) among trace metals (and redox state), fluid (simulated lung or gastric), and by source-downwind location. Iron and potentially toxic elements (As, Cr, Cu, Mn) showed enhanced bioaccessibility in downwind (Caribbean) samples. Fe is of particular interest due to its ability to induce an inflammatory response. Saharan dust air mass samples contain multiple SOCs and biologically active trace metals, raising the question of possible synergistic negative effects on humans and other organisms.

EC05B-1

Re-visiting the modelling of soil-air partitioning, fugacities in soil, and soil-air exchange of persistent organic pollutants

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Soils are the main reservoir of persistent organic pollutants in the environment and air-soil exchange of POPs is a key process affecting the atmospheric occurrence of POPs and the extent of soil as a sink of pollutants. The direction of the air-soil exchange can be determined by comparing the POP fugacity in soil (f_s) and the fugacity in ambient air (f_a). If f_s is higher than f_a , then there is a net volatilization, while if f_s is lower than f_a then there is a net deposition. f_s is usually estimated from the soil-air partition coefficient (K_{SA}), which is often estimated from the octanol water partition coefficient. Lately, polyparameter linear free energy models (pp-LFER) have also become popular for modelling the environmental partitioning of POPs. The recent development and application of a soil fugacity sampler allows for the first time to validate unequivocally these models. The objective of this work is to revisit the modelling of soil-air exchange by fitting this model to the most extensive database available for concurrent measurements of soil concentrations, fugacities in soil and fugacities in ambient air. The application and recommendation for modelling strategies will be provided dependent on the chemical properties, environmental parameters such as temperatures and soil characteristics. Implications for modelling soil-air exchange will also be given.

EC05B-2

Prospects for estimating atmospheric lifetimes of micropollutants using the Junge relationship

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The Junge variability-lifetime relationship relates the relative standard deviation of concentrations of trace gases to their atmospheric lifetime using empirical fitting parameters. Conceptually, the Junge relationship is a consequence of incomplete mixing of gases in the global atmosphere. At the limit, gases with very long lifetimes approach a homogenous distribution in the earth's atmosphere. More generally, variability in atmospheric concentrations of gases with shorter lifetimes measured at remote locations will be greater than variability of gases with longer lifetimes. Here, we present results of a pilot study that aims to take advantage of the ubiquitous presence of cyclic volatile methylsiloxanes (cVMS) in the global atmosphere to calibrate the Junge relationship and estimate atmospheric lifetimes for other pollutants. The cVMS have many advantages

as benchmark substances to calibrate the Junge relationship, namely; 1) They are high-production volume chemicals with high release rates and high concentrations in the atmosphere; 2) Their atmospheric lifetimes are well-understood based on a combination of laboratory and field studies that have been verified with mass balance modeling; and 3) they are emitted from urban areas, which coincides with source areas for many other atmospheric micropollutants of interest and opens the possibility of extrapolating Junge relationships for the cVMS to estimate atmospheric lifetimes of other atmospheric micropollutants. We have analyzed two datasets for evidence of variability consistent with the Junge relationship: Measurements at 12 sites of the Global Atmospheric Passive Sampling (GAPS) network, and measurements made at a rural site in southern Sweden, and found strong Junge relationships in both datasets. We view these relationships as proof-of-concept that the cVMS can be used to calibrate the Junge relationship and enable quantitative estimation of atmospheric lifetimes of organic substances if they are measured simultaneously and have similar source profiles and sink processes. Measurements of siloxanes and other selected pollutants in air at the full complement of over 50 GAPS sites is currently underway, and will provide a more extensive data set for testing the Junge relationships in future work. Particularly intriguing is the potential for applying the relationship to estimate atmospheric lifetimes of substances with significant sinks from dry and wet deposition.

EC05B-3

On the contribution of biomass burning to POPs in air in Africa

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Forest, savannah and agricultural debris fires in the tropics and subtropics are sources for wide spread pollution and release many organic substances into air and soil, including persistent organic pollutants, i.e. polychlorinated dibenzodioxins and -furans (PCDD/Fs) and polycyclic aromatic hydrocarbons (PAHs). The significance of this source for the exposure of humans and the environment is unknown.

The global multicompartiment chemistry-transport model MPI-MCTM [1] is used to predict atmospheric concentrations of selected PCDDs and PAHs. The model large-scale meteorology was constrained by nudging the atmospheric sub-model to re-analysis data. Global emissions of PAHs and PCDDs into air are based on recommended (PCDDs) and selected (PAHs) emission factors applied to fire distributions. Daily real-time fire data are based on satellite-observed fire radiative power measured from satellite (MODIS instrument) [2]. No other primary sources are considered. Model-predicted near-ground concentrations of PCDDs and PAHs are compared with observations during January-June 2008 at a number of stations across Africa [3]. Back-trajectory analyses suggest that some of these had been influenced by fire episodes in the region.

Continental half-year (Jan-June 2008) mean near-ground atmospheric concentrations are 0.0076, 0.51 and 3.25 fg m⁻³ of 2,3,7,8-TCDD, 1,2,3,4,6,7,8-HpCDD and OCDD, respectively. It is found that open fires can explain a major fraction of the air pollution by PCDDs in the background of west, central and southern Africa.

Highest concentrations of PCDD and PAH are predicted in 1-4 km altitude throughout most of the time, sometimes even higher and sometimes near the ground.

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EC05B-4

Tracing contaminants from urban sources to fate

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Cities can be major point sources of chemical emissions through air, surface water and waste water treatment plant discharges. Using Toronto as a case study, we found that the Central Business District held the greatest inventory of PBDEs and PCBs, had the highest air concentrations and the highest emissions to air. These results speak to the importance of non-industrial sources. This source profile is poorly served by pollution emission regulations and hence point to the need for preventative approaches to minimize releases.

EC05B-5

Exposure to airborne pollutants - experience from Danish studies

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DK air pollutant (AP) levels are generally moderate due to windy climate and moderate emissions. Despite this, DK epidemiological studies point at severe negative adverse health effects: stroke, lung cancer, COPD, asthma in adults, wheeze in infants, asthma hospital admission in children, oxidative stress in blood DNA, vascular function in elderly, AP enhance effects of radon on childhood leukaemia, and most recently diabetes. These findings have been possible due to access to precise health data and advanced exposure assessment methods. Unique population and health registries in DK allow detailed health impact assessments to be carried out. Measurements from the DK AP monitoring programmes and AirGIS calculations on address level have been used as exposure proxies in a series of publications to evaluate various negative health outcomes. The later were based either on data from health registers or biomarker measurements. For assessing exposures, a GIS based modelling system, AirGIS (www.AirGIS.dk), has been developed. AirGIS is originally aimed for traffic air pollution, but is under steady improvement and development e.g. also to handle other pollutant emissions. The central part is Operational Street Pollution Model (OSPM), currently applied in >17 countries worldwide. Within the Danish research centre AIRPOLIFE (www.airpolife.dk), the AirGIS system was applied for exposure assessment for a variety of DK cohorts including the diet, cancer, & health cohort of 50,000 people. Wood smoke is the largest source of particles emissions in Denmark, and health effects have been studied in chamber experiments, but not yet in epidemiological studies. Wood smoke and emissions of aeroallergens (e.g. pollen, fungal spores, free allergens etc) from either agricultural activities or from vegetation are among the future aims for further development of AirGIS. Air pollution is believed to increase allergenic potential of airborne pollen and risk of new sensitisation. Traditionally, monitoring of aeroallergens is performed solely through sparse networks. Recent field studies have investigated exposure variability across an urban environment, and related chamber experiments have been applied to dose-response relationships for asthmatic people. Modelling strategies used in air pollution monitoring are currently being modified for aeroallergens, where the long-term goal is to incorporate aeroallergens within the AirGIS.

EC05B-6

Insight into the primary and secondary organic fraction of the organic aerosol in an urban area: Barcelona

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Monitoring and chemical analysis of atmospheric fine particulate matter (PM₁) is important due to its health impact and influence on climate change (1,2). The air quality in the urban area of Barcelona in the Western Mediterranean Basin is assumed to be dominated by traffic related emissions and characterized by high levels of particulate matter and reactive chemical species due to emissions, the weak synoptic conditions and high solar radiation (3,4). Ambient air filter samples were collected during intensive sampling campaigns during 2009 and 2010 (DAURE and SAPUS) on urban background (UB) and road sites (RS) and a rural background at 780 m (RB). The samples were analyzed for organic tracer compounds, e.g. polycyclic aromatic hydrocarbons, hopanes, alkanes, hydrosugars and nicotine, as well as secondary organic aerosol tracer compounds, e.g. dicarboxylic acids, for source characterization and identification of the organic patterns of primary and secondary aerosols. The obtained are compared with "on-line" data, such as those generated with Aerosol-Mass-Spectrometer. The results are discussed in terms of their relation to emission sources and influence of meteorological conditions in order to get an insight on the source contributions to the complex organic aerosol [5].

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EC06 - Sorption and bioavailability in sustainable remediation of organic chemicals

EC06A-1

The role of bioavailability in risk reduction of contaminated sites

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Most regulations and regulatory accepted assessment procedures on soil and sediment contamination are still based on total concentrations. When removal of the contaminant is an expensive exercise and/or there are doubts on the risks posed by the contaminants this often leads to a "wait and see" attitude. Site investigations are often repeated, but no actions are taken to reduce the risks of the contaminants. From a risk-based point of view, contaminations are only a risk if they are or may become (bio)available. This widens the range of management options of contaminated sites and can facilitate more tailor-made solutions for individual sites. In a risk based approach stimulation of biodegradation and/or immobilization and isolation of the contaminant may play a role. In particular bioavailability can be the underlying basis for the description of risks and for determining a solution and can be used to break the infinite circle of new site investigations.

Bioavailability should be more than a concept and including bioavailability in site management asks for methods to measure the bioavailable fraction. Such methods should have an understandable physical base (ISO 17402) and are fortunately available. Using the Tenax method the available and therefore degradable fraction can be measured. Using the different available fractions it is possible to predict the rate of degradation of PAHs and mineral oil and depending on this rate a management plan can be developed. Background and applications will be shown on remediation of heavily and also slightly contaminated soils and sediments.

If contaminants are not biodegradable, the bioavailability can be reduced by immobilization, for instance by adding black carbon or by preventing leaching and physical contact by isolation. Examples will be presented on the management of remote pesticide contaminated areas in Africa using charcoal for immobilization. Natural dune formation and/or stimulation of vegetation to evaporate water thereby preventing leaching have been used for isolation.

EC06A-2

Effect of activated carbon amendments on microbiological communities in PAH contaminated urban soil

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The addition of activated carbon (AC) to contaminated soils is currently being investigated as a cost-effective remediation technique, and an important consideration is the long-term effect of the AC amendments on microbial communities involved in the biodegradation of the organic pollutants. In this work, urban soils impacted by 23±15 mg per kg polycyclic aromatic hydrocarbons were sampled from a remediation field trial, and the long-term effect of 2.0% powder (PAC) or granular (GAC) activated carbon amendments on the microbial community structure and functioning was studied, by using molecular techniques. Denaturing gradient gel electrophoresis (DGGE) analysis showed a statistically significant shift in the predominant microbial community in the soils over time, whereas the effect of PAC or GAC amendments was not statistically significant in an ANOSIM comparison. After three years, the total microbial cell count and soil respiration rates were highest for the GAC amended soils, but cell numbers and respiration rates agreed within a factor three. The sequencing of the predominant DGGE bands, which had similar relative intensity in all soils, revealed the presence of taxa with closest affiliations to known PAH degraders (ie. *Rhodococcus jostii* RHA-1), or taxa known to harbour PAH degraders (ie. *Rhodococcus erythropolis*). The potential of the microbial community to degrade PAHs was evaluated by quantifying specific dioxygenase genes, using real-time polymerase chain reaction (PCR) assays. Similar gene copy numbers were measured in unamended, PAC and GAC amended soils. Polyethylene (PE) passive samples batch studies showed a reduction of the PAHs availability with biodegradation when comparing live and sterile soils. The strongest effect of biodegradation on PAH availability was found in unamended soil, with 75% difference between sterile and live soil slurries, while the lowest PAH availability was measured in PAC amended, live soil. The combination of the chemical and microbial studies suggested that microorganisms with the ability to degrade PAHs persist long-term in soils, regardless of the presence of activated carbons amendments which reduce the PAH availability, presumably because they utilized other soil organic matter as their main carbon source.

EC06A-3

Effects of carbon amendments on ecological responses and PCB bioaccumulation in *Lumbricus variegatus*

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During the recent years synthetic black carbon products (mainly activated carbons AC, but also biochars), have been under an intense study to be used as a potential stabilization method for contaminated sediments. Results from various studies both in laboratory and pilot scale field studies have supported the high efficiency of AC amendments in sorption, and consequential reduction in bioavailability of HOCs. However, recent studies also indicate that AC amendments may inflict adverse ecological effects on aquatic organisms, e.g. sediment avoidance, inhibition in growth and alteration in sediment ingestion. The direct biological effect of AC on organisms may have an influence for both laboratory-scale bioaccumulation and toxicity testing and field-scale ecological impact assessment. The aim of this study was to test the responses of carbon amendments for remedial purposes in oligochaete *Lumbricus variegatus*, a widely used test organism in sediment ecotoxicology. The effects of carbon amendments were tested in PCB contaminated sediments, which do not have acute toxic effect on the test organisms. The measured parameters included PCB bioaccumulation, feeding activity, growth and reproduction.

The AC was more efficient in reducing the bioavailability of PCBs than the tested biochar and the reductions were sediment specific. The results showed a clear AC dose related response in all of the studied ecological parameters. The ecological responses were also sediment dependent. The AC also reduced the biomass of the worms compared to the control; moreover in two sediments out of three the AC addition in the sediment ultimately led the worms to lose weight during the experiment period, whereas in control sediments the worms were gaining weight. The effects of biochar on the biological responses were smaller than that of the AC. The site-specific evaluation is particularly important when remediation measures are designed. Negative effects of carbon amendments to the organisms, such as change in behavior, reduced growth and reduced reproduction needs to be considered, since they are important factors affecting the assessment of toxicity, bioaccumulation and ecological quality of the sediments.

EC06A-4

Effect of activated carbon, biochar and compost on the desorption and the biodegradation of low concentrations of phenanthrene sorbed to different soils

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Polycyclic aromatic hydrocarbons (PAHs) are an important class of soil and groundwater pollutants. Often, a large portion of the PAHs are degraded by soil microorganisms within short times (<100 days), and this is often followed by slower degradation resulting in a non-degradable residual fraction. Such a non-degradable residual fraction is very difficult to remove even by intensive biological and chemical methods. This does imply however, that this non-degradable residual fraction is poorly available to organisms and therefore could perhaps be considered as posing a limited risk.

The addition of PAH sorbing amendments to soil leads to reduced freely dissolved concentrations. On the one hand this limits bioavailability and uptake by organisms leading to reduced toxicity, but on the other hand this might also decrease biodegradation. The aim of this study was to characterize the soil amendments activated charcoal (AC), biochar (charcoal) and compost for their ability to reduce the desorption and biodegradation of phenanthrene as a model PAH in three different sandy loam soils (Outfield, RS, or Olsen).

The extent of abiotic desorption of [9-¹⁴C]phenanthrene from suspensions made up of soil (either Outfield, RS, or Olsen) plus amendment (either AC, charcoal, or compost) was investigated over a period of 24 days by desorption into an infinite silicone sink. The extent of desorption was then compared to the extent of mineralization (*t* = 15 d) of phenanthrene sorbed to the soil plus amendment suspensions by *Sphingomonas sp* (DSM 12247). The total amount of phenanthrene desorbed was 6 to 10% for AC, 38 to 44% for charcoal, 87 to 106% for compost, and 95 to 106% for control without any soil amendments after 24 d. This was more than percentage of initial ¹⁴C found in the CO₂-trap at experiment completion, i.e., amount mineralized. These ranged between 3.0 to 5.4% for AC, 10.4 to 14.8% for charcoal, 14.9 to 21.8% for compost, and 25.5 to 31.2% for control.

The amounts of phenanthrene mineralized were slightly lower than the maximum amounts that were abiotically desorbed, and indicate that sorption to the soil amendments had a stronger inhibitory effect on mineralization than abiotic desorption. Nevertheless, desorption into an infinite silicone sink might be useful as a tool to estimate the maximal extent of mineralization (and biodegradation) in soils polluted with PAHs.

EC06A-5

The influence of field aging of activated carbon in sediment on PCB sorption in field trials

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Bioavailability of hydrophobic organic contaminants of concern such as polychlorinated biphenyls (PCBs) in sediments is strongly influenced by the nature of contaminant binding. This observation is at the foundation of utilizing the sorption capacity of activated carbon (AC) to control risks posed by sediment-associated contaminants. Monitoring over several years at pilot-scale application sites at Hunters Point, CA and Grasse River, NY USA has demonstrated that AC amendment reduces contaminant bioavailability by controlling both chemical accessibility and activity. One important question is the long-term sustainability of this remediation strategy under field conditions. To further evaluate the sorption effectiveness of AC after prolonged exposure in the field, sorption of freshly spiked and native PCBs to 1) AC aged for 2-2.5 years under field conditions and 2) fresh AC amendments to untreated sediments were compared for sediments collected from both pilot sites. Pore water concentrations and sorption coefficients (*K_{oc}*) were determined using passive samplers in batch tests. In a separate study, a mass transfer model simulation of the effectiveness of AC amendment

to reduce pore water concentration at Hunters Point was compared to measured values for up to 5 years of field aging. Insights drawn from this modeling effort help to explain results from the sorption study. Values of K_{ac} for field-aged AC were lower than freshly-added AC for spiked PCBs up to a factor of 10, while the effect was less for native PCBs. For both Hunters Point and Grasse River field-aged AC, similarly diminished sorption compared to fresh AC for the spiked PCBs was observed. However, there was a greater decrease in sorption coefficients compared to values for fresh AC for most of the native PCBs in the Grasse River sediment which is likely due to the nearly ten times higher organic carbon content. Mass transfer modeling for Hunters Point indicates that the mass transfer limitations of PCB sorption may change over time in relation to natural organic matter (NOM). The AC does not lose sorption capacity, yet incremental improvements may slow with time. Site characteristics such as the contaminant desorption kinetics and NOM quality and quantity will be important considerations when designing sustainable remediation strategies with AC amendment. Importantly, these studies show that aged amended AC continues to effectively sorb PCBs several years following field application.

EC06A-6

Impact of biochar on the biodegradation and bioavailability of organic contaminants in soil

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The fate of hydrophobic organic contaminants (HOCs) is often controlled by the organic matter content of the soil. However, organic matter may further be mineralised or degraded by microorganisms and eventually release such organic contaminants. In order to enhance remediation of HOCs, biochar, which is a recalcitrant carbon rich product from the pyrolysis of biomass can be utilised. This study investigated the influence of 0%, 0.1%, 0.5% and 1.0% of two different wood waste biochar (BC1 and BC2), and the influence of 0%, 1%, 5% and 10% of BC1 (2 mm and 3-7 mm) on the relationship between microbial mineralisation and hydroxypropyl- β -cyclodextrin (HPCD), $CaCl_2$ and methanol extractions of ¹⁴C-naphthalene, phenanthrene and azoxystrobin in soil. The amendment conditions were aged for 0, 18, 36 and 72 days (naphthalene), 0, 35, 70 and 140 days (azoxystrobin), 0 and 40 days (phenanthrene). The total extent of ¹⁴C-associated mineralisation was assessed by monitoring ¹⁴C-associated mineralisation over 14 days in respirometric assays and compared to HPCD, $CaCl_2$ and methanol extractions. Results showed that BC1 and BC2 amendments showed significant reduction ($p < 0.01$) in extent of ¹⁴C-naphthalene mineralisation and extractions compared to 0% BC. Both particle sizes showed significant reduction in extent of ¹⁴C-phenanthrene mineralisation and extraction compared to 0%. However, the reduction was greater in 2 mm BC1. There was little or no mineralisation of ¹⁴C-azoxystrobin but BC1 and BC2 showed reduction in extractability. Linear correlation between HPCD extractability and total amount mineralised (¹⁴C-naphthalene and phenanthrene) revealed very good correlation in all concentrations of biochar amendments BC1 ($r^2 = 0.94$, slope = 0.94, intercept = -0.86) and BC2 ($r^2 = 0.94$, slope = 0.90, intercept = -1.34) for naphthalene and ($r^2 = 0.99$, slope = 1.02, intercept = 0.15) for phenanthrene. Additionally, the $CaCl_2$ and methanol extractions underestimated and overestimated extent of mineralisation respectively. This paper thus suggests that biochar used in this study can reduce the bioaccessibility of HOCs and that HPCD extraction strongly predicts the bioaccessibility of naphthalene and phenanthrene in soils amended with biochar. Thus, biochar may be a useful tool in sorption of pesticides to prevent leaching to ground water. However, the production process, particle size, feedstock and soil properties determine the capability of biochar to adsorb organic contaminants.

EC06B-1

Toxicity and bioavailability of geogenic polycyclic aromatic compounds from coal

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Coals contain native polycyclic aromatic compounds (PAC) in varying amounts depending on coal properties, e.g. origin, coal rank or biological precursor material. As a result of longterm mining activities and usage of coal as an energy source, soils and sediments worldwide can be highly contaminated by unburned coal particles. Most polycyclic aromatic hydrocarbons (PAH) that occurred in coal-rich river floodplain soils were associated with coal particles. Studies on the bioavailability of these compounds come to the conclusion of no or very low bioavailability as a result of coal acting as a very strong sorbent. The highly varying properties of coals including the content of heterocyclic aromatic compounds (NSO-PAC) and PAH were not taken into account. Hence, the aim of the present study is (1) to verify the assumption of no or very low bioavailability of coal-derived PAC for different selected coal samples of varying properties and (2) to identify former less known toxic PAC. The results are expected to impact future risk assessment of coal-rich soils and sediments.

Subbituminous (n = 2), bituminous (n = 5) and anthracite (n = 1) coals were used in various contact assays and a bioaccumulation test to assess bioavailability of PAC.

Fractionated extracts of the coals were additionally used in bioassays to assess the potential toxicity.

EPA-PAH analysis of the aromatic fraction of the coal extracts revealed highest concentrations in bituminous coal extracts (up to 120.1 mg/kg). Both fractions of all coal extracts (except the anthracite) exhibited dioxin-like activity and cytotoxic effects. The aromatic fractions of all coals (except the anthracite) and the heterocyclic fractions of bituminous coals were mutagenic. Both fractions of all coal extracts (except the anthracite) were toxic in assays in liquid medium for *D. rerio*, the aromatic fractions were toxic for *C. elegans*. Results of the contact assays using whole coal particles with the same organisms were different: While the whole coal samples led to no mortality in the fish embryo assay (0 to 2.5%), inhibition of *C. elegans* reproduction was 83 to 96% induced by the different coal samples.

We conclude that there is no bioavailability of PAC from coals that leads to mortality in the fish embryo assay. Effects in the nematode contact assay are suspected to result from damages caused by the physical properties of the particles.

Further results will be presented.

EC06B-2

Adsorption of organic contaminant from aqueous solution on natural porous material

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In 2000, the European Union adopted the Water Framework Directive (WFD) which project to obtain a good status of water and aquatic system by 2015. Substances posing health risk to human and to the aquatic environment are listed in two categories: substances that should be reduced and substances that should be eliminated. Pharmaceuticals and pesticides are concerned by this regulation project. Particularly hazardous, some of these compounds have tendency to accumulate in living organism where they exert toxic effects.

The wastewater treatment process used currently are not efficient enough to remove all of these compounds and residues of these chemicals are persisting in surface water. During the last years, biological, chemical and physical methods have been developed in order to remove organic contaminant from water. Among these processes (nanofiltration, reverse osmosis, biological degradation, ozonation), sorption is one of the most attractive especially when the adsorbent is low-cost.

In this study, we propose to investigate the adsorption capacity of different natural adsorbents for pharmaceuticals and pesticides. Zeolites and clays are probably the most promising alternatives to high cost adsorbents. Zeolites belong to the family of hydrated aluminosilicates. Their structure is formed with SiO_4 and AlO_4 tetrahedra conferring them a large internal and external surface area and high porosity. Sepiolite is a hydrated silicate of magnesium presenting a fibrous structure. So we have selected three zeolites among the most abundant in nature (clinoptilolite, mordenite and chabazite) and one clay (sepiolite) in order to investigate and compare their adsorption capacity for drugs and pesticides.

EC06B-3

Novel pathways in the adsorption of weak organic acids by black carbon leading to ionization constant shifts on the surface

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Black carbon (BC) is a natural component of soils and sediments, and the use of manufactured BC (activated carbons, biochars) has been proposed as a remediation tool. A fundamental understanding of the adsorption mechanisms of contaminants to BC is a prerequisite to technological control and an accurate evaluation of bioavailability. Although many contaminants are ionic or ionizable over the normal environmental pH range, little attention has been paid to the interaction of such compounds with BC. Here I show examples of weak organic acids that adsorb to BC by unconventional mechanisms. We studied the adsorption to biochars of the allelopathic aromatic acids AA, cinnamic (pKa 4.44) and coumaric (pKa 4.39) and the veterinary antibiotic sulfamethazine SMT (pKa1 2.28, pKa2 7.42; existing as SMT0, SMT+, SMT- or SMT+/-). Extending several units above the pKa, the anionic forms AA- or SMT- adsorb by first undergoing proton exchange of the carboxylate or sulfonamide group, respectively, with water, liberating hydroxide ion. Proton exchange is followed by adsorption of the corresponding free acid, AA or SMT0. This results, essentially, in a pKa shift of the organic acid on the surface of +5.3 units (AA) or +3.0 units (SMT) relative to the corresponding pKa in water. Since the increase in hydrophobicity is insufficient to compensate for the proton exchange penalty, the driving force for this reaction we believe is the formation of a strong H bond between the organic acid and a surface carboxylate or phenolate group having a comparable pKa. Such "low barrier H bonds" rank among the strongest known in organic chemistry. The bonds are depicted as $[RCO_2[3DOTS]H[3DOTS]O-surf]$ and $[-SO_2N[3DOTS]H[3DOTS]O-surf]$ where the proton is shared almost evenly between the heteroatoms. At pH values where SMT is protonated, the charged p-aminobenzene group undergoes pi-pi-electron donor-acceptor interaction coupled with cation-pi interaction with the pi-electron rich graphene

surface (termed $\pi^+ - \pi$ EDA). At intermediate pH values, the $\pi^+ - \pi$ EDA bond drives a positive shift in the pK_{a1} of SMT and an increase in the stability constant for the zwitterion SMT $^{+/-}$ on the surface relative to the respective value in water. We conclude that adsorption of weak organic acids on black carbon surfaces can result in appreciable shift in ionization constants on the surface driven by the formation of strong complexes, such as low barrier H bonds and $\pi^+ - \pi$ EDA interactions.

EC06B-4

Determining sorption of cationic surfactant to organic matters and clay minerals- An application of Ion-Exchange SPME method

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Sorption property is crucial for the mobility of organic compounds in environment and hence is highly relevant to the bioavailability of those chemicals. The freely dissolved is mostly regarded as the bioavailable fraction and often measured by passive sampling techniques based on equilibrium partitioning processes. In this study, a 7- μ m polyacrylate SPME fiber was successfully used as an Ion-Exchange passive sampler for measuring freely dissolved cationic surfactant. Cationic surfactants are permanently charged organics and have high affinity to negatively charged environmental media (e.g. surface of clays and humics). By employing the SPME method, we studied the sorption behavior of benzyl dimethyl dodecyl ammonium chloride (C12-BAC) to different sorbents, including humic acids, clays minerals and an artificial sediment. By identifying the composition of the artificial sediment and measuring the sorption coefficient of the individual sediment component, the sorption behavior to the whole sediment could be estimated, which was comparable to the data for sediment-only sorption experiment. Whether the sorption of cationic surfactant to field sediment could also be extrapolated via such a method requires further research.

EC06B-5

Walking the tightrope of bioavailability: growth dynamics of PAH degraders on vapour-phase PAH

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Effective biodegradation of soil contaminants requires both adequate environmental conditions and a suitable contaminant bioavailability to degrading microbes. Compound bioavailability however, is 'Janus-faced'; i.e. is essential and likely promoted by the organism for assimilative uptake, whereas, when too high, may lead to toxic effects and provoke avoidance strategies (e.g. tactic responses) of the target organisms. Although the bioaccessible compound pool for both effects may be identical, the exposure of individual organisms to environmental chemicals has opposite consequences and may particularly interfere, when a metabolisable substrate causes toxicity at high bioavailability. Such situation leads to a microbial tightrope walk that is often overlooked in bioremediation studies where one tends to assume that effective pollutant-utilizing bacteria tolerate any exposure to these substrates. The highlights of this paper give evidence of down-gradient, i.e. negative (chemo-)tactic movement of *Pseudomonas putida* (NAH7) away from a NAH point when exposed to vapour phase NAH (surprisingly at even at gaseous concentrations lower than aqueous concentrations that clearly induce chemo-attraction). They secondly elucidate the tightrope walk of substrate bioavailability for assimilative growth and growth inhibition of strain PpG7 in vapour-phase NAPH gradients: Microcosm experiments revealed that high cell densities increased growth rates close (< 2 cm) to the NAH source, whereas intercellular competition for NAH decreased growth rates and biomass at larger distances despite the high NAH gas phase diffusivity. Finally, such varying growth kinetics is explained by a combination of bioavailability restrictions and NAH-based inhibition. To account for this balance, a novel, integrated 'Best-equation' describing microbial growth influenced by both substrate availability and inhibition is presented. Our work clearly demonstrates the importance of bacteria to degrade vapour-phase compounds and to influence vapour-phase PAH concentration gradients even at the centimeter-scale. It further underlines the importance of high active biomass and concomitant effective reduction of their exposure to inhibitory substrates in order to create environments favourable for survival, which may influence the exposure dynamics and ecology of entire microbial communities and hence actively shape environments beneficial for enhanced biodegradation.

EC06B-6

Contribution of microbial biomass to non-extractable residue formation from an organic contaminant

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Biodegradation of pesticides in soil is actually understood as their transformation into various metabolites, microbial biomass, mineralisation products and non-extractable residues (NER). NER are believed to mainly consist of hazardous parent compounds or primary metabolites sorbed to or sequestered by soil organic matter (SOM). Up to date, however, their chemical composition remains still unclear. This is because numerous studies on NER formation are limited to quantitative analyses in soils or to simple humic acids-contaminant systems. During biodegradation of organic contaminants, the C is used by microorganisms for their biomass synthesis. After cell death and lysis, biomass components are stabilised in SOM ultimately forming harmless biogenic residues. We investigated the formation of biogenic residues during biodegradation of 13C-labelled herbicide (2,4-dichlorophenoxyacetic acid) in soil over 64 days. We prove for the first time that nearly all NER formed from this readily biodegradable herbicide in soil contained only non-hazardous microbial biomass components. Therefore, for the proper assessment of the potential risks of a target contaminant in soil to environment it is necessary to consider a possible biogenic origin of NER in the mass balances of contaminants in soils.

EM01 - Collection and use of monitoring data for environmental risk assessment of chemicals

EM01A-1

Update on the global monitoring plan (GMP) in the UNEP Stockholm Convention on persistent organic pollutants (POPs), technical guidance, data analysis, modeling, assessment and workplan

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The GMP established under Article 16 of the Stockholm Convention on POPs (SC) has been active since 2004 and produced a first global assessment in 2009. It is currently revising the technical guidelines, an extensive document describing recommended methods and procedures to monitor POPs in the GMP core media, air and human tissues (milk, serum). The work under the GMP has identified the relevance of modeling long range atmospheric transport including meteorological and climatic variability to understand better the effects of a changing climate on POPs. Important results have been published in the United Nations Economic Commission for Europe's (UNECE's) report on Hemispheric Transport of Air Pollution (HTAP) 2010 Part C and the United Nations Environment Programme (UNEP)/ Arctic Monitoring and Assessment Programme (AMAP) report of "Climate Change and POPs: Predicting the Impacts" Challenges for future work include long term stability and coordination of the networks, the analytical and data quality assurance/quality control (QA/QC), data management and archiving, the development and use of models dealing with atmospheric and marine long range transport, as well as modeling ecological and physiological pathways and time lags in exposure leading to health impacts. This paper describes the main results and identified challenges that will define the future work of the GMP, an international cooperative knowledge building process focused on the fate of POPs released into the environment and the possible ways to decrease or eliminate them.

EM01A-2

Adapting monitoring strategy to the contaminant source characteristic - chromium in the upper Dunajec River watershed

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Application of the monitoring data instead of estimated or predicted values for contaminant emissions and concentrations is crucial for environmental risk assessment. Therefore, representativeness and reliability of these data should be assured. Enhanced knowledge about the temporal and spatial patterns of contaminant distribution improves effectiveness of monitoring data collection. Since these patterns are not uniform in aquatic environments carefully designed sampling regime should be considered for different types of aquatic systems, especially in the operational monitoring under WDF. A 19-week continuous water sampling in the upper Dunajec river watershed (Southern Poland) has proved that chromium contamination in this river, originating from the local tanneries, is variable and discontinuous. To compare two monitoring strategies instantaneous (discrete) and integrated (composed) samples were collected. Sampling was performed in three sites, encompassing the local impoundment reservoir system, which allowed also for estimations of chromium load introduced into the reservoir. Discrete samples were collected weekly by hand, while integrated samples were collected using automatic water samplers (ISCO) in the time-integrating mode. This mode rather than discharge proportional mode was chosen for a better comparison with the discrete sampling. To assess impact of monitoring strategy on load computations dissolved and particulate Cr loads in instantaneous and integrated samples were estimated using averaging calculation methods. Study results showed that discrete sampling can lead to an underestimation of chromium contamination level and load, especially when illegal/unexpected discharges occur in the watershed. From chromium load computations based on results for both types of samples we also concluded that integrated sampling produces more reliable data, with acceptable range of estimated errors. Instantaneous sampling, even if frequent, should not be used for contaminant budget calculations in the localities where strong variability of contamination is anticipated.

EM01A-3

Factors influencing the quality of river monitoring data used for environmental risk assessment of particulate/hydrophobic chemicals

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From 2000 to 2009, about 300 chemicals were analysed in water, bed sediment and suspended particulate matter (SPM) at 17 sites throughout the Rhône river network by the regional water authority, providing a very rich and useful dataset. This work focuses on factors influencing the quality of this database. Few studies have been carried out on the analysis of hydrophobic organic contaminants and metals associated with SPM, we thus favoured critical review of these data. Data processing revealed several issues that can weaken the dataset quality. While the constancy of analytical methods is of utmost importance, data processing highlighted major temporal discontinuity for some chemical concentrations, either due to changes of the analytical methods, or due to inappropriate or shifting quantification limits. Furthermore, despite the fact that this dataset refers to a river network, no chemical concentrations were recorded during flood events, biasing any annual average concentration evaluation, especially for contaminants associated with SPM. Thereby, the sampling frequency or strategy should be carefully chosen in order to obtain information on potential variations of concentrations linked to the river hydrological cycle. Finally, sampling methods should be characterised and compared. In a recent study of the Rhone Sediment Observatory we focused on the comparison of different SPM sampling techniques. A difference in the particle size distribution of SPM sampled with the various techniques was noticed, which could have an effect on the concentrations of contaminants associated with SPM. To conclude, this study revealed that factors such as analytical methods, sampling frequency and sampling methods impact concentrations or annual average concentrations of chemicals in SPM, bed sediment and water, and thus impact the monitoring dataset quality. Consequently, the outcome of an environmental risk assessment based on such a monitoring dataset might be significantly affected. Interlaboratory comparison tests, quality controls, as well as a complete documentation on sampling and analytical methods are among possible solutions to build relevant datasets of chemical concentrations in rivers.

EM01A-4

Making management decisions with imperfect data: assessing potential aquatic metal risks with biotic ligand models

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Accounting for bioavailability, through the use of sophisticated Biotic Ligand Models (BLMs), represents the most technically robust method assessing potential metal risks in the freshwater aquatic environment. New user-friendly BLMs (ufBLM) are now available to facilitate their regulatory use. These new models are based on the outputs of the more sophisticated BLMs but require data on fewer water physicochemical parameters to run (i.e. limited to just pH, calcium and dissolved organic carbon). However, there remain obstacles to using the tools, specifically the lack of site-specific physicochemical input data. Absence of required input data means the models cannot be run. Yet, while not always starting with the perfect dataset for all sites of interest, it is rarely the situation that there is a complete absence of "fit of purpose" input data. There are several ways by which input data gaps can be filled in a robust, precautionary, manner to deliver a screening level assessment which can then be used to develop focussed monitoring programmes, identify sensitive sites and broadly characterise risks. This presentation will give an example of how, through the use of imperfect data, management decisions can be made in relation to the assessment of potential aquatic risks of metals.

EM01A-5

Mapping the chemical environment of London: the London earth project

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The British Geological Survey has been involved in a number of programmes to systematically map the concentrations of chemical elements in the surface environment of the United Kingdom. These include both European-wide EuroGeoSurveys' projects (e.g. FOREGS geochemical atlas and GEMAS metals in agricultural soils) and regional projects in rural and urban areas of England and Scotland. In May 2010 the results for the largest urban mapping survey undertaken, 'London Earth', were released. Soils were sampled at a density of four samples per kilometre square from the Greater London Authority Area. This represents one of the world's largest systematic geochemical mapping exercises in an urban area. Over 6,400 topsoil samples were determined for more than 50 elements by X-ray fluorescence spectrometry (XRF) and other soil parameters such as pH and loss on ignition were determined.

Since the publication of the results further analyses have been done on subsets of the samples including mercury (Hg) and organic contaminant studies, profile lines mapping the occurrence of Au and PGEs (platinum group elements), and bioaccessibility studies. Applications of the London Earth data to environment and health issues will be presented.

EM01A-6

Active local sources of PCBs in the Arctic

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Studies of PCBs in local sources, including building materials such as paint and concrete, small electrical capacitors, and local surface soil were initiated on Svalbard, Norway in 2007. Previous to these studies increasing levels of various pollutants in marine sediments outside specific settlements were discovered, suggesting an active, local source to PCBs.

During 2007-2010 more than 1100 single samples of paint, concrete, soil and small capacitors from 12 different active and abandoned settlements were collected. 78 soil samples from 24 background sites were also collected. The exterior paint in the settlements can be regarded as a primary source of PCBs. In a dry and extreme climate such as exists on Svalbard, paint will eventually flake off and fall to the ground. The settlement soil can then be regarded as a secondary source of PCBs, which can spread the pollution to terrestrial or marine ecosystems by wind and/or water erosion. The background sites will be affected by a mixture of long-range transported PCBs and PCBs from local sources.

This work shows active, local sources to PCBs in the Arctic. The levels of PCBs in the primary sources are up to permille levels and the risk of dispersion is evident. These finds should be taken into account in the ongoing discussion of local sources versus and long-range transport sources. From an administrative point of view, this gives an opportunity to actually take care of the pollution sources before they are spread to the environment.

In this presentation the work done at Svalbard will be emphasized, but also other similar work will be shortly reviewed.

EM01B-1

GEMAS: Geochemical mapping of agricultural and grazing land soils at the European and national scales

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Geochemical mapping of agricultural and grazing land soils (GEMAS) is a cooperative project between the Geochemistry Expert Group of EuroGeoSurveys (EGS) and Eurometaux. REACH specifies that industry must prove that it can produce and use its substances safely. During 2008 more than 4000 samples of arable (0-20 cm) and grazing (0-10 cm) land soil were collected at a density of 1 site per 2500 km² from 33 European countries. The sample density is based on previous experience of the Geological Surveys with comparable projects. The < 2mm-fractions of all samples were analysed for 52 chemical elements in an aqua regia (AR) extraction, 41 elements by XRF and soil properties like CEC, TOC, pH (CaCl₂) following tight external quality control procedures. Natural variation is large (3 - 4 orders of magnitude for most elements) and results can be reliably mapped. Distribution maps of all parameters and elements are produced and demonstrate that the chosen scale is fit for purpose. The data allow the study of the spatial distribution of chemical elements and soil properties at the European scale, and better understanding of processes driving the observed patterns. The distribution patterns of selected elements will be discussed based at the European and national scale maps (e.g., Germany, Ukraine). All elements and parameters display clear geographical distribution patterns that can be linked to a variety of natural features, geology and climate playing a key role. Many elements show substantially lower concentrations in Northern than in Southern European soils. The project will provide background values for a large variety of elements in agricultural and grazing land soils at the European scale.

EM01B-2

Metal bioaccessibility in Canadian soils: using the North American soil geochemical landscapes project

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The North American Soils Geochemical Landscapes Project (NASGLP), a collaborative effort among the US Geological Survey (USGS), the Geological Survey of Canada (GSC) and the Mexican Geological Survey, was initiated to provide a soil geochemical database for a broad-based group of users in the field of environment and human health. This Tri-national Survey will ultimately produce a database of the regional natural-occurring differences in concentrations and physicochemical characteristics of soil which can be used to assess background conditions and identify anthropogenic impacts. The survey is based on low-density sampling (within a 40 km by 40 km grid) yielding 13,487 sites across North America. Soil sampling and analysis in Canada was initiated by the GSC in partnership with other provincial and federal agencies including Health

Canada in 2004. Analytical parameters included total metals, pH, total organic carbon, inorganic carbon and loss on ignition. A sub-set of the samples collected in Canada from the surface (0-5 cm, referred to as the public health layer) and the C horizons were analyzed for metal bioaccessibility using a simplified physiologically based extraction test as a surrogate for bioavailability. Results will be presented on the bioaccessibility of naturally-occurring metals in the soil public health layer and the C horizon, along with relationships of bioaccessibility with soil geochemical data. The data obtained indicated some differences in mean elemental bioaccessibility between the public health layer (0 - 5 cm) and the C horizon (parent material) for each province. There were also some statistically significant relationships between metals bioaccessibility and soil pH, total organic carbon and loss on ignition for a sub-set of the samples. Comparison of the elemental bioaccessibility in the natural background soils to literature information on bioaccessibility from contaminated sites (e.g., mine sites) in Canada will also be shown, along with how the data might influence derivation of generic soil quality guidelines in Canada.

EM01B-3

Quality assurance in the GEMAS project and results of the connected proficiency test

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Quality assurance is one of the keystones to the success of any European scale geochemical mapping project requiring harmonized data. Therefore a set of stringent quality control (QC) measures were introduced into the analytical program of the GEMAS (geochemical mapping of agricultural and grazing land soils) project including field duplicates, analytical duplicates, insertion of two project standards (Ap and Gr) as internal references between true samples, and randomization of all samples prior to analysis. X-Charts proved to be helpful in immediately solving some technical problems as exchange samples or time trends. The within-lab-reproducibility of QC data for the standards Ap and Gr is generally good, but strongly dependent on the analyte. The nested design of duplicate field and duplicate analytical samples allowed carrying out a statistical analysis of variance (ANOVA) to identify the relative contributions of regional, sampling and analytical variances. The ANOVA demonstrates that the technical variability is low enough to detect regional differences in geochemical maps.

Additionally a proficiency test (PT) based on the project standards was performed in 2011 to check the comparability of individual national laboratory data with the data used for mapping, and to confirm the trueness of the mapping data. In total 21 institutions from 16 countries submitted analytical data, including the mean values from QC analyses as "normal participants". Evaluation of data and laboratory assessment was done using robust statistics, limited standard deviations and zu scores. Results are presented for between-lab-reproducibilities, Horwitz ratios as indicator for PT performance, and inter-method discrepancies between analytical methods and sample preparation techniques. The assessment of the QC data for the standards Ap and Gr in this PT confirms the trueness of total element contents and most AR contents.

EM01B-4

Comparison of XRF and Aqua Regia data from agricultural soil in Europe: results from the GEMAS project

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The comparison of analytical results from aqua regia (AR) and X-ray fluorescence spectroscopy (XRF) can provide information on soil processes controlling the element distribution in soil. The GEMAS (Geochemical Mapping of Agricultural and grazing land Soils) agricultural soil database, consisting of 2 x ca. 2100 samples spread evenly over 33 European countries, is used for this comparison. The GEMAS project was designed to fulfil REACH (Registration, Evaluation and Authorisation of Chemicals) requirements (e.g., land use, sampling depth). It minimised critical error sources by standardisation of sampling procedures, sample preparation and analysis. Analyses for the same suite of elements and parameters were carried out in the same laboratory under strict quality control procedures. Sample preparation has been conducted at the laboratory of the Geological Survey of the Slovak Republic, AR analyses were carried out at ACME Labs, and XRF analyses at the Federal Institute for Geosciences and Natural Resources, Germany.

Element recovery by AR is very different, ranging from <1% (e.g. Na, Zr) to > 80% (e.g. Mn, P, Co). Recovery is controlled by mineralogy of the parent material, but geographic and climatic factors and the weathering history of the soils are also important. Nonetheless, even the very low recovery elements show wide ranges of variation and spatial patterns that are affected by other factors than soil parent material. For many elements soil pH have a clear influence on AR extractability: under acidic soil conditions almost all elements tend to be leached and their extractability is generally low. It progressively increases with increasing pH and is highest in the pH range 7-8. Critical is the clay content of the soil that almost for all elements correspond to higher extractability with increasing clay abundance. Also other factors such as organic matter content of soil, Fe and Mn occurrence are important for certain elements or in selected areas.

This work illustrates that there are significant differences in the extractability of elements from soils and addresses important influencing factors. Consequently, soil risk assessments for elements or metals should be made using effects and exposure values, based on the same extraction method.

EM01B-5

Use of monitoring data for environmental risk assessment of metals in soil

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A sound risk assessment for trace elements in soil preferentially takes into account the spatial variation in natural background concentration and in metal bioavailability. Data availability for both aspects differs largely across different countries or regions, hampering a consistent approach on a large (e.g. regional or continental) scale. It is therefore often difficult to compare results for regional risk assessments among different countries. The GEMAS (geochemical mapping of agricultural and grazing land soil) project provides good quality and comparable exposure data of metals in agricultural and grazing land soil at the European scale. In addition soil properties known to influence the bioavailability and toxicity of metals (and other elements) were determined in the same soils. The GEMAS project was carried out by the EuroGeoSurveys (EGS) Geochemistry Expert Group in cooperation with Eurometaux and the aim of this project was to produce harmonised data with respect to the spatial scale (sampling density), analytical methodology and land-use (comparable level of diffuse emissions). The results allow a consistent risk characterisation across Europe and country-specific results can be directly compared. The data also provide a strong basis for taking into account the spatial variability of both exposure (metal concentrations) and effect concentrations (considering bioavailability through variation in soil properties) in a risk assessment for metals in soils and therefore avoid the need for (worst-case) assumptions on both aspects. The use of these GEMAS monitoring data for regional and local risk assessments of metals in soil will be discussed based on examples for several metals.

EM02A - Fate and exposure modelling

EM02A-1

Estimating and evaluating cumulative human exposures to ubiquitous pollutants: integration of outdoor, food web, and indoor fate models with exposure biomarkers

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Widespread observations of environmental contaminants in house dust, food, vegetation, soil, animals, and human tissue have motivated research on better understanding of exposure pathways for a broad range of contaminants over indoor, urban, regional, continental, and global scales. This presentation addresses how fate models at different levels of geographic scale combined with environmental and biomarker measurements can be used to interpret and predict cumulative human exposure from multiple pollutants and emissions sources. We consider three case studies to explore the insights gained by simple but informative integration strategies. First we look at regional scale pesticide exposures in a farming community and compare local contributions to exposure from pesticide use on fields with contributions from food produced within and outside the region. For this study we have a reliable source data and robust biomarker data. Here we find that the integration of outdoor and indoor fate models is key to tracking overall intake. We next look at cumulative exposures to combustion-produced polycyclic aromatic hydrocarbons across ~3000 counties in the United States for which emissions data are available. For this case the variations of county emissions track well with the variations in national-scale biomarker data. But there are clear indications that cumulative intake is dominated by food and indoor exposures. Finally, we look at commercial buildings with filtered and re-circulated air to assess how indoor exposures to particle-bound semi-volatile organic contaminants (SVOCs) are impacted by the level of air recirculation. For this case the reduction of SVOC exposure indoors relative to outdoor levels is sensitive to both the octanol-air partition coefficient and the fraction of air re-circulated. These three cases reveal that the relative contributions to cumulative pollutant intake via different exposure pathways depend on (a) persistence of chemicals at different levels of integration (regional, urban-scale, food-web, indoors), (b) basic chemical properties, (c) the retention of chemicals in food webs, and (d) the retention of chemicals by indoor surfaces.

EM02A-2

Reducing empirical data need in fate and exposure models by overall (scaling) principles

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Environmental chemistry and toxicology face the immense challenge of protecting thousands of species from thousands of substances released into thousands of different landscapes. Empirical studies are limited because of financial, practical, ethical and time-space restrictions. To cover all relevant cases, fate and exposure models have been developed. Yet, such models too, tend to become data hungry because of parameterization. Properties of chemicals have extensively been used to extrapolate knowledge, allowing one to estimate default values for parameters in the absence of experimental data. The objective of the present paper is to extend this approach to species traits and landscape characteristic. In particular, we aim to identify similarities and differences in size scaling in the various disciplines involved, i.e. hydrology, chemistry, biology and technology.

Confining ourselves to lakes and organisms, we will show similarities and differences in the turnover of water, energy and chemicals. Both data and theory suggest that inflow in lakes scales geometrically while inflow in organisms (ventilation, consumption etc.) increases allometrically.

Understanding the overall principles determining the cycling of water and biomass helps us to predict the fate and accumulation more accurately. Rather than choosing a "typical" value for run-off in fate models, we might choose the average and standard-deviations noted for catchments of a certain size. Instead of picking an uptake rate constant for accumulation from a more or less related species, we can now estimate the kinetics in poorly investigated specimens from their body size. In addition, overall principles as size scaling open up opportunities to design generic models.

While we focus on the approach for size scaling, other variables can help to refine explanations. Estimations can be improved by adding temperature as an explaining variable, allowing one to distinguish between temperate and tropical regions or between cold-blooded and warm-blooded animals. In addition, size scaling can also be applied to other parts of the environmental cause-effect chain. As an example for emissions of carbon dioxide and polycyclic aromatic hydrocarbon, fuel use by engines scaled similarly to running organisms. At the same time turnover and species abundance (size spectra) in ecosystems can be related to environmental stressors.

EM02A-3

Not PBT, but LRTP - Identification of substances with possible very high concern

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In the European chemicals legislation REACH, a substance is considered of very high concern if it is persistent, bioaccumulative and toxic (PBT). A substance's long range transport potential (LRTP) and the subsequent hazard to remote regions are not explicitly included but can give cause for serious concern. To identify compounds, which would not be classified as PBT substances but are prone to LRTP (non-PBT-L substances), we screened the Canadian Domestic Substance List (CDSL), which contains 22,438 compounds in use on the Canadian market.

In a first step, the CDSL was searched for organic compounds which are to more than 95% in the neutral form at an environmentally relevant pH range. The identified compounds were run through EPI Suite v4.0 such that substance properties were quantified by the implemented estimation methods. Next, the list was edited in two ways to identify compounds with LRTP. On the one hand, the half-life criterion for long-range transport in air as defined in the Stockholm Convention was applied (half-life in air > 2 days). On the other hand, all compounds were simulated with the multimedia model ELPOS v2, which calculates relative indicators for persistence and LRTP (Matthies et al. 2009). Applying the half-life criterion of 2 days in air as an indicator for LRTP, we identified 621 substances, which are prone to LRT; 594 of these are not PBT. In contrast, investigations with ELPOS lead to a shorter list of 188 substances which are not PBT but show LRTP (non-PBT-L substances). All 188 non-PBT-L compounds are persistent, but not bioaccumulative. However, in order to decide if these substances are of very high concern the list was compared with potential Arctic contaminants identified by Muir and Howard (2006) and by Brown and Wania (2008), respectively. This combination of different screening methods allows to focus on a subset of eleven organic substances, which are not PBT but identified to be characterized by LRTP. An additional literature research on substance properties elucidates possible hints on the reliability of the screening procedure.

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EM02A-4

New read-across model to estimate the BCF for fish from similar chemicals

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The bioconcentration factor (BCF) is a key property to assess the bioaccumulation of organic chemicals. Existing estimation models relate the BCF for fish to the octanol/water partition coefficient. Available experimental data from literature have been collected and thoroughly validated, resulting in a high quality data set of BCF for fish covering 1000 organic chemicals.

Theread-across approach calculates the BCF from similar compounds by a similarity weighted averaging. The selection of the similar compounds and the numerical calculation of the similarity weights are achieved by atom centred fragments (ACF).

The similarity threshold to consider reference compounds can be fine-tuned to balance between accuracy and generality. With the default threshold, the model yields valid results for ca. ¾ of the chemicals. The statistics clearly demonstrate the reliability of the new approach. The model reliability is further investigated by inspecting the value diversity and the molecule size ranges for the selected compounds. A relationship between the estimation errors and these ranges can be shown.

The model performance roughly compares to the literature models. The errors of the new approach are not correlated individually per compound to the respective errors of the other models. However, it can be shown that generally agreement of the prediction results is related to small estimation errors. This offers the opportunity to apply a suite of models for consensus modelling.

The new model as well as the studied literature models is already implemented in the software system ChemProp. The software offers uncertainty estimation for the result in addition. ChemProp is publicly available for free, based on a bilateral license agreement.

The development of this method has been supported by the EU Integrated Project OSIRIS (Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Test Information, contract No. 037017). A part of the data was compiled within the former EU project CAESAR (Computer Assisted Evaluation of industrial chemical Substances According to Regulations, contract No. 022674).

EM02A-5

Modelling the impact of dietary transitions on human exposure to bioaccumulating organic contaminants

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By combining models of a contaminant's fate in the physical environment with models of bioaccumulation through the food chain, it is now possible to describe mechanistically and dynamically the entire sequence of events linking emissions and body burden in humans. Here we rely on such a model combination to examine the role of dietary transitions on human contaminant levels. Concentrations of persistent organic pollutants (POPs) in Inuit populations have been observed to decrease over the last decade or so. Are these decreases an indication of the success of international efforts to reduce the emissions of POPs globally or did they occur because the diet of the Inuit has shifted away from traditional food items toward store-bought food? It is assumed that the diet of the Inuit is composed of regionally harvested traditional food and of food imported from the South. The global contaminant fate model is used to calculate time trends of contamination in the physical environment of the Arctic and the Northern temperate zone over the past 100 years. Using the calculated contamination time trends in the Arctic environment as input, the contamination of the traditional country food is calculated using the ACC-Human bioaccumulation model. The contamination of the imported food is similarly calculated using ACC-human and the calculated contamination time trend in the temperate environment. Longitudinal body burden age trends of a number of individuals representing different sections of Inuit society are simulated: Those maintaining a predominantly traditional diet throughout their life-time, and others who underwent dietary transitions at different times, ages, and to a different extent. By combining multiple longitudinal body burden age trends calculated for individuals born in different years, cross-sectional body burden age trends can be generated and compared with those obtained from cross-sectional human biomonitoring studies. The model predicted concentrations of POPs decline over the past decade both in Individuals who maintained a traditional diet as well as those that increasingly relied on store bought food from the South for sustenance. The latter group is predicted to have experienced a faster decrease in exposure. Transitions in the diet from traditional to store-bought food may contribute at least as much to declines in contaminant concentrations in Inuit as declines in environmental contaminant concentrations.

EM02A-6

Modelling approach to estimating the source strength of cyclic volatile methylsiloxanes from the city of Zurich, Switzerland

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The cyclic volatile methylsiloxanes (cVMS), octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6), are widely used in personal care products. Given their high volatility resulting in a large fraction of the used amounts being emitted to the atmosphere, their persistence in the environment, and their potential for long-range transport, it is important to improve knowledge about emission sources to provide a basis for future environmental fate modelling and for the risk assessment and management of cVMS. In this study, we present a novel approach to estimating the urban source strength of cVMS by employing a multimedia mass balance model in combination with measured cVMS concentrations in air, and apply it to D4, D5 and D6.

Diel variations of ambient air concentrations of D4, D5 and D6 were measured at two sites in Zurich, Switzerland, in winter 2011. One site was located in the valley near the city center and the other site was on a nearby hill. A period with a temperature inversion was chosen for the sampling campaign in order to investigate the enrichment of cVMS in the boundary layer. A multimedia mass balance model was developed and employed to (i) explain the concentration trends observed at both sites in terms of dominant processes and (ii) to estimate urban source strengths. Choosing the emission rate as an adjustable model parameter made it possible to estimate the source strength based on empirical data gathered during the study or available in literature. Key parameters include the measured cVMS concentrations, inversion layer height, temperature, wind speed, and physicochemical substance properties of D4, D5 and D6.

Levels of D4, D5 and D6 in air in the city center were 14-110, 67-450 and 7-57 ng/m³, respectively. Concentrations on the hill were lower (3-49, 29-110, 5-12 ng/m³). There was an increase in the concentrations by about a factor of three in the city during our sampling period, and this could be attributed by the model to the enrichment of the chemicals in the boundary layer. Boundary layer height, which varied considerably throughout the sampling campaign, was determined to be the dominating factor. Degradation by OH radicals and deposition were of less importance. For D5, our estimated source strength compares well with estimated emission rates derived from studies on emission rates from individual buildings or emissions estimates based on market information.

EM02B-1

Scenario-study of the expected cumulative impact of European Union environmental legislation on the aquatic discharges from land - an example with three priority substances

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In order to support the implementation of the Marine Strategy Framework Directive, it was carried out an evaluation of the impact of EU environmental legislation on the quality of the marine environment, with reference to discharges to the European seas. The assessment focused on current trends and future options for reduction of inland-based chemical emissions. The evaluation was intended to check a methodology developed at JRC-IES (MAPPE-Europe model) for the identification of hot-spots in Europe and its capability to estimate the likely chemical loads to European coastal waters under different scenarios.

The work describes outcome of scenario analyses up to 2020 for Lindane (gamma-HCH), Trifluralin and Perfluorooctane sulfonate (PFOS) taken as pilot substances. We assumed different types of legislative measures (business as usual, ban, phase out, etc.) or aimed at specific targets (total or disaggregated load to European seas and possible "cleaning-up" of soil in Europe). When considering the outputs from the scenarios, it is important to take into account the fact that the project aimed to test the applicability of the modelling platforms and that, since limited datasets were used and certain assumptions had to be made, the outputs should be regarded as approximative.

For Lindane, the model estimated sea load of 745 tons per 1995, based on the official emission data provided by EMEP, appears to be reduced by 98.3% in 2005, ten years after the start of the EU regulations for gamma-HCH. Besides, under the BAU scenario, a Lindane sea load of ca.12.5 tons per year should be expected. The trend and ban scenarios support, respectively, a reduction of the load to the European seas in 2020 by 74% and 95% when compared to the BAU estimate.

Looking at Trifluralin, according to the BAU scenario, an annual load of ca.61.7 tones is estimated in 2020. However, this is an overestimation of sea load, because the aggregated emission data of EUROSTAT for the agriculture use of the entire group of dinitroaniline herbicides for EU25 have been considered as model input data. The ban scenario forecasts ca. 0.07 t/y, a negligible level that, due to degradation in soil, in practice eliminates the concern about loads of Trifluralin to European seas.

Considering PFOS under BAU scenario conditions, the total sea load from European countries is estimated to be 5.8 tons per year. The model forecasts approximately a half of this amount when a 50% reduction of emissions takes place.

EM02B-2

Modelling assessment of climate change-induced effects on the fate of POPs in the Baltic Sea region

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The recently released report of the UNEP/AMAP Expert Group stresses that global climate change may cause redistribution of some legacy persistent organic pollutants (POPs) and possibly counteract the efforts which have been made to reduce releases of POPs under the Stockholm Convention. In the present study we assess how predictions of the future variation in three climate change parameters, temperature, wind speed and precipitation, affect the environmental fate and distribution of POPs in the Baltic Sea region using the POPCYCLING model. The model was applied to the entire range of possible perfectly persistent POPs within the following property ranges; $0 < \log K_{OW} < 12$, $5 < \log K_{OA} < 14$, and $-6 < \log K_{AW} < 4$. Perfect persistence was assumed for all the POPs to represent a bounding scenario in which the effects of redistribution of POPs due to a changing climate are maximized. Two greenhouse gas emission scenarios were adopted which are referred to as A2 and B2 representing high and low CO₂ emissions, respectively. A baseline ("present day") scenario was adopted as a control scenario for comparison with the future climate change scenarios. Quotients were calculated between predicted POP concentrations in air based on the A2 or B2 climate change scenarios versus the baseline scenario. The results show that the more extreme climate change scenario (i.e. the A2 scenario) causes relatively more pronounced changes in the modelled air concentrations irrespective of to which media the emissions are assumed to occur. Climate change-induced variations in the modelled concentrations in air vary with changing emission mode. Greater changes in modelled concentrations are observed when emission is assumed to occur to the water or soil compartment, i.e., the quotients between predicted air concentrations under the A2 or B2 scenario versus the baseline scenario range from 0.6 to 2.0, but the quotients range from 0.6 to 1.7 when emission is assumed to occur to the air. Our results thus indicate that the influence of climate change on the concentrations of POPs in air is strongly dependent on the partitioning properties and emission characteristics of the substances, and that although climate change-induced effects on partitioning of POPs may work to counteract emission reductions efforts for some POPs, in other cases climate change may hasten the removal of the POPs from the atmosphere.

EM02B-3

Persistent organic pollutants in the North Sea in the 21st century: simulations with a combined hydrodynamic and fate and transport ocean model

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The fate and cycling of two selected persistent organic pollutants (POPs), PCB 153 and gamma-HCH in the North Sea in the 21st century is modelled with a combined hydrodynamic and Fate and Transport Ocean Model (FANTOM). Large amounts of POPs enter the North Sea system through atmospheric deposition and river inputs, with additional contributions coming from bottom sediments and adjacent seas. To investigate the impact of climate variability on POPs in the North Sea in the 21st century, future scenario model runs for three 10 year periods to the year 2100 are performed. Based on the IPCC A1B (SRES) scenario for the 21st century, our hydrodynamic model results show that average water temperature in the North Sea increases by 2 °C, while salinity is reduced by about 0.59 psu. In the atmosphere, temperatures at 2m increase by 2.38 °C. For wind speeds (10 m), mean values do not change significantly but increases in local maxima values and duration of gale wind conditions are significant. It is the effect of these changes on POPs that are analysed. Hydrodynamic variables are calculated with the Hamburg Shelf Ocean Model HAMSOM model and POPs processes are calculated with the FANTOM model. Since estimates of future concentration levels of POPs in the atmosphere, oceans and rivers are not available, our approach was to reutilise 2005 values in the ocean, atmosphere and rivers for every year of the simulations, while using the (final) result of our 1996 - 2005 runs as the initial condition for all three future runs. In this way, we attribute differences between the three 10-year simulations to climate change only. A cursory look at the results show that for gamma-HCH total mass is highest in summer in both water and sediment with a clear seasonal cycle; total mass in water remains fairly steady for each of the 10-year runs but decreases in each of the future runs relative to the first; total mass in sediment increases over time with future runs increasing faster. For PCB 153, total mass in water remains steady while decreasing linearly in sediment; total mass is greatest in water and least in sediment during the winter months when winter storms cause resuspension from the sediment into the water column (this process is also seen in gamma-HCH). Presently, we investigate to which components of the climate system these changes can be attributed. It appears the North Sea acts as a sink for gamma-HCH but as a source to the atmosphere for PCB 153.

EM02B-4

Geo-referenced exposure modelling of pharmaceuticals in river basins supports selection of mitigation strategies

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The geo-referenced model GREAT-ER can be used to simulate expected concentrations of pharmaceutically active chemicals (PhAC) in surface water. The model was amended by features for simple menu-controlled definition of measures to reduce PhAC emissions into surface water. Scenario results can be displayed as concentration ratios against a reference scenario. Predictions of spatially resolved concentrations of the antibiotic Clarithromycin in the Main river basin (Germany) are presented to demonstrate how the model can support the selection of promising mitigation strategies. A reference scenario was prepared representing the current state of the contamination with Clarithromycin. Two different scenarios were selected to demonstrate the feature of the model for a priori evaluations of the effect of measures: (i) a local scenario in which two of the larger WWTPs discharging directly into river Main were arbitrarily selected to be equipped with an additional ozonation treatment step with 90% removal efficiency for Clarithromycin; and (ii) regional scenario for the eastern part of the Main catchment in which we assumed that at least 10% less consumption of Clarithromycin can be

achieved in the area due to a shift in prescription and consumption behavior.

The effect of the scenarios is evaluated by comparison of the concentration profiles of Clarithromycin along River Main. The effect of the local technical measure at the upstream WWTP is evident from much lower simulated concentrations. The additional ozonation at another WWTP more downstream only leads to a small decrease in concentration. The regional measure proved to be much less effective in the upstream part, but results in almost the same improvement more downstream. From the simulation data at the mouth of river Main into the Rhine, it can be seen that both measures obviously removed only a small fraction of the total emission in the catchment. This is an effect of the overlying multi-point emissions from the several hundred wastewater treatment plants of which only a small number have been included in the respective measures. Concluding, the model allows for the identification of local "hot spots" and also an a priori evaluation of potential mitigation strategies. The analysis given shows that for pharmaceuticals sustainable overall reduction of surface water concentrations is hardly to be achieved by local measures, even if some of the largest WWTPs are technically refitted.

EM02B-5

Spatiotemporal exposure assessment of pesticides in flowing waters - results for predicted environmental concentrations in some brooks in Germany

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In 2011 the "Georisk"- project of the German Federal Environmental Agency was finalised. Objective of this project was to form the scientific basis for an integration of more realistic landscape based scenarios into the process of pesticide registration. Here, results of spatiotemporal simulations of PECTwa in flowing water systems are presented. The objective of the simulations was to predict initial environmental concentrations in flowing water bodies resulting from spray drift entries. Based on this the downstream development of these concentrations over space and time with regard to dispersion processes was simulated (PECTwa, Time over Threshold) including different application pattern within two days using a random generator (application time slot 2 days from 8 a.m. to 6 p.m.). We calculated 25 different application scenarios. An adequate GIS-based software-environment and a functional workflow have been developed which make use of high and medium resolution geodata (water bodies, application areas, mitigating vegetation). The observed spatial entity here is a brook in the Hallertau Region, Germany. All hydrological parameters were derived from ground truthing data. Additionally a more generalised approach using artificial scenario-based landscape definitions will also be presented.

While the risk assessment assuming lentic water bodies is based on the comparison of the PECini with the RAC, the local exposure pattern predicted by this dynamic model is summarized to the maximum TWA (i.e. 1 h, PECTWA(1h)) and the total duration when the PEC is above the RAC (ToTh). Because the local PEC is depending on the variable timing and magnitude of the pesticide entries upstream, Monte-Carlo distributions provide a set of possible exposure patterns for each segment from which different PECTWA(1h) can be extracted (e.g. minimum, maximum, median). The exposure duration (as ToTh) is based on the calculation of a RACdyn to consider that effect thresholds are higher if the exposure duration is shorter.

The results show a continuous downstream increase of ToTh and a downstream increasing TWA strongly correlated to application patterns and the hydrological parameters. The artificial scenario-based results show thresholds of hydrological parameters of the flowing water systems where the influence of the hydrological conditions are the most important influencing parameters related to the simulated PECTwa.

EM02B-6

High resolution GIS maps for predicting the POPs contamination in soil and comparison with measured data

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The assessment of POPs contamination requires reliable spatial maps for burden and flux assessment. In this work, contamination maps were developed and validated at a space resolution of 1[GREEKX]1 m with a time frame of one day, in an experimental area located in the central Alps, where direct measurements of PCB concentrations in soil and environmental parameters were available for the year 2008.

Physical algorithms calibrated on experimental data were set up for temperature and organic carbon estimation, along the soil profile and across the year, in order to deduce the horizontal, vertical and seasonal distribution of the contamination potential for PCBs in soil (Ksa maps).

The developed maps were validated with an external set of PCB contamination data, giving very good results (e.g. for CB-153, R² = 0.80, p-value $\leq 2.2 \cdot 10^{-06}$). The obtained regression coefficients were used for the mapping of the actual soil contamination (concentration maps), taking into account the temporal shifts in soil concentrations from the equilibrium (as defined by Ksa values). These maps offer the opportunity to evaluate burden and fluxes with highly resolved temporal and spatial detail, and therefore with a high degree of ecological realism (emission maps).

EM02C-1

Comparison of far field and near field exposure from consumer products for chemical exposure prioritization

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Traditionally fate and exposure modelling for Risk Assessment and Life Cycle Assessment of products has been focusing its effort on multimedia modeling of far field exposure. However, direct exposure of consumer products via either indoor air (for e.g. indoor furniture or flooring) or via direct dermal contact (e.g. for cosmetics or toys) may represent a dominant exposure. The present project therefore aims at developing metrics and method to consistently compare near field and far field exposure to chemical in products and at testing them on a serie of case studies of three consumer products.

Direct releases from consumer products are characterized by a new metric - the Product Intake Fraction - that determines the fraction of a chemical in a product that is taken up by humans during its use and disposal phase.

Measured releases for a pacifier plasticizer used in typical average conditions leads to relatively high product intake fraction of 6-10-2 and modeled product intake fraction for different shampoo ingredients are in the range of 10-3.to 10-1 depending on the considered chemical properties. For indoor releases of the flooring material, indoor intake fraction is high in the range of 10-4.to 10-2.

Comparison between impacts of direct consumer exposure to those linked to far field life cycle emissions shows that direct impacts are of the same magnitude as or higher than indirect life cycle impacts. Similarly the impacts of indoor releases of a flooring material are of the same order of magnitude as the respiratory effects of outdoor emissions. An uncertainty analysis shows that that the extrapolation from acute to chronic toxicity was the major source of uncertainty (54.1%) and that the three main substances contributing to the impacts also dominated the uncertainty with 84.0% of the total uncertainty.

EM02C-2

Assessment of local impacts of municipal solid waste management using life cycle assessment (LCA)

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This research takes part in a PhD work whose aim is to develop a methodology to assess locally environmental impacts of municipal waste management (MSW) in order to provide environmental elements to local decision makers and stakeholders.

MSW is a local issue managed under the responsibility of local authorities. To integrate environmental considerations, decision-makers often use Life Cycle Assessment (LCA). However, the LCA methodology does not take into account the characteristics of the territory involved and consequently not allow local assessment. Nevertheless, such a consideration appears necessary for local issues such as toxicity and odours. To solve the problem of assessing local impacts, the SETAC (Society of Environmental Toxicology and Chemistry) recommends the use of the Site dependent approach. This approach allows considering, in the characterization step, some spatial and temporal conditions of releases and some characteristics of the potentially affected environment in order to realize fate, exposure and effect analysis.

Our methodological research focuses on fate analysis step and its integration in the classification step. The novelty of our approach is the consideration of local environmental characteristics, through the USEtox model in a modified version, to locally assess toxicity and odours impacts. USEtox is a multimedia model which describes the fate, the exposition and the effect of substances released in the environment through three boxes (global, continental and urban). For spatial assessment of substances fate, we propose to change in the dimensions of the environmental boxes, compartments and in local relevant parameters which are determined by default in USEtox and not suitable for local assessment.

This work leads to the construction of a new set of spatial elements for classification and characterization steps for toxicity and odours impacts. The new classification step will be based on USEtox results from the new version and the consideration of spatiotemporal conditions of emission. The characterization step will be limited to the consideration of health effect and olfactory thresholds. The final objective is to compare classical LCA results (without taking into account spatial and temporal parameters) with located toxicity and odour LCA results. The spatial assessment of local impacts should allow policy makers and stakeholders to get "personalized" answers to debate within the decision-making.

EM02C-3

Coupled modelling of plant uptake, soil water balance and soil solute transport for estimating the fate of cadmium and lead in amended agrosystems

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Soil water balance, solute transport, leaching to groundwater and plant uptake of solute and water are coupled processes. Recently, a coupled plant and groundwater transport model for NaCl could simulate the transpiration-induced changes in groundwater salinity. However, for metals, no models that simultaneously predict plant uptake as well as leaching to groundwater were found. In this study, the water budget of soil, the uptake into plants and the leaching to groundwater of cadmium (Cd) and lead (Pb) were simulated simultaneously using a physiological plant uptake model and a tipping buckets water and solute transport model for soil.

Factors affecting uptake of trace metals into vegetation are type of metal, plant species and cultivar, plant-related parameters such as transpiration and growth, and soil parameters like pH, organic matter, soil texture and redox status. Robust tools for predicting the transfers of metals from soil and air to plants are scarce and often incorrect due to the large variability of metal uptake in plants.

The objective of this work is to present and test a model framework for the simulation of the coupled transport of water and dissolved trace metals, the uptake of both into crops, and leaching of solute and water to groundwater. The model is parameterized with data derived from a ten-year field study where four organic amendments were applied every two years.

Simulation results were tested on measured concentrations of lead (Pb) and cadmium (Cd) in soil and plants from the ten-year field study. Predicted concentrations slightly decreased with time in control soils, but increased in amended soils by about 10% (Cd) and 6% to 18% (Pb). Estimated plant uptake was lower in amended plots, due to an increase of Kd. Predicted concentrations in plants were close to measured levels in plant residues (straw), but higher than measured concentrations in grains. Initially, according to the model, lead was mainly deposited from air into plants (82% in 1998); later uptake from soil was dominating (30% from air in 2006), because of decreasing levels in air. For cadmium, uptake from air into plants was negligible (1-5%).

EM02C-4

Ranking of emissions of plant protection products from protected crops to environmental receptors

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A number of EU Member States requested EFSA to develop guidance on how to carry out environmental risk assessment of Plant Protection Products (PPPs) emitting from covered crops. In response, EFSA established a working group and commissioned supporting research to establish an inventory of protected crop systems and to determine the importance of emissions from these systems to relevant environmental compartments. The working group developed a classification system, consisting of 13 categories ranging from mulching and direct cover to high-tech greenhouses and closed buildings, in order to categorise the enormous variability in covered crop systems. The work on emissions performed so far revealed that emissions from covered crops may be substantial and might have been underestimated in the past. For the small and more open systems, it is expected that emissions do not substantially differ in comparison to open field systems. Comparison of emissions from selected closed walk-in structures with emissions from open field identified greenhouses and walk in tunnels having priority for scenario development for risk assessment. For these covered crop systems, the Panel on Plant Protection products and their Residues (PPR Panel) recommends the development of tiered risk assessment procedures. Further investigation is necessary to find out whether risk assessment for open field may be used as the first tier in these procedures. Furthermore, elements were identified that should be considered in deriving the risk assessment scenarios, for both soil-bound and soil-less cultivations.

EM02C-5

EU scenarios for exposure of soil organisms resulting from spray applications of pesticides to annual crops

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Based on a Member State consultation EFSA initiated a revision of the guidance for the soil exposure assessment including development of EU scenarios for numerical models. The aim of the exposure assessment was to obtain a 90th percentile of the PEC in space and time considering all fields in the regulatory zones North, Centre and South grown with the target crop where this active substance is applied. The types of ecotoxicologically relevant concentrations considered were both the concentration in total soil and the concentration in pore water averaged over the top 1, 2.5, 5 or 20 cm of soil (both peak and TWA concentrations for time windows up to 56 d). The scenario selection was based on a simple analytical model that calculates the concentration in soil assuming that degradation (first-order kinetics at a constant rate) is the only loss process from the 20-cm plough layer. The concentration in the pore water was calculated assuming a linear sorption isotherm assuming that sorption is proportional to organic matter. Concentration maps were calculated at a resolution of 1x1 km² for the whole area of annual crops in each of the regulatory zones North, Centre and South. Simulations including uncertainty in the dry bulk density and in substance properties such as the Kom and the DegT50 showed that a spatial 95th percentile has to be selected for obtaining an overall 90th percentile when median or geomean values of these substance properties are used as input for the scenario calculations in the regulatory process. So the target was to select a spatial 95th percentile. Scenario were selected based on maps of the peak concentrations in total soil and in pore water that were generated for 19 substances and averaging depths of 1 and 20 cm. The selected scenarios for concentration in total soil have all relatively high organic matter contents and low temperatures. However, the selected scenarios for the pore water concentration have all relatively low organic matter contents because the pore water concentration is almost directly proportional to the inverse of the organic matter content for substances that show significant sorption. Calculations with the simple analytical model for a number of example substances showed that the concentration in total soil increased in the order South-Centre-North and that the concentration in the pore water increased in the order North-Centre-South (so opposite orders for these two types of concentration).

EM02C-6

Mechanisms behind conservatism in tiered soil risk assessment

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In recent EFSA opinions on assessment of exposure of organisms to substances in soil a rigorous scientific concept was presented to describe the spatio-temporal distribution of chemical substances in soil. Further ecologically relevant soil layers were derived. These layers represent the habitat of specific soil organisms used for risk assessment. For example the soil layer 0-1 cm is proposed for epigeic and anecic earthworms. The mean concentration for this layer (Ecologically Relevant Concentration) can be identified from the predicted concentration depth distribution. It is finally used for lower tier risk assessment (RA) in combination with an effect endpoint, e.g. a NOEC.

Though this approach appears straightforward it produces a surprising result. Compared to current soil RA it leads to dramatically increased ERC for a number of test organisms. On the other hand there are several publications comparing the current lower tier soil RA to higher tier (field) studies serving as reference tier. These came to the conclusion that the current soil RA is appropriate, i.e. it discriminates between critical and uncritical substances in sufficiently conservative way.

Why does the soil RA proposed by EFSA become so conservative and is this justified? A concept is proposed which analyses the whole process (design of lower tier effect study, derivation of endpoint, link to the exposure situation under real-world conditions) in order to provide answers to the above question.

The RA for earthworms is used as example because of the large number of studies available. Several factors may be considered to attribute to the different occurrence of effects in lower (lab) and reference tier (field) studies.

- Time to effect. Duration of study at reference tier typically 1 year, usually 56 days at lower tier (earthworm reproduction).

- Likelihood of proposed ecologically relevant soil layer, e.g. 0-1 cm. This habitat is very narrow. Extremes of soil moisture and temperature may render it temporarily to a hostile environment

- Impact of initial concentration. NOEC values usually in terms of initial concentration. Often experimental period at reference tier is long compared to DT50 of compound. Thus mean concentration is likely to be much lower than initial one.

These factors are investigated employing detailed analysis of the spatio-temporal concentration distribution in soil. Based on this information local effects can be estimated and aggregated on population level.

EM02D-1

Variability and estimation of pesticide half-lives in vegetation

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Half-lives in plants are among the most important drivers for the fate and behavior of pesticides in the environment, but also among the most uncertain parameters in multimedia models. We therefore aim at characterizing the variability of measured half-lives in plants collated from peer-reviewed literature and identify important predictor variables to provide an estimation model to predict bulk removal half-lives in various plants and plant components for a wide range of pesticides. We analyzed more than 1000 data points covering a variety of reported half-lives in plants of almost 200 pesticides applied to at least one of 100+ plant species collected from 270+ peer-reviewed references published between 1956 and 2012.

Reported half-lives for synthetic pesticides range from 4.8 hours for dichlorvos sprayed on chrysanthemum flowers to 54 days for tebuconazole sprayed on wheat. The geometric mean half-life of all data points aggregated over pesticides and plant species is 4.1 days with a geometric standard deviation of 6.25. We identified a typical variability of less than a factor of 20 between reported half-lives. We also identified differences between removal from plant surfaces (usually leaves) and plant interior compartments, thereby giving insight into different contributing physical processes, such as photo-degradation exclusively important for plant surface removal. The number of data points per pesticide ranges from a single measurement for 28 pesticides to 55 analyzed samples for the well-studied insecticide endosulfan. We identified molecular weight, K_{ow}, K_{oc}, air and soil temperature, air and soil humidity, wind speed, surface-roughness, plant water and lipid content, soil pH and radiation intensity as potential predictor variables to be further analyzed for their influence on the degradation and other removal processes and for their reliability to be included into the final regression model.

EM02D-2

Predicting herbicide leaching to field drains in a clay dominated headwater catchment using MACRO

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The one-dimensional non-steady state MACRO model was used to help interpret observed propyzamide and carbetamide losses in an 8.6 ha underdrained headwater sub-catchment of the Upper Cherwell, with strongly undulating topography. Although the catchment is dominated by heavy clay soil, a small area of lighter and better drained soil over sandstone generates some groundwater contribution. Estimates of recharge to the sandstone aquifer were made using soil moisture balance calculations. A one-dimensional time-variant groundwater model was used to represent groundwater flow. Significant transfers of both herbicides to the drain network occurred soon after application. Peak concentration coincided with peak drain flow and concentrations then decreased gradually in a quasi-exponential fashion, mirroring the receding hydrograph. Observed carbetamide concentrations were about an order of magnitude higher than those observed for propyzamide due to a combination of a higher application rate and lower K_{OC}. For propyzamide, total observed loss over the study period was estimated to be 1.1% of the applied mass and for carbetamide the loss was estimated to be 8.6%. MACRO predictions of the timing and magnitude of peaks in drainflow and herbicide concentrations were reasonable, particularly when corrected for baseflow contributions, although a period of a month when snow covered the ground led to a failure to represent peak flow. Hydrograph and chemograph recession was also reasonably well predicted. Nearly 100% of herbicide leaching to drains in this heavy clay soil is predicted to occur via macropore flow for both propyzamide and carbetamide. The results demonstrate the utility of one dimensional models as an explanatory framework for processes operating in larger undulating fields with significant topography. The results also confirm that drainflow is probably the dominant pathway for the transfer of these herbicides to the catchment outlet. This imposes considerable constraints on the management options available to reduce problematic herbicide exposure in this catchment.

EM02D-3

Nano-pesticides: state of knowledge, impacts on fate and exposure assessment

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Nano-formulations are already used in the pharmaceutical and food industries. In comparison, applications in the agrochemical sector are only emerging and a rapid growth is predicted in the upcoming years. Deliberate application of nanoparticles as within agricultural practices could be one of the rare intentional diffuse inputs of engineered nanoparticles into the environment (e.g. nano-silver). It is thus essential that risks and benefits to human and environmental health are adequately evaluated.

The aim of the present study is to (i) review the current state of knowledge on nano-pesticides, (ii) identify possible impacts on environmental fate and (iii) analyse the suitability of current pesticide exposure assessment procedures to account for novel properties within the EU regulatory context.

Nano-pesticides encompass a great variety of products and cannot be considered as a single category. The aims of nano-formulations are generally common to other pesticide formulations and consist in increasing the apparent solubility of poorly soluble active ingredient (a.i.), releasing the a.i. in a slow/targeted manner and/or protecting against premature degradation. Some nano-formulations are expected to have significant impacts on the fate of a.i. and/or to introduce new ingredients whose environmental fate is still poorly understood.

Current exposure modelling procedures are probably satisfactory for assessing the fate of most nano-formulations, provided that (i) realistic sorption and degradation parameters can be determined and used as model inputs, (ii) changes with time can be accounted for, if necessary, or (iii) the separate assessment of the various ingredients is proved to be a protective approach in all cases.

For formulations containing particles that may exhibit colloidal behaviour, further issues need to be considered since the transport of solutes and nano-particles cannot be described by the same approaches. The potential retarded/facilitated transport of the a.i. due to its association with a nano-carrier (e.g., polymer based or nano oxides associated with a.i.) should also be considered.

Further research is thus required in order to (i) identify the assumptions currently applied that are not valid in the case of nano-pesticides, (ii) evaluate the points or situations in which differences may impact significantly on the exposure assessment outcomes, and (iii) refine or adapt current protocols as required.

EM02D-4

On the effect of local pesticide reduction programmes in large river basins

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Large river basins have multiple sources of pesticides, which may be spread over more than 100-1000 km². To effectively reduce the amount of pesticides entering surface water bodies, reduction programmes are implemented. The cumulative effect of pesticides entering the river system in upstream areas can compromise downstream water use e.g. raw water quality for drinking water abstractions. Local reduction programmes that target certain uses of pesticides may clearly affect local concentrations while the expected impact on the larger scale may fail to appear. For larger scale assessments pesticide fluxes coming from other sub basins and even transnational fluxes need to be taken into account. We illustrate the effect of a local reduction programme for the use of glyphosate on hard surfaces in the Meuse river basin. We used a long-term data set of glyphosate concentrations in the Meuse river to derive the trends in the concentrations before and after the implementation of the reduction programme. The results indicate spatial difference in the course of concentrations over time. In some areas of the river basin trend reversal is found, which might be possibly linked to programmes of measures but insufficient evidence is provided by the trend analysis to found this assumed action-effect relation.

Further modelling results complementing the monitoring data indicate spatial heterogeneity in the contribution of the sub basins in the targeted management area. The obtained regional insights are important for policy makers in prioritizing certain areas or approaches. In the Meuse river case, the hydrology of the catchment is such that the contribution from hard surfaces during summer is expected to have a larger impact on the downstream water quality because of low river discharges. Future recommendations are targeted monitoring in sub basins and at the outlets of waste water treatment plants and modelling the whole catchment to distinguish between sources and to derive cost-effective programme of measures.

EM02D-5

Application of a process based metamodel (MetaPEARL) to support vulnerability assessments of leaching to groundwater on different geospatial scales

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1. Introduction

A process based metamodel (MetaPEARL) is applied to support vulnerability assessments for leaching of pesticides to groundwater on different geospatial scales.

2. Materials and methods

The MetaPEARL model is based on an analytical model for pesticide leaching and a statistical evaluation of the simulations for 1000 scenarios and 56 pesticides with the numerical model EuroPEARL (Tiktak et al 2006). MetaPEARL requires a limited set of spatial data (precipitation, temperature, organic matter and texture) and takes into account the most important substance properties, available in registration dossiers (Kom and DT50). Geospatial information in form of homogeneous, EU-wide raster data are used in our exercise for MetaPEARL. The raster data, provided by the Joint Research Centre of the European Commission in cooperation with the European Food Safety Authority (EFSA), are the most recent and comprehensive information (<http://eusoils.jrc.ec.europa.eu/library/Data/EFSA/>). The maps contain information on soil, climate, land use, land cover and crops with a cell size of 1 km.

3. Results and discussion

We use the MetaPEARL model with the JRC/EFSA maps to determine the relative potential of pesticide leaching in the use areas of specific pesticides and crops in the EU. Taking into account the simplifications of the metamodel we provide relative vulnerabilities of scenarios to example pesticides rather than predicting concentrations.

Furthermore setting leaching studies into context of the area of use are explored by GIS analysis. The approach may support the evaluation of leaching studies generated under certain conditions in one region by setting them into context (on a relative scale) to the environmental conditions in other regions.

4. References

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EM02D-6

PEARL-BE: combining a meta-model and a proces-based pesticide leaching model to develop groundwater scenarios for Belgium

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Registration of pesticides on the European market requires a risk assessment of groundwater contamination following guidelines of the FOCUS workgroup. Nine worst-case standard FOCUS scenarios representing different soil types and climatic conditions are developed for Europe. In this study, the pertinence of standard FOCUS scenarios for Belgian conditions is evaluated and more specific scenarios are developed for each agricultural region using local information on soil and climate.

Local soil profiles were constructed using information from the Aardewerk-database (13 000 soil profile descriptions for Belgium) and the digital Belgian soil map.

Within each agricultural region, soil profiles were parameterised using median soil properties for each soil type. Hydraulic soil properties were determined using pedotransferfunctions derived for Belgian soils. Daily meteo data for a 20-year period were available for 10 by 10 km grid cells. Crop calendars were parameterised according to the FOCUS Châteaudun scenario.

Modelling of pesticide leaching was done in a two-step approach. First Meta-PEARL was applied to all soil profiles to delineate vulnerable soils using median yearly net precipitation for each agricultural region. Soils with a predicted groundwater concentration close to the 80th percentile of PECs were considered as vulnerable soils. Next FOCUS PEARL was applied to the selected sensitive soils using daily meteo data for the centroid of each agricultural region. At the scale of Belgium, the predicted pesticide groundwater concentrations for 10 sensitive soils were compared to the results of 4 FOCUS scenarios (Châteaudun, Hamburg, Kremsmunster, Okehampton) to evaluate the pertinence of the FOCUS scenarios for Belgian conditions. Spring and autumn application of FOCUS substance B to sugar beet and winter cereal were compared. The Belgian scenarios were more critical than the FOCUS scenarios in all cases. At the scale of the agricultural region, FOCUS PEARL was applied to 3 sensitive soils and the scenario with the highest PEC was selected as the sensitive local scenario for the region.

The study shows that the results from the FOCUS scenarios are quite different from the results obtained with scenarios adapted to the local situation and based on more detailed information on soil characteristics. For Belgium, the use of local scenarios derived according to FOCUS guidelines is more conservative with respect to the protection of groundwater resources.

EM03 - Global scale modeling of environmental cause-effect chains for risk assessment and life cycle assessment: Quo vadis?

EM03-1

GIS-based regionalized Life Cycle Assessment: how big is small enough? Methodology and case study of electricity generation

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Few regionalized life cycle impact assessment method developers have justified their choice of spatial scale. In order for the promise of regionalized impact assessment to be realized, spatial scale should be chosen in a systematic fashion, and should match the spatial scale of the environmental phenomena that drive changes in characterization factors across space.

We propose the minimization of global spatial autocorrelation (AC) as a systematic technique to choose the spatial scale of impact assessment methods. Spatial autocorrelation is present when a data value can be inferred with minimal error from the data values of its spatial neighbors. The presence of AC can be interpreted as the presence of phenomena which are influencing CFs on a different spatial scale than the chosen one. The minimization of autocorrelation is therefore the choice of a spatial scale which best matches the spatial scale of the environmental or societal phenomena which are driving significant changes in CFs.

We calculated changes in global spatial autocorrelation at various spatial scales by using different techniques to aggregate disaggregated data. Our hypothesis is that the minimal spatial autocorrelation can be found by varying the number of spatial areas in the impact assessment method. In a case study for ecosystem damage due to freshwater consumption, global spatial autocorrelation was minimized at a spatial scale of 12.000 spatial units.

The choice of spatial scale is quite important in understanding, interpreting, and applying regionalized impact assessment methods correctly and with minimal uncertainty.

Because the method proposed here makes no assumptions about spatial pattern or number of spatial areas, it can be applied to all regionalized impact assessment methods.

EM03-2

Spatially-explicit global scale modelling of acidifying air emissions

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Terrestrial acidification is the process in which atmospheric deposition, related to emissions of SO₂/SO₄, NO_x and NH₃, cause changes in soil acidity that can eventually harm terrestrial flora. Life cycle impact assessment (LCIA) uses characterization factors (CFs) to evaluate the consequences of terrestrial acidification. Current available LCIA methods modeling relies on regional to continental spatially-resolved models to evaluate atmospheric fate, soil sensitivity and the effect on the ecosystem, respectively. This represents a major limitation when assessing commodities produced and traded throughout a global economy. Consequently, this work shows a novel global scale approach to assess pollutant atmospheric transport and deposition, its consequences on soils, and its ultimate effect on plant species richness. CFs include an atmospheric fate factor (FF), a soil sensitivity factor (SF), and an effect factor (EF). FF describes the atmospheric impact pathway from the emission location to the corresponding deposition location, SF represents the soil pH change due to a marginal change in deposition and EF quantifies the biome-specific vegetation's "potentially not occurring fraction of species" (PNOF) due to a change in soil pH. FF derivation was based on the global tropospheric GEOS Chem chemical model. Changes in soil pH were evaluated with a geochemical steady-state model parameterized with a novel set of regional parameters at a global scale. EF was calculated from published regression and coefficients as a function of terrestrial biomes. CFs were then calculated through the sum of products of these three factors. CFs were obtained at a resolution of 2°x2.5° for SO₂/SO₄, NO_x, NH₃ and emissions. The obtained 2°x2.5° CFs results were also aggregated to coarser spatial resolutions: worldwide, continental and country levels. The outcome of this study is particularly useful for LCA practitioners who can now assess the consequences of a global economy while still maintaining the ability to differentiate emissions occurring between and within countries and thus enhance the environmental relevance of LCIA results. Furthermore, as worldwide spatial variability can now be assessed coherently, it gives the practitioners an idea of the uncertainty incurred from the choice of using coarser resolved (i.e. world, continental, country) CFs when the emission location is not "exactly" known.

EM03-3

Spatially-dependent freshwater concentration-species richness response functions for nitrogen and phosphorus

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Up to now, there are no concentration-species richness response functions for nitrogen (N) and phosphorus (P) in freshwater systems on a global scale. Reduced data availability and strong spatial specificity are reasons that challenge global scale modelling. The objective of our study is to (1) derive dose-response functions of aquatic species richness along both N and P concentration gradients for freshwater ecoregions worldwide and (2) compare the potential gain in precision and the increase in uncertainty by simulating different levels of spatial detail.

This was attained by gathering data from the literature (total of 472 studies) on the occurrence of aquatic species (diatoms, invertebrates, and bacteria, among others) along N and P concentration gradients. We gathered data on a total of 3707 species distributed across 110 freshwater ecoregions. In the presentation, we will (1) show results of the logistic regressions based on the empirical data we attained from the literature and (2) address the issues of increasing uncertainty and loss in precision by grouping ecoregions with similar characteristics with each other. This is a necessary step to assess the risk of nutrient pollution to world's regions for which very little data is available.

Our study tackles the issue of reduced data availability by performing a thorough empirical data gathering on a global scale. It also maintains spatial specificity and with the use of an ecoregion level of spatial resolution. Ultimately, our study provides a consistent method to compare the effects of N and P concentration to different aquatic organisms and different freshwater systems worldwide. Our results allow for ecologists to identify world's freshwaters that are most susceptible to nutrient concentration shifts. In addition, it is a valuable method to environmental risk and life cycle impact assessments since our results can be easily integrated to fate and exposure modelling so as to provide a cause-effect chain of freshwater eutrophication.

EM03-4

Including metal speciation in LCA terrestrial ecotoxicity: new regionalised characterization factors

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At present, ecotoxicological impacts of metals are often the main contributors to LCA results. This domination does not necessarily reflect a real toxicity but rather indicates the misuse of fate and effect models, initially developed for organic compounds and not accounting for metal speciation. A framework for including metal speciation in freshwater ecotoxicity in LCA has been developed recently. It relies on the use of a commercially available speciation model to define the bioavailable metal fraction and of freshwater archetypes to account for the influence of freshwater physicochemical properties on speciation. Although really interesting, this approach is not directly applicable to soils because speciation models were developed for aquatic environments. Soils greater heterogeneity hinders generalization and could invalidate hypothesis made in aquatic geochemical modelling.

The main goal of this project is thus to develop a method specific to soils, including defining the appropriate regionalization scale, in order to obtain terrestrial ecotoxicity CFs.

The Harmonized World Soil Database (HWSD) version 1.1 (FAO/IIASA/ISRIC/ISS-CAS/JRC, 2009) was used and enhanced to gather data on soil properties. The soil bioavailable metal fraction is computed with a soil specific parameterization of two available aquatic speciation models (WHAM 6.0 and MINEQL+), for each of the 5200 possible combination of soil properties of the world listed in the enhanced HWSD. The model parameterization is validated using field data. Multiple linear regressions are performed to detect the most influential soil properties on bioavailable metal fraction and soils are grouped in archetypes according to these properties. According to the framework proposed for freshwater ecotoxicity, a bioavailable factor is added to the definition of the CF and new effect factors are computed in terms of bioavailable fraction. Using the new regionalization scale here defined, new CFs are calculated for Zn, Ni and Cu for terrestrial ecotoxicity.

Results obtained for Zn indicate that it is possible to group soil types of the world in 70 different archetypes when considering the 9 most influential soil properties on speciation ($R^2=0,776$). Model predictions are in good agreement with measured values (a difference of not more than one order of magnitude). Zinc bioavailability factors, with a variability of 20 orders of magnitude, show the importance of integrating speciation in LCA.

EM03-5

Evaluation of spatial variability of metal bioavailability in soils using geostatistics

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Soil properties show significant spatial variability at local, regional and continental scales. This is a challenge for life cycle impact assessment (LCIA) of metals, because fate, bioavailability and effect factors are controlled by environmental chemistry and can vary orders of magnitude for different soils. Here, variography is employed to analyse spatial variability of bioavailability factors (BFs) of metals at the global scale. First, published empirical regressions are employed to calculate BFs of metals for 7180 topsoil profiles. Next, geostatistical interpretation of calculated BFs is performed using ArcGIS Geostatistical Analyst. Results show that BFs of copper span a range of 6 orders of magnitude, and have significant spatial variability at local and continental scales. The model nugget variance is significantly higher than zero, suggesting the presence of spatial variability at lags smaller than those in the data set. Geostatistical analyses indicate however, that BFs exhibit no significant spatial correlation at a range beyond 3200 km. Because BF is spatially correlated, its values at unsampled locations can be predicted, as demonstrated using ordinary kriging method. Similar approach can be employed for analyzing spatial variability of terrestrial ecotoxicity characterization factors of metals. Predicted maps can be used to provide a set of regionalized factors at spatial scales that are both scientifically relevant and practically feasible in LCIA.

EM03-6

A global assessment of the temporal and spatial variability of national dilution factors

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Of the many factors which influence the exposure of the freshwater aquatic environment to contaminating chemicals none has a more dramatic effect than dilution. Both where and when a chemical enters surface water will make an enormous difference to its impact on wildlife. This is of particular importance for down-the-drain chemicals as these substances are discharged to freshwaters via sewer systems after consumer use. However, too often, this dilution capacity is fixed to a "generic" value. Although the spatial variability of dilution factors is often acknowledged, temporal variability is often unaccounted for which may potentially lead to underestimating the environmental risk. To address the magnitude of these dilution differences across the world, estimates of dilution factors were developed globally at a 0.5 degree resolution using gridded data. Thus, the focus here is on the numbers and location of the human population and the river water available to dilute their waste. The river flows estimates are calculated at both annual and monthly resolution based on readily available annual and monthly runoff estimates. The domestic waste water effluent is derived from combining gridded population and national per capita domestic water use estimates. For each grid cell both annual and monthly dilution factors were generated. This approach allowed the quantification of temporal and spatial variability of dilution factors not only at a catchment level but also at a national level, by means of statistical measures such as median and percentiles. This method revealed the dramatic differences in available dilution of chemicals both within and between countries, for example Canada has on average 4-orders of magnitude more dilution available than Tunisia, and Finland 3-orders of magnitude more than Spain. Over the course of a year, national dilution could vary between 10 and a 1000-fold depending on the country.

The work presented here is a significant step forward in terms of understanding the impact of river flow temporal variability on dilution factors at a national and global scale. The proposed methodology has great potential for scientists and decision makers across the globe, as it provides the means to improve screening-level chemical risk assessments.

EM0B1-6

Lead and lead isotopes in agricultural soils of Europe: natural distribution or contamination?

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In environmental sciences the $^{206}\text{Pb}/^{207}\text{Pb}$ isotope ratio is routinely used to argue for Pb contamination of different compartments of the environment. However, the lead-isotope background variation of a continent has never been established for any sample material. For the first time, a map of a Pb isotope landscape, based on samples of agricultural soils at the continental-scale, is presented. Agricultural soil samples (Ap-horizon, 0-20 cm), collected at an average density of 1 site/2500 km² (2211 samples in total) from 33 European countries, were analysed for Pb concentration and Pb isotopes (^{206}Pb , ^{207}Pb , ^{208}Pb). Lead concentrations vary from 1.6 to 1309 mg/kg, with a median of 16 mg/kg. Isotopic ratios of $^{206}\text{Pb}/^{207}\text{Pb}$ range from 1.116 to 1.727, with a median of 1.202.

The new data define the soil geochemical Pb background for European arable land, providing crucial information for geological, environmental and forensic sciences, public health, environmental policy and mineral exploration. The European continental-scale patterns of Pb concentrations and Pb isotopes show a high variability dominated by geology and influenced by climate. Lead concentration anomalies mark most of the known mineralised areas in Europe. At the continental scale of this survey, it is difficult to distinguish between natural anomalies, due to ore occurrences, and an amplified signal caused by mining and smelting. With the exception of some very local anomalies (related to cities, smelters), none of the observed patterns can be directly related to contamination, and all anomalies rapidly decrease to background values with distance to source. Combining the regional distribution of Pb concentrations with Pb isotopes, the conclusion is that the majority of lead in European agricultural land is still of natural origin.

EP01 - Antimicrobial resistance in the environment

EP01A-1

Characterization and comparison of multi-drug resistant methicillin-resistant *Staphylococcus aureus* [MRSA] from recreational beaches and high touch surfaces at a university and surrounding community

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Over the last decade community-acquired methicillin-resistant *Staphylococcus aureus* [MRSA] has emerged as a major cause of disease in the general population with no health care exposure or known classical risk factors for MRSA infections. MRSA colonization is a known risk factor for developing MRSA infections and MRSA are spread from fomite to person and from person to person. In a 2009 study we isolated and characterized 6 MRSA from public marine recreational beaches. The data suggested that there were multi-drug resistant MRSA in the beach environment and thus may be a reservoir for transmission of MRSA to beach visitors as well as a reservoir for macrolide and tetracycline resistance genes. The current study sampled and identified MRSA from local marine and fresh water recreational beaches which included sand, fresh and marine waters samples (n=296); from high touch surfaces on the University of Washington campus (n=294), in UW undergraduate housing (n=85), and the

local community (n=130). The presence of type I-VII of mobile Staphylococcal Cassette Chromosome [SCCmec], multilocus sequence typing [MLST] of the allelic profile of seven housekeeping genes, and the presence of aminoglycoside resistance gene, *aadD*; macrolide resistance genes, *erm(A)*, *erm(B)* and *erm(C)*, and *msr(A)*; and tetracycline resistance genes, *tet(M)*, *tet(K)*, were determined by PCR assays and sequencing. Pulse field gel electrophoresis was done and the genetic relatedness of the isolates to USA300 was determined by Dice coefficient, UPGMA using the GelCompar II software. Strains that had > 80% homology with USA300 were classified as USA300. The highest level of MRSA positive samples [15.3%] were found in fresh water running into the marine beaches, where we frequently observed children playing during sampling, and at the fresh water beach on Lake Washington, while 11.7% of the surfaces from 5 of 8 undergraduate homes were MRSA positive. A surprising 98% of the 55 MRSA isolates were resistant to other classes of antibiotics and most likely represent reservoirs for these genes in the environment. In North America the majority of community acquired MRSA infections are due to USA300. This clone was found at the UW, in student housing and in the community but not in the recreational beach samples.

EP01A-2

Unique distribution of sulfonamide resistance genes, *sul*, in the Philippines aquatic environment

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EP01A-3

Tetracycline and mercury resistance genes in aquaculture sediments

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Farming fish in cages is an open system which has an effect on the chemical and ecological balance of the farming area. Fish feed, medicines and anti-foulants can affect microbial communities in the sediments under the cages. Considerable part of the substances used ends up to the water outside the actual aquaculture facility either directly or via excrement of the fish. The aim of this study was to characterise the effects of aquaculture to the surrounding waters and sediments. In particular we were interested amount and diversity of tetracycline resistance genes (*tet*) and mercury resistance (*merA*) gene in comparison to pristine sediments. Four fish farms in the Finnish and Swedish archipelago were sampled during years 2006-2011.

Total DNA from top sediment samples was isolated with Fast DNA spin kit, Quantification of bioavailable tetracyclines and mercury was done by bioreporter bacteria. Total tetracycline content by was analysed by HPLC and total mercury by ICP-MS The resistance genes (*tetA*, *tetC*, *tetH*, *tetM* and *merA*) were quantified by qPCR.

No bioavailable tetracycline or mercury was found in any of the samples before 2011. LODs were 0,3 µg g⁻¹ sediment for tetracycline and 0,04 ng g⁻¹ sediment for mercury.

The total amount of mercury, tetracycline and oxytetracycline was very low in all samples taken before 2011. However, there was high amount (more than 2 ng g⁻¹) of oxytetracycline present in one sample taken on 2011. The number of tetracycline resistance genes was clearly elevated in all fish farm samples until 2009 and the number of mercury resistance genes was also slightly elevated. Samples taken after that are currently under analysis. Diversity of *tetC* gene was non existent and diversity of *tetM* was relatively low. In contrast, the diversity of *merA* was considerable. The aquaculture farms had used tetracyclines but ceased that about a decade ago so the reasons for the elevated tetracycline resistance genes is not obvious, like are the reasons for accumulation of *merA* gene. Very low diversity of tetracycline resistance genes may implicate a common source for the genes. The *merA* sequence diversity in both fish farm samples was similar, phylogenetic analyses showed that there was a little difference between sequences from different sampling sites.

EP01A-4

Abundance of streptomycin and tetracycline resistance genes in apple orchards treated with streptomycin in comparison to untreated apple orchards

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Streptomycin is the only antibiotic authorized for use in plant agriculture within the EU and Switzerland. Its use is authorized on an annual basis for the prophylactic treatment of apple and pear orchards against the bacterial disease fire blight. Tetracycline is currently the only other viable alternative should resistance to streptomycin emerge in the fire blight pathogen; *Erwinia amylovora*. We have developed a multiplex qRT-PCR for the relative quantification of streptomycin and tetracycline resistance genes, with the 16S rRNA genes as the endogenous control. Using these multiplex qRT-PCRs we have monitored the abundance of streptomycin and tetracycline resistance genes in streptomycin treated and untreated orchards in 2010 and 2011.

2. Materials and methods

Sample of flowers, leaves and soil were collected from three orchard sites at each time-point (265 samples per year). The time-points consisted of prior to streptomycin spraying, one day after the streptomycin spraying, two weeks after streptomycin spraying and at apple harvest. The DNA extraction method and the relative abundance of resistance genes were detected as previously described.

3. Results and discussion

The abundances of *strA* and *strB* genes increased in the flower and leaf samples over time in comparison to the untreated samples in 2010 and 2011. However, the harvest samples contained a similar abundance of *strA* and *strB* genes to the samples prior to streptomycin spraying. There were no streptomycin influenced changes in the abundance of streptomycin resistance genes in the soil samples. The relative abundances of the tetracycline resistance genes *tetB*, *tetM* and *tetW* were not affected by the treatment with streptomycin in the flower, leaf or soil samples. There were low fluctuations within the abundances of the streptomycin and tetracycline resistance genes within the samples isolated from the untreated orchards over time.

4. Conclusions

There were short term increases in the abundances of *strA* and *strB* genes associated with streptomycin treatment in the flower and leaf samples. However, the abundance of these resistance genes returned to pre-treatment level at harvest. Streptomycin treatment did not influence the abundance of streptomycin resistance genes in the soil samples nor did it influence the abundance of tetracycline resistance genes within the orchard samples. Thus, the streptomycin associated increases in resistance gene abundances are temporary.

EP01A-5

Antimicrobial resistance and their selectors in pig manure and sewage sludge

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Antimicrobial resistance is a pestering problem, and the solution still remains to be found. Due to a supposed reservoir function of the environment, environmental contamination with antibiotics and / or resistant bacteria is a serious issue. In addition, bacteria are hard to trace, once spread into water or soil. Therefore, it is the more necessary to monitor contaminated effluents before they enter the environment.

We investigated the antimicrobial resistance of bacteria - comprising *E. coli*, enterococci, lactobacilli and clostridia - which were isolated from sewage sludge and pig manure.

The statistical association of antimicrobial resistance with different potentially selective factors was assessed in a linear model.

Chemical precipitation in sewage plants was significantly associated with increased bacterial resistance in sewage sludge. Antibiotic contamination and heavy metal contents were significantly associated with bacterial resistance in pig manure. *E. coli* from manure samples with supermedian contents of copper (> 11.8 mg / kg manure wetweight) were significantly more often resistant against ampicillin and piperacillin. *E. coli* from manure samples with zinc contents > 22.75 mg / kg manure wetweight were significantly more often resistant against ampicillin, doxycycline and piperacillin.

In order to track whether environmental isolates might be transferred to humans, we investigated by ERIC-PCR the genetic relatedness of *E. coli* from pig manure, sewage plants, and human hospitals. In general, different phylogenetic groups prevailed among isolates from pig manure and human hospitals; isolates from sewage sludge resembled partly isolates from pig manure, partly isolates of human hospitals. Two closely (or clonally) related *E. coli* isolates were found in a sample of pig manure and a human stationary patient. Interestingly, despite the close phylogenetic relatedness, both isolates carried different resistance genes. On the contrary, identical resistance gene profiles were present in phylogenetically unrelated isolates from humans and pigs. These findings indicate a major role of horizontal spread in the distribution of antimicrobial resistance among *E. coli*.

EP01A-6

Occurrence and dissemination of antibiotic resistance genes in anthropic environments

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Dissemination of antibiotic resistance genes is recognized to occur in various environmental compartments although it remains difficult to demonstrate in complex environmental matrices. Some environments have been defined as putative hot spots for gene transfer as they can sustain high microbial cell densities and combine both antibiotic and antibiotic resistance bacteria. In this study we compare the occurrence of class 1, 2 and 3 integrons (mobile genetic elements mobilizing different resistance cassettes according to their respective class) from wastewater, sludges, farm slurry and manure. Same or equivalent environmental matrices were also evaluated for their propensity to support the transfer of a model integron-bearing plasmid (pB10).

In farm samples, only class 1 and class 2 integrons could be detected. Prevalence of integrons was significantly more important in the farmyard manure than in the slurry. In

WWTP influents, the occurrence of class 1 integrons was significantly more important than the other classes. We could show that WWTPs as a process reduced the relative concentrations of integrons by 2 log. In dynamic point of view, plasmid pB10 did not appear to disseminate in farm manure microcosms while maintaining steadily over the course of the experiment. In activated sludge microcosms, pB10 did not persist because of an apparent loss of the donor bacteria. Nevertheless, the dissemination of the plasmid appeared as an increasing plasmid to donor ratio in microcosms setup with sludge from anaerobic digesters or fixed biofilm reactors. Regarding integrons, the difference of occurrence may reflect more or less homogenous anthropic selective pressure on antibiotic resistances. On the other hand, propensity for pB10 to transfer sludge microcosms seems to rely on the origin of the sludge ecosystem from a given treatment process.

EP01B-1

Detecting evolutionary hot spots of antibiotic resistances in Europe (DARE)

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Antibiotic resistances (ARs) are a great threat to human health, which has meant that research in this area has focused primarily on their role within clinical settings. This is in strong contrast to the research in nonclinical environments, such as the natural and urban environment, where this research has only recently received more attention. Here the first results of a European wide network COST Action are presented. The Action consists of 84 members of 18 European Nations and Israel which try to identify the gaps of knowledge, which need to be investigated in order to propose interventions to curb the spread of AR evolution and AR microbes within the environment. The Action has developed criteria for antibiotics and bacterial organisms and environmental hot spots of AR evolution, which should be investigated as first priority. Furthermore, in order to assess the risk of antibiotic resistance evolution a new approach of risk assessment is needed. However, the Action has also identified here the largest gap of knowledge. Currently, no systems exist which will allow to assess the risk of spread or evolution of new antibiotic resistances. Therefore investigations to fill this gap of knowledge are strongly called for and this is also the case for research resulting in a first geographic pattern on AR resistance in Europe. Such an epidemiological base information exists already for clinical and veterinary data, but is missing for environmental data.

EP01B-2

Selective pressure of antibiotic pollution on bacteria of importance to public health

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Many bacteria of clinical importance survive and under certain conditions may grow in different environments. Antibiotic pollution may exert on them a selective pressure leading to an increase in the prevalence of resistance. The objective of this study is to determine whether measured environmental concentrations (MEC) of antibiotics and concentrations used as action limits in environmental risk assessment (ERA) may exert a selective pressure on bacteria of importance to public health in the environment. Species sensitivity distributions were derived for ciprofloxacin, erythromycin and tetracycline using endpoints calculated from minimum inhibitory concentration distributions of clinically relevant bacteria. The potentially affected fraction (PAF) of bacterial taxa at MEC of antibiotics and ERA action limits was used as a proxy for antibiotic selective pressure. MEC and ERA action limits were also directly compared to wild-type cut-off values. The PAF of bacterial genera at antibiotic concentrations measured in water environments is estimated not to exceed 6%. MEC in river sediments, swine feces lagoons, liquid manure and farmed soil are estimated to inhibit wild-type populations in up to 49%, 84%, 100% and 23% of bacterial genera, respectively. At concentrations used as action limits in ERA, erythromycin and ciprofloxacin are estimated to inhibit wild-type populations in up to 15% and 65% of bacterial genera. Concentrations of antibiotics measured in different environments and used as action limits in ERA are high enough to exert a selective pressure on clinically relevant bacteria that may lead to an increase in the prevalence of resistance.

EP01B-3

Monitoring and management of antibiotic resistance of veterinary drugs in Germany

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The use of antibiotic drugs in veterinary therapy results in a target conflict of demand for therapy vs. antimicrobial resistance. Advantages such as secure animal health of companion and farm animals, avoidance of economical losses in animal husbandry and prevention of bacterial zoonoses are opposed by the risk of the enhancing antimicrobial resistance in humans and animals. A management approach is the responsible use of antimicrobials applying the "ONE HEALTH Principle" (animals + humans = one health) of the WHO and EU (animal health strategy 2007-2013). This strategy needs information on the current status of antimicrobial resistance. This information is obtained from monitoring programs conducted and improved in Germany for more than 10 years.

These include:

National resistance monitoring - animal pathogens

National food monitoring program

Monitoring of resistance for zoonosis germs

National Monitoring of resistance for commensal germs

Additional information is obtained from the collection of postmarketing data (ADRs, PSURs etc.) and since 2011 from data of antibiotic disposal to be registered by law.

This talk presents some of these data and shows management actions drawn from these data and put into force both nationally and within the whole EU.

EP01B-4

Reduction of pathogens and antibiotic resistance genes by Soil Aquifer Treatment (SAT) in the Mézquital Valley, Mexico

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Aquifer recharge presents advantages for integrated water management, namely, treatment of reclaimed water and additional dilution of pollutants due to mixing with natural groundwater. Nevertheless, this practice can represent a health and environmental hazard because of the presence of pharmaceuticals, pathogens and antibiotic resistance genes. The Mézquital Valley, 60 km north of Mexico City, is the world's largest wastewater (WW) irrigation area. The WW from Mexico City is transported without treatment to the Mézquital. There, approximately 800-900 km² of agricultural land are irrigated with this untreated WW. We followed the WW on its way to the Mézquital taking samples from seven sites starting with the effluent of a big hospital in Mexico City and ending with water from a spring that arises after SAT in the Mézquital. Total DNA was extracted from the water and applied to quantitative real-time PCR (qPCR) targeted to the 16S rRNA gene of bacteria, the 23S rRNA gene of Enterococcus and to six resistance genes mediating resistance to fluoroquinolones, sulphonamides and Glactam antibiotics. Total bacterial load was reduced by almost three logs from 1.75 [GREEKX] 10⁹ 16 S rRNA gene copies / 100 ml WW to a final concentration of 3.73 [GREEKX] 10⁶ 16 S rRNA gene copies / 100 ml spring water destined for drinking water purification. A four log reduction to 1.66 [GREEKX] 10³ copies / 100 ml water was detected for the nosocomial pathogen, Enterococcus. For the resistance genes, the highest concentration was found for the sulphonamide resistance gene sul1 with a concentration of 1.14 [GREEKX] 10⁹ gene copies / 100 ml WW, which decreased to 1.38 x 10⁴ copies in the spring water. The fluoroquinolone resistance genes qnrA and qnrB were present in the WW at 5 x 10⁵ copies / 100 ml; both were completely eliminated on their way to the spring. In summary, two of the six resistance genes were completely eliminated, the others were reduced by three to five logs from the WW to the spring. Mixing of WW with rainwater, passage through a dam, and sedimentation of particles suspended in WW hardly affected bacterial load and resistance gene abundance. The major cleaning effect was exerted by SAT in the Mézquital. Natural WW treatment, like sedimentation, mixing with rainwater and SAT, can considerably decrease biological contaminants in water. However, total bacteria, nosocomial pathogens and resistance genes were not satisfactorily removed to use this water without further treatment as drinking water.

EP01B-5

Impact of treated wastewater irrigation on antibiotic resistance in agricultural soils

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Antibiotic resistance (AR) is a global phenomenon with severe epidemiological ramifications. It is becoming increasingly clear that anthropogenically-impacted natural aquatic and terrestrial "hotspots" may be playing a significant role in the global proliferation of AR because these environments serve as gene reservoirs which can be transferred to clinically relevant (potentially pathogenic) organisms via water and food webs. Treated-wastewater (TWW) irrigation is becoming increasingly prevalent in arid regions of the world, due to growing demand and decline in freshwater supplies. The release of residual antibiotic compounds, antibiotic resistant bacteria (ARB), and antibiotic resistance genes (ARGs) from wastewater effluent may result in proliferation of AR in irrigated soil microcosms. The aim of this study was to assess the impact of TWW-irrigation on soil ARB and ARG reservoirs. Tetracycline, erythromycin, sulfonamide and ciprofloxacin resistance was assessed in agricultural soils irrigated in tandem with either freshwater (FW) or TWW, using standard culture-based isolation methods and culture-independent molecular analysis using quantitative real-time PCR (QPCR). Significant

levels of native bacterial antibiotic resistance were detected in both FW- and TWW-irrigated soils. Nonetheless, ARB and ARG levels in TWW-irrigated soils were on the whole identical (or sometimes even lower) than the FW-irrigated soils, despite detection of relatively high levels of both ARB and ARG in the TWW. This indicates that the high numbers of resistant bacteria that enter the soils from the TWW are not able to compete or survive in the soil environment; and suggests that the impact of the TWW-associated bacteria on the soil microbiome is on the whole negligible. We therefore conclude that AR in the soil is primarily associated with native resistance of the indigenous soil microbiome and not with introduced strains or genetic elements.

EP01B-6

Urban wastewater disinfection and solar irradiation of wastewater polluted stream: effect on antibiotic photo-degradation, inactivation of antibiotic resistant *E. coli* strains and their resistance

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Two wastewater disinfection processes (UV radiation and chlorination) were tested in the inactivation of two previously selected *E. coli* strains and their resistance to three antibiotics (Amoxicillin (AMX), ciprofloxacin (CPX), and sulfamethoxazole (SMZ)). Next, disposal of urban wastewater treatment plant (UWWTP) effluent into surface water was simulated and the effect of solar irradiation on the photodegradation of target antibiotics, the inactivation of antibiotic resistance *E. coli* strains and their resistance were investigated. Two different strains of *E. coli* resistant to the target antibiotics (higher antibiotic resistance, HAR; lower antibiotic resistance, LAR) were isolated from wastewater sample and subsequently inoculated in previously autoclaved wastewater samples. Total inactivation by UV radiation (250 W wide spectrum UV lamp) was observed after 60 min in LAR *E. coli* strain inoculated wastewater (4.0 10⁶ UFC/100 mL) but not after chlorination process. Additionally, unlike of chlorination process, UV radiation effected resistance of LAR *E. coli* strain to CPX (MIC decreased from 12 to 8 µg/L). When simulated solar irradiation effect was investigated on wastewater polluted surface water, *E. coli* strains inactivation was found to be really low (26 and 50% respectively). According to wastewater disinfection tests, solar irradiation effected resistance of LAR strain to CPX too, but MIC was decreased to 8 µg/L only after 180 min irradiation. Finally, the effect of solar irradiation on antibiotics (1 mg/L initial concentration respectively) photodegradation rate resulted in the following order (half-life time): CPX (t_{1/2}= 24 min) < AMX (t_{1/2}= 99 min) < SMX (t_{1/2}= 577 min). In conclusion, antibiotics and ARB may be released into the stream from UWWTP effluents partially unaffected, and because of the lower efficiency of solar irradiation, the risk of horizontal transfer and selection of antimicrobial resistance genes among bacteria can increase.

EP02 - Endocrine disrupting chemicals: recent developments

EP02A-1

Zebrafish eleutheroembryos provides a suitable vertebrate model for screening thyroid gland disrupting chemicals

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Thyroxine-immunofluorescence quantitative disruption test (TIQDT) was designed to provide a simple, rapid, alternative bioassay for assessing the potential of chemical pollutants and drugs to disrupt thyroid gland function. This study demonstrated that zebrafish eleutheroembryos provided a suitable vertebrate model, not only for screening the potential thyroid disrupting effect of molecules, but also for estimating the potential hazards associated with exposure to chemicals directly impairing thyroxine (T4) synthesis. Amitrole, potassium perchlorate, potassium thiocyanate, methimazole (MMI), phloroglucinol, 6-propyl-2-thiouracil, ethylenethiourea, benzophenone-2, resorcinol, pyrazole, sulfamethoxazole, sodium bromide, mancozeb, and genistein were classified as thyroid gland function disruptors. Concordance between TIQDT on zebrafish and mammalian published data was very high for those chemicals with a direct effect on the thyroid gland function, as the sodium-iodide symporter (NIS) and the thyroid peroxidase (TPO) inhibitors. The physiological relevance of T4-intrafollicular content was clearly higher than regulation at the transcriptional level of tg or slc5a5. Moreover, concentration-response analysis provided information about the thyroid disrupting potency and hazard of selected positive compounds. Finally, the effect of perchlorate, a NIS-inhibitor, but not MMI, a TPO- inhibitor, was completely rescued by low-micromolar amounts of iodide. TIQDT performed on zebrafish eleutheroembryos is an alternative whole-organism screening assay that provides relevant information for environmental and human risk assessments.

EP02A-2

All mixed up: phenotypic plasticity in a genotypic world

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The processes of sexual differentiation and development are dependent upon an intricate cascade of molecular signals and deviations from normal can have catastrophic effects on individual fertility and fitness. Previously, several studies have shown that exposure of *Xenopus laevis* to sufficiently potent estrogens at critical times during development results in feminization and/or demasculinization, including male to female phenotypic sex reversal at adequate doses. However, given that genotyping of *X. laevis* has only recently become possible, studies performed in the past were rarely able to make concrete linkages between genetic and phenotypic sex. Therefore, to further characterize the relationship between genotype and phenotype, *X. laevis* tadpoles were exposed to 0.1, 1, or 10 µg/L 17α-ethynylestradiol (EE2), the estrogen analog commonly used in oral contraceptives, from 12 h post-oviposition through 13 wks post-hatch. All EE2 treatments resulted in significant delays in time to metamorphosis. Genotyping showed that genetic sex ratios were similar among treatments. However, morphological evaluation revealed that phenotypic sex ratios were altered in all EE2 treatments. Interestingly, complete male to female phenotypic sex reversal was rare at the concentrations tested, and a surprising number of individuals displayed intersex gonads, abnormal gonads, and atypical vitellogenin over-expression, that were only diagnosed upon histological examination. The impacts of these conditions on fertility and fitness are not known but are likely to be adverse and more complex than complete sex reversal. In all likelihood, the relatively great number of intersex and abnormal animals is a result of estrogens functioning downstream of the initial molecular signals of sexual differentiation. Thus, genetically male animals receive mixed endogenous male and exogenous female signals that cause disordered sexual development. The vitellogenin over-expression was probably temporally independent from primary effects on sexual differentiation and likely drove the significant delays to metamorphosis that were observed in all EE2 treatments. Expression analysis of mRNA, as determined by *Illumina* transcriptome sequencing, is ongoing to help characterize the transcriptional signature of abnormal sexual development and to allow further assemblage of the molecular pathway that leads to the disordered development.

EP02A-3

Androgen-induced kidney hypertrophy in the European bullhead (*Cottus sp.*): a potential biomarker of androgen exposure

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The present work was designed to identify an androgen-regulated signal in the kidney of the European bullhead (*Cottus sp.*) and to characterise response patterns prior to laboratory and field applications to address androgenic potential of chemicals. In a first step, gender dependent ultrastructural modifications of kidney were quantified using the Kidney Epithelium Height (KEH) measurement. During breeding period, male bullheads exhibited an increased KEH value of 20.96 ± 3.57 µm compared to female (16.96 ± 1.28 µm). No gender difference was recorded in non-breeding bullheads. In a second step, bullheads out of breeding period were exposed 14 or 21 days to different concentrations of androgens (0.5 or 5 µg/L of trenbolone (Tb), 1 or 10 µg/L of 11-ketotestosterone (11KT), 0.5 or 5 µg/L of Spironolactone (SPI), 0.5, 5 or 50 µg/L of Dihydrotestosterone (DHT)), estrogen (0.05 or 0.5 µg/L of Ethinyl-estradiol (EE2)) or heavy metal (1 or 10 µg/L of Cadmium (Cd)). Results showed that androgens are able to induce kidney hypertrophy. Trenbolone induced a dose-dependent hypertrophy (KEH= 16.59 ± 0.81 µm at 0.5 µg/L and 21.01 ± 1.76 µm at 5 µg/L vs 15.58 ± 0.85 µm for the controls) as the higher concentration of 11-KT (KEH = 17.55 ± 1.48 µm at 10 µg/L) and DHT (17.43 ± 1.77 µm at 50 µg/L). SPI and Cd have no effect on kidney ultrastructure. In bullheads exposed to EE2, a trend to decrease of KEH was observed. In a next step, wild bullheads were electrofished in 9 streams located in the North of France and characterised by various contamination levels. KEH responses don't allow to discriminate both reference (KEH = 16.03 ± 1.25 µm) and contaminated (KEH = 16.17 ± 1.08 µm) sites. This work highlights an androgen-regulated signal in the kidney of the European bullhead. However, kidney hypertrophy appears as few sensitive and time consuming indicators. Also, identification of associated protein and/or RNA modifications could provide a relevant biomarker of androgen exposure.

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EP02A-4

Endocrine disrupting compounds as potential obesogens: musk compounds as a case study

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Since the discovery of leptin and other adipokines it is now clear that the adipose tissue is not only a storage place for excessive fat but a real endocrine organ, making it possibly sensitive to endocrine disrupting compounds (EDCs). Recently 'the environmental obesogen hypothesis' stated that environmental pollutants such as EDCs could

play a role during the development of metabolic diseases such as obesity, broadening the endocrine disrupting concept to a more physiological disruption concept including endocrine, neural and metabolic disruption. This hypothesis could, together with an increased caloric intake, a sedentary lifestyle and genetic predisposition, give potential complementary explanation to the recent epidemic proportions of obese and diabetic patients.

Musk compounds are a group of synthetic EDCs used in a variety of personal care products and therefore often daily used. Synthetic musks enter the systemic circulation mainly through dermal absorption and have been detected in human adipose tissue due to their high lipophilicity (Log *kow* = ± 5). During this study we evaluated the potential obesogenic properties of three musk compounds belonging to the different classes of musks: the nitromusk Musk xylene; the polycyclic musk Tonalide® and the macrocyclic musk Ethylene Brassylate.

The adipogenic potential of the test compounds were evaluated using the 3T3-L1 cell line, a model in vitro cell system for the study of adipogenesis. These cells are fibroblastic and can differentiate into adipocytes after a ten-day exposure with an adipogenic cocktail (isobutylmethylxanthin, dexamethasone and insulin). The Adipored assay, a fluorescent staining for the quantification of lipid droplets associated with the phenotype of mature adipocytes, was used to screen the three musk compounds for their adipogenic potential. Tonalide® was the only compound inducing a dose dependent increase of the lipid droplet formation. The effect of Tonalide® on the differentiation of adipocytes was further confirmed by measuring the gene expression of an adipocyte specific marker gene adipocyte specific protein 2 (aP2) during exposure.

The Adipored assay as well as the expression of the adipocyte specific marker gene aP2 show that Tonalide® is the only musk compound, from the three tested, inducing the differentiation of adipocytes in vitro. In the next step, microarray analysis and RNAi technology will be applied to unravel the adipogenic mechanisms of Tonalide®.

EP02A-5

Development of gene expression biomarkers in cetaceans skin biopsies exposed to bisphenol A (BPA) and perfluorooctanoic acid (PFOA): new tools for emerging contaminants assessment

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Currently, one of the main toxicological issues, in the management and conservation of the marine environment, is the study of the potential impact of compounds released from plastics, such as bisphenol A (BPA) and industrial derivatives, such as perfluorooctanoic acid (PFOA). On this regard, the assessment of toxicological risk in wildlife requires the development of sensitive biomarkers including those based on the use of in vitro systems. BPA is one of the most distributed compounds in the world, both in the aquatic and terrestrial ecosystems acting as agonist or antagonist for endocrine receptors. The perfluorinated compounds are used as surfactant and in the surface treatments, they are persistent and it has been shown that they can act as endocrine disruptors as well. To develop new gene expression biomarkers in cetaceans we exposed skin biopsies of three odontocetes species (sperm whale, killer whale, and bottlenose dolphin) to BPA and PFOA. We selected two potential biomarker genes such as the peroxisome proliferator-activated receptors α and γ (PPAR α and γ). The PPARs belong to a superfamily of ligand-dependent nuclear receptor (PPAR α , β and γ) which regulates physiological processes of lipids homeostasis, inflammation, adipogenesis, reproduction, etc. PPAR α and PPAR γ seem to be modulated by the presence of BPA and PFOA respectively. The two genes of interest (PPAR α , PPAR γ) were sequenced in three odontocetes species. The mRNA levels were quantified in response to the two different treatments in the slice samples. Four genes (PPAR α , PPAR γ , and the previously developed ER α and E2F1) are modulated by the treatments in all the three species. In particular, the results of this set of experiments, revealed that the BPA treatments induce the expression of the genes PPAR α and PPAR γ showing a dose-response trend. Increasing the BPA concentration increases the bottlenose dolphin, killer whale and sperm whale slices mRNA levels, as well as for E2F1 apart from the killer whale. On the opposite, the PFOA exposure shows a down-regulation of the PPAR α and PPAR γ both in sperm whale and killer whale slices, while ER α and E2F1 are poorly induced by PFOA in both species. These data represent the first evidence of emerging contaminants effect on cetaceans based on an in vitro experiment and suggest the potential use of this diagnostic markers as early warning signal of exposure to plastic released compounds and emerging contaminants in marine wildlife monitoring.

EP02A-6

Detection of estrogenic and androgenic chemicals in waste waters and peri-urban water bodies in Bulawayo, Zimbabwe

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Endocrine disrupting chemicals (EDCs) as environmental contaminants have received considerable attention since their discovery. In this study, the yeast estrogen and androgen screen tests were adopted to detect the presence and potencies of estrogenic and androgenic endocrine disruptors in treated effluents from sewage treatment plants (STPs), Thorngrove (THORN) and Aisleby (AIS) STPs, an urban river (Matsheumhlope River), and effluent receiving peri-urban dams (Umguzi Dam and Khami Dam) in the industrialised and semi-arid City of Bulawayo, Zimbabwe. Pollutants in water samples were extracted by C18 Solid phase extraction (SPE). Estrogenic activities of extracts were expressed as 17 β -estradiol equivalence quantities (EEQs). Estrogenic activity was detected in the effluents of both STPs with EEQ values of 32.8 ng/L and 55.3 ng/L for THORN and AIS STP respectively. In Umguzi Dam water, estrogenic activity was extremely high, showing the highest EEQ of 236.5 ng/L while Khami Dam had EEQ of 8.5 ng/L. Matsheumhlope River, an urban river, had the least EEQ value of 2.2 ng/L. Androgenic activity was only detected in AIS STP, with dihydrotestosterone (DHT) equivalence quantity (DHTEQ) of 93.1 ng/L. The results indicate that the STP effluents have high concentrations of estrogenic chemicals and urban/ peri-urban dams, the ultimate sink of all waste waters from the city, are laden with estrogens with potential to cause reproductive disorders in fish and other aquatic organisms. The study can also indicate the potential adverse effects of inadequate management practices in developing countries; which are rarely given much attention in urban xeno-biotic pollution studies. We recommend 'follow-up' field based studies to determine the effects of these xeno-estrogens on reproduction of fish and other aquatic organisms in the peri-urban water bodies.

EP02B-1

Assessment of exposure to estrogenic contaminants in bile extracts of red mullet from Western Mediterranean: an integrated chemical and biological approach using the ER-LUC assay.

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In this work we have identified and measured the concentrations of major metabolites of polycyclic aromatic hydrocarbons (PAHs) and alkylphenols in field collected bile extracts of red mullet (*Mullus barbatus*) from Mediterranean Spanish waters. Hydroxylated-PAHs (1-naphthol, 9-phenanthrol, 9-fluoreneol, 1-pyreneol, 1OH-BaP and 3OH-BaP) and alkylphenols levels (4-n-nonylphenol (NP) and 4-tert-octylphenol (OP)) were quantified in fish bile samples by gas chromatography-mass spectrometry in electron ionization mode (GC-ESI-MS). In addition we have applied the estrogen responsive luciferase (ER-LUC) reporter gene assay to measure total estrogenic activity in the same bile extracts of male fish. By integrating the results of both analytical chemical and bio-analytical approaches, we have attempted to explain the measured ER-LUC activity by the calculated potencies based on chemical analysis of hydroxylated PAH and AP metabolites. Our results showed consistent spatial differences in concentrations of hydroxylated-PAHs (Σ OH-PAHs), alkylphenols and estrogenic activity in bile samples, which are presented and discussed.

EP02B-2

Mixtures of the most common antiandrogenic pesticides act additively in vitro: implications for risk assessment

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Evidence suggests that there is a widespread decline in male reproductive health and that mixtures of pesticides may be an important causal factor. At present, 1252 active plant protection products are registered in the US and 411 in Europe, with another 72 "pending". With such a high number of products on the market, it would be practically impossible to test enough combinations to make robust conclusions about their potential to impact the environment and humans. Therefore, the accurate predictability of mixture effects using modelling approaches is essential for risk assessment. We tested mixtures of pesticides with androgen receptor (AR) antagonist properties using a fixed-mixture ratio design. The selection criteria for inclusion in the mixture was based on estimated exposure (high), antiandrogenic activity (active), registration status (current) and toxicity (less than 10 μ M) and led us to select 13 pesticides. We used the MDA-kb2 assay to test 3 mixtures: 1. Pure antagonists (8mix: fludioxonil, fenhexamid, ortho-phenylphenol, tebuconazole, dimethomorph, methiocarb, pirimiphos-methyl, p,p'-DDE); 2. Dual function agonists/antagonists (5mix: cyprodinil, pyrimethanil, vinclozolin, chlorpropham, linuron); 3. All (13mix). Concentration addition (CA) and independent action (IA) models were calculated to predict mixture responses. All the mixtures were within the "prediction window" of CA and IA, whereby CA tended to overestimate, and IA to underestimate, effect. The 8mix agreed with CA, but not IA, whereas the 5mix and 13mix agreed with either CA or IA, or both, depending on inhibition level (10% or 50%) and mixture ratio. For the first time, this shows that multi-component mixtures of widely used pesticides can act together in a predictable and additive manner. Significantly, the mixture tested here is composed of some of the most widely used pesticides found on foods in Europe and the USA, mostly fungicides, indicating that the potential for exposure to mixtures of these test pesticides is high. Considering that risk assessment procedure does not incorporate mixture scenarios at present, it is possible that risk to human health and environment is being underestimated, and therefore, effects of mixtures should be taken into account.

*Orton, F., E. Rosivatz, M. Scholze, and A. Kortenkamp, 2011. Widely used pesticides with previously unknown endocrine activity revealed as in vitro anti-androgens. *Environ Health Perspect*, 119: p. 794-800.

EP02B-3

Endocrine and anti-androgenic potency of by-products in flame retardants

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Some brominated flame retardants (BFRs) have unintended negative effects on the environment and human health (e.g. endocrine effects). Some of them show a strong bioaccumulation in aquatic and terrestrial food chains, some are very persistent, and some show serious toxicological effects such as endocrine disruption [1-5]. During the last decade an increasing number of reports have presented evidence of these negative effects caused by some BFRs. Less toxic alternatives appear to be available already but comprehensive information on their possible toxicological effects and exposure are lacking. The European Commission-funded project ENFIRO investigates halogen-free substitution options for some BFRs resulting in a comprehensive dataset on viability of production and application, environmental safety, risk assessment, and life cycle assessment. In total 15 halogen-free flame retardants (HFFRs), consisting of metal-, organic-, and nano-based FRs, as alternatives for decaBDE, TBBP-A, and brominated polystyrenes were selected. These HFFRs were assessed in a toxicity screening to provide information on the hazards. This paper presents the results of the hazard characterisation and shows that impurities in some technical HFFR products were responsible for in vitro estrogenic and anti-androgenic activity. The identity of the impurities that were responsible for the endocrine effects could be resolved with LC-HRTOF-MS and NMR.

EP02B-4

Investigation of endocrine disruption in Australian aquatic environments - stage 1

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This project uses an integrated approach consisting of multiple in vitro and in vivo bioassays, in situ sampling and trace chemical analysis to compare endocrine disruption at 73 sites in mainland Australia. Sample sites were selected to include waterways impacted by a variety of sources such as wastewater discharge, agricultural run-off, industrial effluent, urban drains and pristine reference sites. Duplicate 1 L discrete water samples were taken quarterly over a one-year period. Samples were concentrated using solid-phase extraction (SPE) and split into two aliquots, one for in vitro bioassay analysis and the other for chemical analysis. A battery of CALUX assays (estrogen receptor, ER; androgen receptor, AR; progesterone receptor, PR; and glucocorticoid receptor, GR) was used to determine the classes of EDCs present in water extracts. Chemical analysis was used to identify causative compounds as well as for point source confirmation. Preliminary results have identified at least 11 sites that have estrogenic (or anti-androgenic) EDCs. Analysis with the AR-, PR-, and GR-CALUX assays is currently underway. Chemical analysis has confirmed estrogen mimics (such as bisphenol A, t-octylphenol), the pesticide atrazine, an industrial compound tris(2-carboxyethyl)phosphine (TCEP), and numerous pharmaceuticals and personal care products (e.g., atenolol, dilantin, triclosan, carbamazepine, ibuprofen). Hormone analysis is currently underway. Based on in vitro and chemical data, several stock solutions representative of a selection of exposure concentrations found in the environment will be created for in vivo laboratory fish exposures using a native species (rainbowfish, *Melanotaenia fluviatilis*) and a widespread exotic pest species (mosquitofish, *Gambusia holbrooki*) in stage 2. An estrogenic biomarker (vitellogenin) and an androgenic biomarker (in development) will be used to assess whole organism endocrine disruption. The same endpoints will be utilized with in situ techniques by sampling fish from polluted aquatic environments identified in stage 1 to assess the amount of endocrine disruption present in the most impacted natural aquatic environments. Finally, in stage 3, a risk assessment will be generated using in vitro, chemical, in vivo, and in situ data to assess the potential risk to aquatic ecosystem health.

EP02B-5

Applying tissue-burden based quality benchmarks to assess the ecological risks of endocrine disrupting organotin compounds in Hong Kong waters

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Since their application as biocides in the 1960s, organotins (OTs), in particular tributyltin (TBT) and triphenyltin (TPT), have caused widespread adverse effects on marine organisms such as the induction of imposex (i.e., superimposition of male sexual characteristics - penis and vas deferens - on females) in over 200 species of neogastropods, and growth inhibition and deformities in oysters. A mandatory global ban on the use of OT-based antifouling systems has been enacted since September 2008. It is, therefore, anticipated that there will be a reduction of OT pollution in marine environments around the world. In this study, we measured the imposex status and tissue concentrations of OTs (i.e., mono-BT, di-BT and TBT, mono-PT, di-PT and TPT) in the rock shell *Thais clavigera* collected from 28 coastal sites of Hong Kong during summer 2010. The results indicated that *T. clavigera* from a number of sites contained high TPT concentrations and suffered from high degree of imposex. The average TPT tissue concentration was 11,108 µg kg⁻¹ dry weight (dw) in *T. clavigera* collected from Aberdeen, which was 26 times higher than the maximum TBT tissue concentration in the animals obtained from Kadoorie Beach (i.e., 422 µg kg⁻¹ dw). Using the Monte Carlo approach, an ecological risk assessment was conducted by computing the distribution of risk quotients (RQs); RQ is the ratio between a measured tissue concentration of the target pollutant (MTC) and predicted effects tissue concentration (PETC) (i.e., RQ = MTC/PETC). The results showed that 11.1% of *T. clavigera* across all sites in Hong Kong waters was at risk with RQ > 1 due to exposure to TPT, whereas the risk associated with TBT was relatively low (0.7% with RQ > 1). Kadoorie Beach, Butterfly Beach, Waterfall Bay and Aberdeen, the four sites which are close to the shipping facilities, were severely impacted by TBT, while all 28 sites were alarmingly heavily contaminated by TPT. As TPT is highly toxic and may trigger imposex in *T. clavigera*, the local rock shells are still under considerable threat associated with this pollutant even after the global ban of OTs in antifouling systems. This study highlights that TPT is a major environmental concern which deserves immediate actions to control its use and release, and to remediate its pollution in the marine environment of Hong Kong and the Pearl River Estuary.

EP02B-6

Multi-parameter assessment of endocrine disruption in Irish marine waters using biological effects measurements, chemical analysis and passive sampling

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An integrated study examining the uptake and concentrations of selected endocrine disrupting compounds (EDCs) in transplanted *Mytilus edulis* was carried out at 3 sites on the coast of Ireland. The study combined the biological effects alkali-labile phosphate assay (ALP) and the estrogen luciferase reporter assay (ER LUC); chemical analysis using liquid chromatography tandem mass spectrometry (LCMSMS), and the Polar Organic Chemical Integrative Sampler (POCIS). The exposure was conducted over a three month period with mussels transplanted from a reference site to cages at three coastal locations; Dublin Bay and Galway Bay impacted by secondary treated wastewater effluent and a reference site with little to no anthropogenic input in Galway County on the Irish West coast. Results from the exposure study showed that levels of selected EDCs and their resultant effects in the Irish marine and estuarine waters studied were low. Mussels and water samples were analysed for the estrogenic EDCs estrone, 17β estradiol, 17α ethynyl estradiol, nonylphenol, octylphenol and bisphenol A using liquid chromatography/ tandem mass spectrometry (LC-MS/MS). A similar ratio of the steroid estrogens estrone and 17β estradiol was detected in water samples and POCIS. The levels detected were in the low ng/L range for water samples, and ng/device for POCIS. The highest concentrations of steroid estrogens were detected in POCIS in July at all sites, with lowest levels observed in September. EDC levels in mussel tissue were detected at low ng/g concentrations. ALP and ER LUC results varied between sites. The natural cycle of seasonal ALP and ER LUC responses in the transplanted mussel stock was used to normalise levels detected in the mussels after transplantation and exposure. Results from this integrated study provide a comprehensive assessment of the levels and potential for biological effects of selected EDCs in Irish marine waters. The suitability of transplanted mussels as biomarkers in environmental monitoring programs is discussed. Transplantation of bio-monitor species, the collection of multi-parameter biological effects and analytical data in addition to collection of data from 'less impacted' areas, when corrected for natural processes, will be vital for the derivation of appropriate future assessment criteria and may be suitable to further support legislative objectives under Marine Strategy Framework and Water Framework Directive monitoring.

EP02C-1

Reproductive responses of two native freshwater fish species populations of Chile, after 5 years of bleached kraft mill effluent discharge and discharge diverting to sea

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Between 2007 and 2011, we monitored the reproductive status of wild populations of native fish in a basin of central Chile, the potential effect of exposure to effluent discharges from ECF pulp plant cellulose with a modern production and processing system (greenfield mill). We have confirmed endocrine disruption at different levels of biological organization in populations of two species of native freshwater fishes of Chile (*Percilia gillissi* and *Trichomycterus areolatus*) downstream the discharge during periods of 2007 to 2009. Depending on the seasonality of monitoring have shown an induction or inhibition of the production of sex steroids, gonadal maturation and histological level increased or decreased during periods of recrudescence and spawning. In January 2010, the plant began to discharge into the sea, but due to the earthquake

of February 27, 2010, the plant discharged into the river again briefly. Despite there was a high variability in the size of the gonad of *P. gillissi*, we observed a decrease in gonad size, along with induction of EROD activity downstream of the discharge. *T. areolatus* showed no differences with the reference sites for the same period. During periods of 2010 and 2011, there have been recoveries of populations downstream of the discharge, especially in *T. areolatus*. While there is a difference in the sensitivity and resilience of the species, is showing greater resilience in *T. areolatus*. Both species appear to exhibit an overstimulation of the reproductive system during exposure to effluents from pulp, which carries a positive and/or negative feedback of the reproductive system, dependent on the gonadal maturation period to be monitored. These changes have not reached a critical threshold of disturbance, as there is evidence of resilience in both species, by suspending the discharge of effluents from pulp to its habitat.

EP02C-2

Polycyclic aromatic hydrocarbons and endocrine disruption: the role of junctional intercellular communication

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Male reproductive function in animals and humans is considered to be a system highly sensitive to many chemicals and physical agents generated by industrial or agricultural activities. Recently, many disturbing trends have been observed in male fertility, such as decreasing sperm counts, deteriorating semen quality and increasing frequencies of malformations of testis and incidence of testicular cancer. Endocrine-disrupting Chemicals (EDCs) are discussed as possible cause of these adverse trends in male reproductive health. In addition, to estrogen- and androgen-mediated processes, there is a strong evidence that testicular cell-to-cell communication mediated by gap junctions, termed Gap Junctional Intercellular Communication (GJIC), is involved in testicular development, regulation of hormone release, cell differentiation, initiation, and maintenance of spermatogenesis. Thus, inhibition of GJIC during critical stages of development may result in male reproductive dysfunction leading to infertility. Indeed, many chemicals known to be EDCs modulate GJIC and/or impair connexin expression in gonadal or non-gonadal cells. However, there is limited information on the detailed role of GJIC in adverse reproductive effects caused by specific EDCs. Recent studies indicate that anthropogenic air pollutants can possibly impair reproduction of human and wildlife. It has been reported that Polycyclic Aromatic Hydrocarbons (PAHs) on airborne particulate particles compromised sperm functions and altered endocrine hormone levels in exposed animals. Our study addressed the endocrine-disrupting potential of air pollution as a source of compounds that may alter male fertility. The inhibition of GJIC by PAHs and air samples was assessed in testicular cells in this experiment, in order to determine whether PAHs could cause endocrine disruptive effects by closing gap junction channels. This research was supported by GACR P503/10/P249, the SoMoPro project number 2SGA2764 (funded from the European Community within the Seventh Framework Programme (FP/2007-2013) under Grant Agreement no. 229603 and co-financed by the South Moravian Region) and by the Brno PhD Talent Financial Aid from Statutory city of Brno to Petra Kubincova.

EP02C-3

Improving the in vivo predictive value of in vitro estrogenicity bioassays by combining in vitro results with kinetic characteristics of estrogenic compounds

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Stably transfected reporter gene In vitro bioassays such as the ER-Luc bioassay (T47D or U2OS cell-based) or the transcriptional activation assay (using HeLa-9903 cells) provide rapid and sensitive, effect-directed tools for quantifying the estrogenic potency of compounds and sample extracts. A limitation when using these in vitro bioassays for in vivo hazard assessment, however, is that the ADME (absorption, distribution, metabolism and excretion) characteristics of compounds are not taken into account, which can hamper the in vivo predictive value of these bioassays. Our aim was to alleviate this limitation by taking specific ADME characteristics into account.

Our present work compares the in vitro estrogenicity of a suite of selected (xeno-)estrogenic compounds relative to the reference compound ethinylestradiol (EE2) and literature-derived in vivo estrogenic responses obtained with the uterotrophic assay using subcutaneously exposed rats. The selection of compounds includes alkylphenols, benzophenone derivatives, isoflavones, phenyl methanes and steroids. A methodology was applied that calculates EE2 equivalencies by not only taking the in vitro derived estrogenic potency of these compounds into account, but also compound-specific, in vitro differences in hepatic availability (the dose escaping hepatic clearance) relative to EE2 as determined by incubations with rat liver microsomes and cytosol. In addition, compound-specific differences in serum protein binding were assessed using equilibrium dialysis in order to determine the available concentrations of the selected compounds.

The studies demonstrate that combining in vitro estrogenic potency with in vitro determined compound-specific kinetic characteristics for hepatic clearance and protein binding does improve the correlation with the in vivo effect doses obtained with the rat uterotrophic assay, and quantifies the impact of these factors. The improved in vivo predictive value of the in vitro estrogenicity bioassays contributes to the derivation of an in vitro effect-based benchmark dose needed for risk assessment of e.g. drinking water samples containing estrogenic compounds. The approach also offers future perspectives for other in vivo predictions based on in vitro determined toxic potencies.

EP02C-4

Quantifying thyroid hormones and steroids in wastewater effluents by using a Mode of Action-based tool-box

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Study of Endocrine Disrupting Chemicals has focused largely on estrogenic compounds. However, it has not been until recently when researchers expanded the scope of their work to xenobiotics that may perturb thyroid hormone homeostasis and/or interact with nuclear steroid receptors for steroids (i.e. estrogens, androgen, progesterone, and glucocorticoid receptors). Simultaneously to the advances in the Environmental Sciences (chemistry, and (eco)-toxicology) and Engineering to address the risks associated with the presence of EDCs in the environment, there has been an increased public awareness and demand of information by citizens and water utilities.

Whole Effluent Toxicity (WET) is a permit requirement in the U.S. that EPA uses to implement the Clean Water Act's prohibition of the discharge of toxic pollutants in toxic amounts. WET refers to the aggregate toxic effect to aquatic organisms from all pollutants contained in a facility's wastewater (effluent). WET tests measure wastewater's effects on specific test organisms' ability to survive, grow and reproduce. In anticipation of WET requirements of wastewater facilities to monitor the increasing number of EDCs in wastewater and meet the pertinent regulations, a comprehensive set of tools needs to be implemented. A Mode Of Action-based approach, in which a battery of bioassays and chemical analysis are combined to identify toxic effects and their causing agents, is proposed for this purpose.

The current work encompasses a multi-laboratory and multi-disciplinary effort to monitor and characterize the endocrine disrupting activity of effluents from wastewater treatment plants, and the specific chemicals that may underlie these effects. The primary endpoints targeted in this project are thyroid disruption and estrogenic and progestagenic effects.

In this presentation, the results from monitoring selected steroids (estrogens, androgens, and progestins) and thyroid hormones, as part of the initial chemical characterization of a set wastewater effluents, will be shown. Additionally, the equivalent disrupting potency calculated from the measured concentrations by chemical analysis will be compared to the results from a battery of ex vivo and in vitro bioassays (i.e. thyroid gland explant culture assay, thyroid peroxidase inhibition assay, rat and human pregnane-X and progesterone cell-based nuclear receptor reporter assays, and vitellogenin gene expression assay with larvae Fathead Minnows).

EP02C-5

Statistical analysis of histopathology endpoints

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Histopathology experiments, especially in the aquatic environment, are usually conducted with multiple subjects (e.g., fish) in each of multiple vessels (e.g., tanks) within a water control and each of several doses or concentrations of the test material. Responses are typically ordered severity scores on each subject with values 0 for no effect, 1 for minimal effect, through 4 or 5 for severe effect. The usual analysis for such responses from a 1-generation study is to do separate Mann-Whitney or Dunn's nonparametric comparisons of treatment groups to control, where the data are either replicate means or medians, or else replicates are ignored and all subjects are analyzed as independent observations. The mean score is statistically unsound and both it and the median score ignore much important information. Furthermore, these tests on median scores make no allowance for the common phenomenon of varying numbers of subjects in different vessels. On the other hand, ignoring replicates and treating all subjects as independent ignores the experimental design and frequently observed phenomenon that subjects in the same vessel tend to have responses correlated differently from subjects in different vessels. These pairwise approaches also ignore the biological expectation that severity of effect tends to increase with increasing concentrations. A step-down Jonckheere-Terpstra test, applied either to replicate medians or ignoring replicates, uses the presumed monotone concentration-response but otherwise suffers from the same limitations as pairwise methods.

A new test, the Rao-Scott Cochran-Armitage by Slices, or RSCABS, is proposed that incorporates the replicate vessel experimental design and the biological expectation that severity of effect tends to increase with increasing doses or concentrations, while retaining the individual subject scores and revealing the severity of any effect found.

A statistical protocol is proposed for analyzing both 1-generation and multi-generation histopathology studies. It is illustrated with data from three recent multi-generation medaka studies done by a joint Japan-USA project under the auspices of the OECD Validation Management Group for Ecotoxicity.

EP03A-2

Stability of functionalized gold nanoparticles in water systems with different composition

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EP03A-3

Application of field-flow-fractionation for the analysis of engineered nanoparticles in complex matrices

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EP03A-4

Quantitative analysis of fullerenes in soil samples

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Fullerenes are carbon-based nanomaterials present in the environment due to several natural events including volcanic activity, meteoritic impacts, lightnings and wildfires, and due to the human activity, including combustion processes, car-brakes, etc. During the recent years a new source of fullerenes to the environment can be expected through the increasing development of nanotechnology. Thus, a general consensus exists in considering fullerenes as a new family of emerging pollutants.

Recent studies have identified the presence of C60 and C70 fullerenes in atmospheric particulate [1] and wastewater samples [2]. Nevertheless, analytical methods for these compounds are somehow scarce, especially in the case of solid matrices. Traditional extraction techniques have been applied for this purpose with limited success [3] and laser-desorption ionization sources have been extensively applied for their mass spectrometric analysis despite little quantitative information can be achieved and they entail risk of fullerene self-generation. There is a need of developing reliable, robust, accurate quantitative methods which allow to assess the occurrence of fullerenes in soil and sediment matrices.

In the present work, ultrasound assisted extraction and accelerated solvent extraction techniques have been explored and both methods have been optimized and validated for the analysis of eight functionalized and unfunctionalized fullerenes in soils. The method allows the detection and quantification of fullerenes in the pg/g order. In order to assess the presence of fullerenes in real soil matrices, 58 real soils have been analyzed. The results and their relation to fuel-burning/industrial/urban activity will be further discussed.

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EP03A-5

Sedimentation of nanomaterials in natural waters

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There is a growing need for risk assessment of different nanomaterials in order to support their safe production and use. For this we need to estimate the exposure concentration of nanomaterials in the environment. We focus on estimating the exposure concentration in water based on removal process such as sedimentation and dissolution. In this study we measured the sedimentation and dissolution of different nanoparticles, PVP capped Ag, Silica coated Ag, CeO2 and C60. In order to cover a broad range of particle properties metal, metaloxide and carbon particles were used. Due to use of HR-ICPMS relatively low concentrations of metal particles could be tested. These particle related properties are combined with the environmental properties of 6 different water types which range in ionic strength, dissolved organic carbon, acidity and suspended solids. Additionally the effect of the suspended solids is taken into account by comparing sedimentation in filtered and unfiltered water samples. Sedimentation rates and residual concentrations are derived from the observed data. The dissolution measurements show that removal of nanoparticles from the water phase due to dissolution is negligible, except in sea water where up to 10% of added Ag particles dissolved. The removal due to sedimentation ranges from less than 8% to almost 100%. This depends greatly on water characteristics, but large difference are also found between particle types, e.g. PVP vs Silica coated Ag particles. In general high dissolved organic carbon concentration increased stabilization against sedimentation. The description of sedimentation by first order removal kinetics towards a residual concentration seems valid. This is valuable for predicting the exposure concentration of nanoparticles in the aquatic environment.

EP03A-6

River biofilms and silver nanoparticles: de novo nanoparticle formation and nanoparticle weathering

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This work focusses on the effects of the extracellular matrix of river biofilms on silver nanoparticles (Ag NPs) and introduces new possibilities to visualize nanoparticles in environmental samples.

River biofilms are the main producers of biomass and oxygen in freshwater ecosystems. Ag NPs will first interact with the extracellular matrix (EM) of periphytic organism before the latter are actually exposed. EM components may change the properties of the nanoparticles and thus influence the interaction with and effects on the organisms. EM is extracted from biofilms colonized in indoor channels in a flow-through system fed with river water and is analyzed for cell lysis, protein content, TOC/DOC content, DOC size distribution.

Ag NPs and AgNO3 are diluted in NaHCO3 with 10mg/L EM in the dark or the light. The samples are characterized for the presence of NPs and the size (dynamic light scattering (DLS)), NP tracking analysis (NTA), electron microscopy (EM)), stability (electrophoretic mobility), Plasmon resonance (UV-VIS), dissolution (ICP-MS), and chemical composition (ESRF).

AgNPs: Dispersions remained stable in NaHCO3 at pH 6- 8.6 over one week in both light and dark. The addition of EM lead to a slight size increase but dispersions remained stable in the dark. Exposure of Ag NPs to EM and light lead to pH-dependent weathering over time.

AgNO3: There was no significant nanoparticle formation in the dark in the absence of EM and a low rate of particle formation in the light. In the presence of EM and light, de novo particle formation was observed within minutes and at a slower rate in the dark. Particle size was pH dependent. The presence of a Plasmon resonance signal shows that at least parts of the NPs are elemental Ag.

We are currently measuring the chemical composition of the weathered and newly formed particles.

In situ characterization: With a new approach we use CLSM and NTA to characterize NPs in biofilms. The size of Ag NPs was thus determined in intact biofilm samples. We are now going to track particle size and formation in biofilms over time. The chemical identity of the NPs in the biofilms will be analysed via CLSM-Raman Spectroscopy.

Ag NPs, Ag+, and EM components from river biofilms form a dynamic system which is influenced by pH and light. Our results indicate that wherever there is Ag+ and EM in the environment, nanoparticles may form. This is essential information for the assessment of the potential environmental effects of AgNPs.

EP03B-2

Respiratory distress and biochemical changes in brain but no impact on behaviours of rainbow trout exposed to nanoparticulate titanium dioxide

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Since the advent of nano-ecotoxicology a number of studies, including several from our research group, have demonstrated very subtle biochemical changes in brains of fish exposed to engineered nanoparticles (NPs). This has led us to investigate the implications of these effects for fish behaviour; behaviours link physiological and ecological processes and are key determinants of fish population structure. In this study investigating the effects of TiO₂ NPs on locomotor and social behaviour of fish, juvenile rainbow trout (23.7 ± 3.7 g) were exposed to 1 mg/L TiO₂ NPs (24.5 ± 10.6 nm, primary particle size) or an equivalent concentration of TiO₂ bulk material (134.1 ± 42.5 nm). Spontaneous fish movements were quantified with Ethovision video tracking software (Noldus Information Technology) and outcome of interactions between fish scored by direct observation; juvenile rainbow trout are aggressive and outcome of paired interactions between trout from different treatment groups may reveal fitness disparities. Following 14 days exposure fish from both treatment groups exhibited morphological changes to gill tissue, most notably a characteristic swelling of primary and secondary lamellae, compared to control fish. However, elevated concentration of red blood cells and haemoglobin were only observed in fish exposed to TiO₂ NPs, suggestive of a nano- specific effect. Biochemical changes including elevated (1.5 fold) lipid peroxidation were also observed in the brain of fish in fish exposed to TiO₂ NPs. Element analysis revealed accumulation of Ti at gill of fish but no significant accumulation was observed in internal organs, including brain, suggesting toxicity observed was driven

by interaction of TiO₂ NPs at the gill and TiO₂ NPs are not bioavailable. Whilst these data indicate that exposure to TiO₂ NPs may cause biochemical disturbance in rainbow trout, locomotion was unaffected. Video tracking of movements revealed no significant difference in distance travelled control (195 ± 37 m/h, mean ± SEM, n = 5), bulk TiO₂ (167 ± 31 m/h, n = 5), or TiO₂ NPs (117 ± 26 m/h, n = 6) exposed fish. Outcome of paired interaction was also not affected. These data suggest TiO₂ NPs cause toxicity in rainbow trout but respiratory distress and brain injury exhibited by fish in this study does not impair ecologically relevant behaviours.

EP03B-3

The Four S's of Nanoparticle-Cell Interactions: size, shape, surface chemistry and serum proteins

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There are currently over 1000 consumer products on the market that contain or utilize nanomaterials, and this number is expected to increase exponentially in the near future in the absence of detailed knowledge concerning the interactions of such materials with biological systems. While nanoparticles have been shown to cross cell membranes, little research has examined the influence of particle characteristics on membrane transport. Furthermore, our inadequate understanding of potential human and ecological effects has resulted in uncertainties of the potential risks of this technology, and impeded the advancement of quantitative risk assessments. The goal of this project was to characterize the influence of particle size, shape, and surface chemistry on the movement of gold nanoparticles across mammalian cell membranes, as well as the influence of serum protein concentrations on this uptake. A549 carcinomic human alveolar cells were utilized to characterize the movements of gold nanoparticles across cell membranes. Specifically, the absorption of three different gold nanoparticle shapes was examined at multiple sizes: spheres (5, 20, and 50 nm), cubes (50 and 75 nm) and rods (20x100 and 20x200 nm). The influence of surface chemistry on absorption was examined through surface modifications of these particles of various shapes and sizes by polyethyleneimine, polyethylene glycol, and citrate. Each particle was characterized by TEM, DLS, UV-vis, and zeta potential. In total, comparisons of cell uptake were examined for 21 different modifications of gold nanomaterials. Also, the influence of serum protein concentrations on this uptake was investigated for nanospheres with anionic and cationic surface modifications. Cells were plated in 12-well plates at 100,000 cells per well and exposed to 1 mg/L gold for 0.5, 1, 1.5, and 2 hours. Bioaccumulation of nanoparticles at each time point was quantified by inductively coupled plasma-mass spectrometry (ICP-MS), and movement was visualized via confocal microscopy. This research provides a foundation upon which to predict biological interactions with nanoparticles and facilitate future risk assessment endeavours.

EP03B-4

Trophic transfer of gold nanoparticles in a water food chain

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The risk of nanoparticles is becoming an issue. Nanoparticles are ultrafine particles and can be easily penetrated through cell membrane. To understand their potential risk, we investigated trophic transfer of nanoparticles in a water food chain. The low and high trophic level organisms of this study were green algae *Chlamydomonas reinhardtii* and invertebrate *Daphnia magna*. *Chlamydomonas reinhardtii* was initially exposed to gold nanoparticles (AuNPs; 10 nm), and 2-day old *Daphnia magna* were fed by the exposed cells (2.5 [GREEKX] 10⁵ cell/ml). The results showed that gold nanoparticles can transfer from *Chlamydomonas reinhardtii* to *Daphnia magna* via food exposure. This study demonstrated that nanoparticles can be transferred from the algae to invertebrates in aquatic ecosystem. *This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2011- 0015985).*

EP03B-5

The chronic toxicity of ZnO nanoparticles in *Daphnia magna* and the application of different detection methods to assess their bioavailability

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Zinc oxide nanoparticles are being increasingly produced and will therefore somehow end up in the aquatic environment. Although many different acute toxicity studies have been performed, the number of chronic toxicity data is still very limited. However, when looking at environmental relevant scenarios the knowledge on chronic toxicity is of great importance. Additionally, there are still many conflicting data on whether the toxicity caused by ZnO nanoparticles is due to the ion fraction or to the nanoparticle itself. Therefore, we investigated the chronic toxicity of ZnO nanoparticles in a well characterised model species *Daphnia magna*. During 21 days the daphnids were exposed to a concentration range of ZnO nanoparticles (Alfa Aesar). Different endpoints such as reproduction, mortality and growth were checked. In order to determine the speciation of the nanoparticles the concentrations of total zinc, ZnO nanoparticle and zinc ion were measured in the daphnia test medium. Unfiltered and filtered (0.45 µm filters) medium samples were taken at different times of exposure and the zinc concentration was analysed by ICP-OES. Another technique called Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) was used to measure free Zn²⁺ concentrations. In order to distinguish between the ion, nano and aggregated fraction, ultrafiltration via different filters was used. The different fractions were analysed via ICP-MS. The results of the chronic exposure experiment revealed an EC₅₀ value around 0.02 mg/L for the reproduction of *D. magna*. The growth and mortality of the daphnids were significantly influenced at the highest exposure concentrations. The results of the first filtration technique indicate that there were no significant differences in zinc concentrations between the unfiltered and filtered (0.45 µm) medium samples directly after spiking (0 h). In contrast, after 48 h, the zinc concentration decreased significantly in the filtered samples compared to the unfiltered ones. This indicates a certain aggregation of the particles in the first 48 h after spiking. Preliminary results for the AGNES technique show that only about half of the zinc oxide nanoparticles dissolved into zinc ions. Further research is needed to confirm the chronic toxicity results and to elucidate the link with the bioavailability of the ZnO nanoparticles under different forms.

EP03B-6

Effect of non-ageing and ageing ceria nanoparticles suspensions on fresh water micro-algae

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When assessing the hazards properties of nanomaterials in the environment, the main research challenges are numerous. Firstly, determining if nanomaterials are more or less toxic than the bulk forms of the same materials and the extent to which toxicity is governed or influenced by the physico-chemicals properties of the nanoparticles. Secondly, it appears necessary to study the effect of nanomaterials and nanoparticles throughout their life cycle including both initial forms and physico-chemically modified form (i.e. aggregated or agglomerated forms) resulting from an ageing process.

Our work focused on the effect of commercial ceria nanoparticle (nCeO₂) suspensions, towards freshwater micro-algae assessing the effect nCeO₂ suspensions with different agglomeration/aggregation state obtained by using an artificial ageing process. Both ageing and non-ageing nCeO₂ suspensions were fully characterized using dynamic light scattering (ZetaSizer, Malvern Instruments) or laser diffraction (MasterSizer, Malvern Instruments) and transmission electron microscopy (TEM). In addition, the interaction between NPs and algae were investigated using flow-cytometry and environmental scanning electron microscope technique (E-SEM).

The results obtained showed that the algae growth inhibition was similar after exposure to non-ageing or ageing nCeO₂ suspensions. The results obtained from flow-cytometry and E-SEM proved that the ceria NPs are able to tightly entrap the algae cells, which could in part contribute to the effect recorded. Those results also support the fact that aggregation or agglomeration has a few influences when focusing on the standardized algae ecotoxicity test. Moreover by comparison to our previous studies performed with other ceria suspensions, it was shown that the primary particle size and consequently the particle surface area is a relevant parameter in assessing the ecotoxicity of nanoparticles.

EP03C-1

Bioavailability of carbon nanotubes to aquatic organisms of different trophic levels and the consequences of CNT-cell interactions to vital functions

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Multi-walled carbon nanotubes (CNT) were synthesised using 14C-benzene in order to quantify bioaccumulation of this nanomaterial in different aquatic organisms. Next to uptake and elimination, transfer of CNT along the food chain, the influence of the presence of sediment or dissolved organic carbon (DOC) on its bioavailability, and distribution of incorporated CNT to different fish tissues were investigated. Radioactivity was detected in algae, daphnids, blackworms, and zebrafish, which had been exposed to dispersed 14C-CNT material via the water (1 mg/L). Furthermore, single tubes and small agglomerates were visualised by means of transmission electron microscopy in algal cells, branchial erythrocytes of fish, and gut epithelial cells of daphnids, worms, and fish. Transfer of CNT from the fish gills to the blood current was confirmed by the presence of radioactivity in blood samples and gonads. However, CNT were not detected in the brain of zebrafish, which indicates that they do not pass the blood-brain barrier. CNT were not bioavailable for blackworms when spiked to the sediment. Similarly, ingestion of CNT-containing prey by daphnids (CNT-spiked algae) or by zebrafish (CNT-spiked worms) resulted in lower accumulation in the predator compared to after uptake of equal amounts via the water phase. Although DOC was shown to keep prepared dispersions more stable over time, its presence had no influence on CNT bioavailability. The consequences of the observed interactions of CNT with cells, following water exposure of different organisms, regarding possible damage to vital functions are investigated at the moment. By now, CNT-associated alterations in the cell

biochemistry of exposed algae were observed by attenuated total reflection Fourier-transform infrared spectroscopy. Furthermore, the condition factor of fish that had taken up CNT during four days, and were daily fed for six days afterwards, was significantly lower compared to the one of control organisms. This might indicate that digestion of food is hindered by the observed presence of CNT material in gut epithelial cells. Longer tests with repeated exposure periods are currently performed to verify this finding. The combined results of the present fate and effect studies deliver conclusive insights that can be used to assess the possible risks of CNT release to the aquatic environment.

EP03C-2

Short-term toxicity of silver nanoparticles on litter-associated fungi and bacteria from streams

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Current knowledge of AgNP toxicity is mainly based on laboratory studies with single species, which might not reflect their effects in natural environments. Therefore, it is essential for realistically assessing AgNP effects to apply approaches that take the ecological complexity and variability of natural communities and ecosystems into account. Litter-associated fungi and bacteria provide suitable model systems for community ecotoxicology because they play a fundamental role in the functioning of various ecosystems. They effectively degrade plant litter and produce substantial amounts of biomass that is subsequently channeled to higher trophic levels. This study assessed the short-term toxicity of AgNP on litter-associated stream fungi and bacteria in comparison to that of AgNO₃ (Ag⁺). Prior to use, the nanoparticles were characterized in terms of size, surface charge and aggregation behaviour. Toxicity of AgNP in comparison to Ag⁺ was determined in inhibition tests based on a broad set of functional parameters. The specific sensitivity of fungal communities was assessed as sporulation rate and 14C-acetate incorporation into ergosterol. The specific sensitivity of bacterial communities was assessed by determining rates of 14C-leucine incorporation into protein. Moreover, potential extracellular enzyme activities (phosphatase, -glucosidase and leucine-aminopeptidase), and respiration were measured to evaluate effects on overall microbial activity. Toxic effects of AgNP were variable, depending on the functional parameter used, and distinct from Ag⁺ toxicity. No inhibitory effect of AgNP on microbial respiration was observed, whereas Ag⁺ caused an inhibition. Both AgNP and Ag⁺ exposure stimulated potential phosphatase activity and reduced potential leucine-aminopeptidase activity. -Glucosidase activity was stimulated by AgNP but reduced by Ag⁺. Finally, bacterial and fungal growth and sporulation were more strongly inhibited by AgNP than by Ag⁺. These findings suggest that dissolved ionic Ag (i.e. AgNO₃) does not fully explain the observed toxicity of AgNP on litter-associated fungi and bacteria in streams. Overall, our study confirms the need to consider functional diversity using complementary indicators for ecotoxicological investigations on short-term AgNP toxicity.

EP03C-3

The effect of ageing on the bioavailability and ecotoxicity of ZnO-NP, bulk ZnO and ZnCl₂ in natural soil

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Releases of metal nanoparticles into soils are expected when nanoparticles pass the sewage treatment plant or end up in sewage sludge. Concern has risen about ecotoxicological effects of nanoparticles. Short-term toxicity tests and bioaccumulation studies on zinc oxide nanoparticles (ZnO-NP) have already been performed with isopods, earthworms and springtails. Little information is available on the long-term fate and effects of ZnO-NP in soils.

Here the behaviour of ZnO-NP, bulk ZnO and ZnCl₂ was studied in natural soil to evaluate the influence of ageing time on chemical speciation and stability of the nanoparticles. Dissolution and ecotoxicity to the springtail *Folsomia candida* was determined for the three Zn forms after three, six and twelve months.

Zinc concentrations in the soil pore water ranged from 1.85 to 12.6 mg Zn/l in freshly spiked soil with ZnO-NP and this increase was found to be linear. Porewater concentrations after three, six and twelve months increased in a non-linear manner with increasing soil concentrations for ZnO-NP and bulk ZnO. Zn concentrations in the pore water increased with time, but peaked at intermediate concentrations. The highest Zn concentrations measured after one year ageing were 67.1 and 66.5 mg Zn/l for ZnO-NP and bulk ZnO, respectively. Zn concentrations in pore water, collected from aged soils with ZnCl₂, increased with exposure concentration, but also with time. ZnO-NP and bulk ZnO were toxic in freshly spiked soil with no significant difference between the two ZnO powders. No effect on Collembolan survival or reproduction was found in three, six or twelve months aged soil with ZnO-NP and bulk ZnO. The toxicity of ZnCl₂ decreased with time as shown by the three-fold increase in EC50 value after three months ageing.

The release of Zn ions from ZnO-NP was reduced at high spiking concentrations. Sorption and agglomeration may inhibit dissolution and increased sorption may take longer due to the physical and chemical properties of nanoparticles. An increasing solubility throughout one year was observed, although at very low rate when based on total Zn concentrations.

Several studies show that toxicity to soil organisms is related to the free ions released from metal nanoparticles. Our toxicity tests show that an ageing period of three months or longer is able to reduce toxic effects on survival or reproduction to *F. candida*.

EP03C-4

Effects of C60 nanoparticles on Lumbricus rubellus earthworms: from gene expression to population dynamics

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In the present study the effects of C₆₀ exposure on *Lumbricus rubellus* earthworms was assessed at different levels of biological integration. Two in vivo experiments were carried out, with the same exposure levels of C₆₀ (0, 15 and 154 mg C₆₀/kg soil). In the first experiment adult earthworms were exposed for four weeks and in the second experiment the offspring was exposed lifelong. Effects on the individual level were assessed. C₆₀ exposure caused a decrease of reproductive success in the four weeks exposed earthworms and offspring exposed to C₆₀ demonstrated lower juvenile survival and growth rate. These effect markers were used to model consequences at the population level. The population model showed that C₆₀ exposure could affect population growth rate and life stage distribution. In order to relate these observations to potential modes of action, sub-lethal responses were also studied for these earthworms. At the tissue level, histological examinations demonstrated concentration-dependent effects in both experiments. Damage was noted at the cuticle (accompanied with injuries to underlying tissues) as well as at the gut epithelium. At the molecular level, gene expression analysis was performed for several selected genes. The general stress marker heat shock protein 70 (HSP70) and the cytokine-like coelomic cytolytic factor 1 (CCF-1) were both affected by C₆₀ exposure. As an effect on immune response (CCF-1) was noted, in vitro experiments with immune cells were performed. Coelomocytes, extracted from adult earthworms and exposed in vitro, showed decreasing survival and phagocytic activity with increasing C₆₀ concentrations. Furthermore, C₆₀ will be characterized in the exposure media, to assess actual exposure. In conclusion, this study demonstrates the impact of C₆₀ exposure at the molecular, cellular, tissue, individual and population level. Further research is needed to identify the precise mode of action of C₆₀ exposure to earthworms.

EP03C-5

Effect of Cu-NPs versus Cu-salt in Enchytraeus albidus - survival, reproduction, avoidance, oxidative stress biomarkers, energy allocation and gene expression - a systems biology approach

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Environmental effects of Cu-nanoparticles (Cu-NP) are little studied in terrestrial ecosystems. In the present study, the toxicity of Cu-NPs on the enchytraeid *Enchytraeus albidus* is compared to the toxicity of a copper-salt (CuCl₂). Traditionally, effect assessment is made at the population level [1]: survival and reproduction. Recently, a cDNA microarray has been developed, which allows toxicogenomic studies. The effect was studied including various endpoints towards a systems biology approach: survival, reproductive output, avoidance behaviour, oxidative stress biomarkers, energy budget (lipids, proteins, carbohydrates) and gene expression. The results from survival, reproduction and avoidance showed that Cu-NPs were more toxic to *E. albidus* than the same concentrations of the CuCl₂-salt. The physico-chemical analysis of the particles indicated that only a small fraction was released as ions. Hence, the results indicated a nanoparticle-specific effect - lower reproductive output and higher avoidance. This was observed as 2-8 fold (significant) lower ECx values for Cu-NP exposed organisms compared to CuCl₂ exposed organisms. These results indicated a nanoparticle-specific effect. Oxidative stress biomarkers showed that both salt- and nano-copper caused oxidative stress and damage to *E. albidus*, as confirmed by LPO levels, and effects could be discriminated between the copper forms. Nevertheless and despite the visible discrimination between nano and the salt form (time and exposure dependent), there was no single or a set of biomarkers that provided the best discrimination. The energy allocation levels showed clear differences between worms exposed to control soils and those exposed to Cu for 3 weeks, but no difference after 6 weeks exposure. There was no apparent difference between the impacts of the two Cu exposure forms. The number of differently expressed genes (DEG) decreased with increasing concentration for CuCl₂ exposure, whereas for Cu-NP, the number did not change. The number of common DEG decreased with increasing concentration. Differences were mainly related to transcripts involved in energy metabolism (e.g. monosaccharide transporting ATPase, NADH dehydrogenase subunit 1, cytochrome c). Overall, our results indicated that Cu-salt and Cu-NP exposure induced different gene responses. All results pointed to a

nanoparticle-specific effect, and not due to Cu-ions released from the particles, as also indicated by the ISE measurements.

EP03C-6

Long-term effects of sewage sludge spiked with Ag-NP on soil microorganisms

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Due to their antibacterial and antimicrobial properties silver nanoparticles (Ag-NP) are widely used, e.g. in textiles, medical applications or cleaning products. This means that Ag-NP will inevitably enter wastewater treatment plants (WWTP). In the literature, research is reported on the fate and behavior of Ag-NP in WWTPs. Burkhardt et al. (2010) determined that nearly 95% of silver is bound to the sewage sludge whereas around 5% leaves the WWTPs with the effluent in ecologically negligible concentrations. In the sewage sludge silver ions are transformed to silver sulphide which precipitates. In Germany and other countries sewage sludge is used as a fertilizer in agriculture. Therefore, the goal of the present project was to determine long-term effects of sewage sludge spiked with soluble Ag (silver nitrate) and Ag-NP on soil microorganisms over a period of 180 days. We used sludge from a local WWTP and spiked it with silver nanoparticles - NM-300K from the OECD Sponsorship Programme - and silver nitrate. The test soil was the medium acidic and slightly humic loamy sand "Refesol 01A". Silver concentrations of 1.6 and 3.4 mg/kg d.m. soil for NM-300K and 2.1 and 4.0 mg/kg d.m. soil for silver nitrate were obtained. To investigate potential effects on soil microorganisms standardized test systems were used. Ammonium oxidation (ISO 15685) and carbon transformation tests (CTT; OECD 217) were performed analyzing samples at day 32 / 60 / 100 and 180. Two replicate batches were used for each concentration. A control without sludge and one with non-spiked sludge served as reference. Effects on ammonium oxidation or respiration (CTT) of the microorganisms based on Ag-NP or silver nitrate were not observed after 32 days. Referring to the control with sludge we obtained an inhibition of the microbial respiration activity for the highest concentration of silver nitrate after 60 days, whereas ammonium oxidation showed no effect. After 100 days both concentrations of NM-300K and silver nitrate resulted in a comparable respiration inhibition of around 30%. The ammonium oxidation test showed a statistically significant dose-dependent inhibition for NM-300K and silver nitrate. Results for day 180 are not yet available. Our results indicate that Ag-NP and silver nitrate can become bioavailable after the degradation of sewage sludge and can cause an inhibition of the soil microorganisms.

EP03D-2

An approach to determine appropriate dose metrics for nanomaterials

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Exposure limits for chemicals are traditionally derived from studies in which increasing doses of the substance are administered. For soluble substances, a unique measure of the dose is the total mass of the substance administered or, equivalently, the total number of molecules administered. Consequently, exposure limits are generally based on mass concentrations, such as X mg of chemical substance Y per kg soil.

With nanotechnology a new array of often insoluble chemical entities has made its appearance. The different characteristics of nanomaterials (e.g. size, shape, polymorph form of the crystall structure) all may determine their toxic potential. For example, daily intake of X mg of nanomaterial (NM) with particle size d1 may be more toxic than X mg of the same nanomaterial with particle size d2. In other words, for NMs information on the administered mass of the chemical substance alone may not be a sufficient description of the dose that determines a particular response. As a result, the question arises of what dose description to use when setting exposure limits for NMs. It has been speculated that exposure limits based on particle numbers, such as used for particulate matter, may be more appropriate, while others advocate surface area.

An adequate dose metric for NMs should describe all relevant characteristics that are necessary to explain differences between responses. A minimal criterion for an adequate dose metric is that the dose metric should be able to discriminate doses with different responses. In its most complete form, the dose of a NM can be described by a (distribution) function that specifies the number of particles in the ensemble with specific characteristics. For example $PN(d, \zeta, \alpha)$ may give the number of particles N with a diameter d, surface potential ζ and crystal structure α .

Ideally, a dose metric should be as concise as possible, with as few dimensions as possible. A reduced dose metric (for example requiring only information on administered total number of particles, or total surface area) would be pragmatic for risk assessment purposes, since only one exposure limit would have to be derived for various NMs of the same substance. However, a priori there is no reason why such a reduced dose metric should exist. In this contribution, we present a method to determine whether a reduced dose metric for (a class of) NMs exists. As an illustration, the method is applied to results from experiments with various NM published recently.

EP03D-3

Assessment of environmental risks of nanomaterials throughout the product life cycle

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Estimation of exposure due to release into the environment represents a key challenge in determining nanomaterials safety. It is therefore important to understand to what extent existing risk assessment approaches and tools can be applied or will require modification to take account of the particular properties of nanomaterials. The present work aims to identify methodologies and tools to assess and manage the environmental risks due to manufactured nanomaterials, identify appropriate risk assessment methods, and develop improved methods for risk assessment. Some currently available nano-specific support tools are evaluated, in order to identify gaps where either risk assessment methods and/or input data are insufficient.

A significant problem is that there still remain many outstanding questions regarding the behaviour of nanomaterials in the environment, which could differ from that of materials of similar composition in bulk form. Reducing the level of these uncertainties is essential to proper risk assessment. There is thus an urgent need for reliable data on the physico-chemical properties, toxicokinetics and degradability of nanomaterials to understand their transport, persistence and fate, and exposure potential in the environment. However, there is still a lack of basic information on the possible release routes for nanomaterials during production, use and final disposal or recycling. For this reason, it is necessary to improve our present knowledge on the release of nanomaterials from products during all phases of their life cycle.

The NanoSustain project is developing innovative solutions for the sustainable use, recycling and final treatment of nanotechnology-based products. Experimental work is carried out to investigate the potential for release of nanomaterials from products during industrial operations, such as sanding and grinding, and at the end of life during disposal by incineration and recycling by melting. Data obtained from such experiments should help reduce the level of uncertainty in the risk assessment

EP03D-4

A weight of evidence approach for ranking and prioritization of occupational exposure scenarios for engineered nanomaterials

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It has been recognized that substantial limitations and uncertainties make the conventional risk assessment of chemicals infeasible to apply to engineered nanomaterials (ENMs) today, which raises health and safety concerns. The present deficit of quantitative exposure measurements and nano-specific exposure models will lead in the near term to uncertain and largely qualitative exposure estimations, which may fail to support proper risk assessment and management actions. In this context it is necessary to develop well-validated, easy-to-use approaches for early occupational and consumer safety evaluation [1]. A variety of such tools already exist: e.g. the Swiss Precautionary Matrix for Synthetic Nanomaterials, the Dutch Stoffenmanager Nano, the Danish NANOSAFER, the French Anses system. This is currently a dynamic area of research and several tools are still under development. However, with few exceptions, they all produce qualitative results.

In this context we propose a quantitative model for relative ranking and prioritization of occupational exposure scenarios (ES), specifically tailored for ENMs. The tool is based on a scoring weight-of-evidence method and it uses expert judgement to estimate exposure potential of nanomaterials in the workplace. The conceptual structure of the approach has been defined on the basis of state-of-the-art exposure models for conventional chemicals like the Advanced REACH Tool (ART) and for nanomaterials, such as the Stoffenmanager Nano. In order to apply the tool we used data from the EU FP7-funded NANEX project. Using the model we obtained rankings for 3 groups of inhalation occupational ES and, in order to validate our results, we compared them with parallel rankings of the measured exposure concentrations of the materials, reported for the same scenarios. The results show that the WoE-based model slightly overestimates the exposure in most cases, which proves that it is a conservative approach. In order to test the stability of the model, probabilistic Monte Carlo sensitivity analysis has been applied and it shows that the tool always performs in a stable manner in regard to variations in the input data.

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EP03D-5

Comprehensive environmental assessment of nano-based self decontaminating surfaces

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Engineered nanoparticles are being exploited for a wide variety of military applications. Material science research into the development of new engineered nanoparticles

is far outpacing environmental and human health and safety research, yet the health and safety data are critical for acquisition decisions, regulatory decisions, worker safety, product use and disposal, and public acceptance of nanoparticle-containing products. Traditional life cycle analyses address key steps in nanoparticle synthesis, use, and disposal, but lacks specific information regarding fate and effects in the environment. Conversely, traditional environmental risk assessments address fate and effects of chemical stressors in the environment, but only consider chemicals at specific environmental sites and not throughout the chemical's life cycle. We are developing and applying the comprehensive environmental assessment (CEA) approach, detailed by Davis (2007), which combines life cycle analysis parameters (e.g., manufacture, storage, use, disposal) with traditional risk assessment parameters (e.g., characterization, exposure, effects, assessment) to understand of nanoparticle exposure and effects in different environmental settings. The application of this approach is demonstrated through a case study examining novel nano-based reactive surfaces; a UV-light activated technology. Specifically, we have examined the residue of aged coatings following UV exposure; the most likely release of nanoparticles from this specific nanotechnology. Results of this study represent one of the first documented releases of a nano-sized particulate from a nano-enabled technology. The use for CEA for engineered nanoparticles will improve acquisition, risk, and regulatory decision making and management prior to any unforeseen adverse environment, health, and safety (EHS) events that could dramatically impact the use of these revolutionary new materials.

EP03D-6

Multimedia environmental fate models for engineered nanoparticles - a case study of nano-TiO₂ in the Rhine River

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With the increased production and use of engineered nanoparticles (ENPs) their release to the environment becomes inevitable. Multimedia fate models are valuable tools to assess exposure levels of pollutants in different environmental compartments, both at a regional as well as at a global scale and have the potential to be used in a pro-active risk assessment of ENPs. However, whereas multimedia environmental fate models are well established for organic pollutants, the field of environmental fate modelling is still in its infancy for ENPs. Due to the fundamentally different properties of ENPs compared to normal (i.e. low-molecular weight) organic pollutants, it is necessary to completely revise the process descriptions used in the models and to adjust them to account for ENP-specific properties. Here we present a new framework of multimedia fate models to describe the fate and behaviour of ENPs in aqueous environments and predict exposure levels of ENPs.

As a first case study and illustration of our new multimedia fate modelling concept for ENPs we present a study on TiO₂ nanoparticles (NPs) in the Rhine River. The core of this model is the parameterization of ENP-specific processes. A key process governing the environmental fate of ENPs in aqueous environments is the heteroaggregation of ENPs with naturally occurring suspended particulate matter (SPM) in the nano- and micrometre size range. Deposition of free ENPs by gravitational settling and of ENPs attached to SPM determine the distribution of ENPs between water and sediment compartments.

Our model enables the prediction of steady-state concentrations of TiO₂ NPs along the course of the Rhine River. Varying the parameters affecting the heteroaggregation process allows us to compare their relative importance on the overall fate and transport of TiO₂ NPs in the model system. Overall, the sediment compartment always represents the main reservoir of TiO₂ NPs, but the concentration profile and transport potential of TiO₂ NPs in the water compartment strongly depends on the heteroaggregation attachment efficiency, C_{het}-agg, and the characteristics (size, density and concentration) of the SPM.

EP04 - Greener nanotechnology, an integrative approach to an emerging technology

EP04-1

Green nanotechnology challenges and opportunities

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Nanotechnology is an emerging field, for which there is an unusual opportunity to use science, engineering and policy knowledge to design novel products that are benign as possible to human and environment health. Recognition of this opportunity has led to the development of the "green nanoscience" concept.

Developing an Action Agenda

Green nanotechnology has been making great forward progress, but the challenges it has encountered point to an agenda of actions where involvement by the scientific research community, industry and government is crucial to supporting a more rapid and effective commercialization of green nanotechnology.

The agenda is based on input from experts in green who participated in the GN10 and GN11 Conferences in 2010 and 2011. The first, and most pressing need is for more and better analysis and characterization tools, which are required to support the rest of the agenda. The second item of the agenda, improved mechanistic understanding, is a key part of the foundation for developing green nano design guidelines. Finally, new regulations, as well as outreach to regulators must be based on the analysis, understanding, and design concepts that are the result of the first three items.

The Agenda

1. Discover, uncover and provide key analysis and characterization tools,
2. Develop, characterize and test precision-engineered nanoparticles for biological and toxicological studies needed to guide greener design,
3. Investigate and understand reaction mechanisms to support more efficient and precise synthesis and production techniques,
4. Develop design guidelines for green nanomaterials,
5. Definition of green criteria for new nanomaterials for fast-track approval by the US EPA, and
6. Education and outreach to regulators to ensure regulatory structures for green nanotechnology reflect accurate knowledge of their intended uses and potential impacts.

Nanotechnology does not have to follow the typical path of many past innovations in the chemical industry that, despite providing significant benefits, also turned out to have unanticipated costs to human and environment health. The development and commercialization of viable green nanotechnologies is difficult, and it will require effort from the scientific, research and government communities. But there is a pathway forward, and concrete actions that could construct a solid foundation for a profitable and environmentally sustainable future for nanotechnology.

EP04-2

Rapid in vivo assessment of the nano/bio interface to guide safer nanomaterial design

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The rapid rate of discovery and development in the nanotechnology field will undoubtedly increase both human and environmental exposures to engineered nanomaterials. Whether these exposures pose a significant risk remains uncertain. Despite recent collective progress there remain gaps in our understanding of the nanomaterials physiochemical properties that drive or dictate biological responses. The development and implementation of rapid relevant and efficient testing strategies to assess these emerging materials prior to large-scale exposures could help advance this exciting field. I will present a powerful approach that utilizes a dynamic in vivo zebrafish embryonic assay to rapidly define the biological responses to nanomaterial exposures. Early developmental life stages are often uniquely sensitive to environmental insults, due in part to the enormous changes in cellular differentiation, proliferation and migration required to form the required cell types, tissues and organs. Molecular signaling underlies all of these processes. Most toxic responses result from disruption of proper molecular signaling, thus, early developmental life stages are perhaps the ideal life stage to determine if nanomaterials perturb normal biological pathways. Through automation and rapid throughput approaches, a systematic and iterative strategy has been deployed to help elucidate the nanomaterials properties that drive biological responses.

EP04-3

Ecotoxicology in nanoremediation: n-TiO₂ nanoparticles increase Cd bioaccumulation and toxicity in Mediterranean mussel *Mytilus galloprovincialis*

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The present study investigates the interaction of TiO₂ NP with Cd and how the presence of n-TiO₂ might affect Cd bioavailability and toxicity in a model marine species, the Mediterranean mussel *Mytilus galloprovincialis*. n-TiO₂ is an efficient photocatalyst largely used in several technological applications including water and aqueous wastes remediation. Cd is one of the most toxic metals of interest for remediation. Both compounds have been quite extensively studied in terms of toxicity in the Mediterranean mussel showing distinct pathways of cellular interaction and biological targets. Mussels have been in vivo exposed for 24h and 7 days to n-TiO₂ (1mg/L) and Cd (0,1mg/L) in single and co-incubation. The documented adsorption of Cd onto n-TiO₂ has been confirmed also in our study; the presence of n-TiO₂ enhanced Cd bioaccumulation in mussel's whole soft tissue and increase Cd citotoxicity (reduction of Neutral red retention time in hemocytes). A significant disruption/reversion of Cd capacity to increase the efflux functionality of ABC transport proteins (most probably involved in Cd-resistance) has been also observed in presence of n-TiO₂. Our results indicate that specific NP applied for remediation purposes might exert indirect toxic effects to the biota by affecting both bioavailability and toxicity of the removed toxic contaminant. The present study will promote nano-ecotoxicology as a new strategy in order to develop eco-friendly nanomaterials for specific technological application as nanoremediation.

EP04-4

Nanoparticle properties affecting embryotoxicity: toward a design of safer nano-Zinc oxide

The impact of nano metal-oxides on human and environmental health is predicted to be increasing. Among metal oxides, nano ZnO (nZnO) is retained one of the most dangerous. Recently nZnO has invaded the market for its UV protective and antibacterial properties, that make it suitable for a wide range of application for functional coating formulations to protect wood, plastics, textiles from UV and microbial degradation. Previous data already showed that nZnO has a powerful embryotoxic potential on *X. laevis* and that it was able to mainly affect gut development. It was clearly demonstrated that nZnO produced severe lesions at the intestinal mucosa and potentially cross the gut barrier reaching the underlying tissues. In this work we used *Xenopus laevis* embryos to characterize the embryotoxic and teratogenic potential of nZnO according to the modulation of NP size and surface charge, as well as to the irradiation conditions. To optimize the stability of the NP suspensions and to achieve useful NP-surface functionalization, we worked in strict connection with a private nanotech company with both R&D and commercial activities. The purpose was to provide mechanistic data on nZnO ecotoxicology and to suggest criteria to design safer Zinc oxide NPs.

We demonstrated that nZnO-induced embryotoxicity was mediated by NPs' own reactivity rather than ion dissolution and that it is strongly associated with the modality of the biological interactions at the nano-level, which at last depend upon the physical and chemical NP surface properties. These properties are also at the base of the induced oxidative potential by nZnO, which is also very efficiently modulated by light irradiation. Finally NP dimension, and especially surface charge, played a crucial role in determining the embryotoxic potential and the intestinal translocation and lesions of nZnO.

The present results showed how a comprehensive knowledge of the nZnO physical and chemical properties, affecting the interactions at the bio-interface, may contribute to make nanotoxicology a predictive science and may help chemists and material scientists in the design of safer NPs.

EP04-5

Greener nanomanufacturing: toward low-waste and high-yield synthesis of monodispersed metal oxide nanoparticles

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Metal oxide nanoparticles have attracted much attention due to their wide variety of applications, such as catalysts, energy storage materials, solar cells, light emitting diodes, sensors, transparent electrodes. For practical applications, syntheses should be greener, with low waste and high yields, in order to avoid future environmental health and safety risks and to prevent material resource depletion. If industry plans to use a broad array of metal oxide nanoparticles and employ hundreds of different chemicals to synthesize, we may have to develop a large number of new syntheses and attempt more than hundreds of different toxicological assessments because the specific policies for each environmental impact will be considered including combination of chemicals. To avoid such complicated risks, we require a few generalized synthesis methods of nanoparticles having both technical and environmental performance in order to simplify production and toxicological assessments.

We propose a synthesis route through an pseudo-first-order catalytic esterification using only oleyl alcohol and oleic acid, derived from non-toxic natural oils, as solvents, reagents, and surfactants during a one-step synthesis of metal oxide nanoparticles. This greener approach simplifies the assessment of pollution and toxicity.

We have synthesized metal oxide nanoparticles at 230 °C or less by using an pseudo-first-order catalytic esterification. This approach offers much lower temperature and shorter reaction time than the other methods to produce monodispersed metal oxide nanoparticles with high yield. The produced byproducts are water and oleyl oleate. The oleyl oleate could be recycled to oleyl alcohol and oleic acid again by using hydrolysis.

All of metal oxide nanoparticles are monodispersed and show no aggregation. The average diameters of monodispersed In₂O₃ and ITO nanoparticles are 7.2 nm and 6.3 nm in diameter (with 89 % and 87 % dispersities within ± 1 nm differences from the average diameter), respectively. The yield of nanoparticles at 230 °C is greater than 90 %.

The pseudo-first-order esterification offers a number of advantages for metal oxide nanoparticle synthesis because it is rapid, produces high yields, permits precise doping, and minimizes waste. We will demonstrate syntheses of several different metal oxide nanoparticles.

EP04-6

Cadmium-free quantum dot nanocrystals for lighting and displays

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Quantum dot (QD) semiconductor nanocrystals have been considered in a broad range of applications, from biological tagging to LEDs, lasers, displays, lighting and solar cells. In their photoluminescent mode of operation, QDs are currently in lighting products, and have the promise to be in liquid crystal display products in the near future. In electroluminescent mode, quantum dot light emitting devices (QLEDs) are an emerging class of thin-film hybrid organic-inorganic structures that can potentially achieve best-in-class performance amongst large-area emissive light sources [1]. Market research projects world-wide QD production to increase from ~1kg today to ~1000 kg by 2015. Should solar cell applications of QDs come to fruition, QD production could further increase to several hundred metric tons.

Historically, QDs are made out of a semiconductor core comprising Cadmium Selenide (CdSe). This is due to 1) the early discovery in the 90's of facile synthetic methods for the lab scale production of QDs of this material, 2) the visible wavelength compatibility of this quantum confined system, 3) the general ease of II-VI semiconductor synthesis and 4) the lack of an equally facile and visible-emitting alternative semiconductor. The CdSe system has been ideal for scientific study, and has even led to early product launches most notably in lighting where the environmental net benefit is easily demonstrated. However, due to the combination of known Cadmium toxicity, world-wide regulations, and consumer sentiment, it is desirable to make available an alternative for Cadmium containing QD materials which are Cadmium-free. This green-chemistry guided research and development effort is an ideal example of how nanomaterials afford the opportunity for materials design not just for performance, but simultaneously to minimize the potential for environmental impact. However, when assessing environmental impact, it is crucial to utilize a cradle-to-cradle view of the material's lifecycle.

EP05 - Non-target analysis and identification of toxicologically significant emerging pollutants

EP05-1

Prioritising non-target identification in wastewater effluent: from picking peaks to programs!

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Non-target analysis of wastewater effluent poses many challenges, including a multitude of peaks and potential interferences of matrix constituents with non-target analysis. However, these samples also offer much valuable information about emerging contaminants in the environment, especially those resulting from urban sources. Here, we detail the results of non-target analysis and prioritisation in several wastewater treatment plant effluents from around Switzerland. The program enviMass was used to perform both target and non-target identification of all samples. The list of non-target masses from all samples were then compared and prioritised according to intensity (and thus, approximately concentration in the environment). This way, thousands of non-target peaks could be reduced to a much smaller list of interesting masses for data-dependant MS/MS analysis to provide fragmentation information for identification of the non-targets. Then, using "the more information the merrier" approach, we gathered as much information as possible from the analysis to hone in on the most likely molecular formulas and thus corresponding candidates. Programs used here included MOLGEN-MSMS (formula calculation), MetFrag and MetFusion (database query and fragmentation prediction) and Mass Frontier (rule-based fragmentation prediction), as well as several different calculations for partitioning and retention properties of compounds. Here, we show how the enviMass workflow allowed a quick and effective selection of peaks of interest and comparison of samples, while the combination of features from many programs improved the chances of non-target identification. While the confirmation of tentatively-identified non-targets is very dependent on the analytical information, the number of possible candidates and the availability, we show how the combination of a few key programs can maximise the use of analytical information and streamline these efforts greatly.

EP05-2

Screening and toxicological evaluation of organic micro-pollutants in the Rhine and Meuse river basins

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The rivers Rhine and Meuse serve as drinking water source for millions of people in Europe. In these waters, rapid improvements in chemical and bioanalytical techniques have led to the discovery of all kinds of emerging contaminants at very low concentrations. Some studies also reported the presence of traces of emerging contaminants in drinking water samples. Dutch drinking water companies therefore intensively investigate their water sources for the presence of emerging contaminants and their fate during treatment processes.

Because thousands of compounds are present in the aquatic environment, it is impossible to detect their presence via target analysis in standard monitoring programs. Non-

target screening is therefore used as an important additional tool to obtain information on the types of organic contaminants that are present in the aquatic compartment. It functions not only as an intake monitoring tool, but also as a safety net for new or unknown compounds which are not included in the target analyses. In this study, sensitive GC-MS non-target screening methods were developed (up to LOD 1 ng/L) and applied to different locations in the Dutch part of the Rhine and Meuse river basins in 2010 and 2011.

A first goal of the study was to examine which known and unknown compounds are found in the Rhine and Meuse. By mapping the organic pollution in the river basins, and following the trends of pollution over the year, an integrated picture on the presence of organic pollutants in place and time was obtained. Up to 400 different compounds were found in the water samples. Screening results for the Rhine and Meuse indicated that although there are differences in the occurrence of compounds between the sources, some compounds appear to be widespread (e.g. Surfynol 104 and 5-methyl-1H-benzotriazole). Industrial compounds and flame retardants comprise the largest groups of compounds.

A second goal of the study was to investigate whether there are compounds present that are potentially of concern for the drinking water production. To this aim an integrative ranking system was developed in which the identified compounds were sorted according to aspects as their human toxicological risk, frequency of detection, persistence in drinking water treatment and associated public concern. A top ten of contaminating compounds that are most relevant for drinking water production from the rivers Rhine and Meuse was compiled. This list will be nominated for political action.

EP05-3

Integrated characterization of a mutagenic waste water treatment plant effluent combining advanced screening techniques and biological assessment

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Increasing numbers of emerging contaminants have been detected in surface water over the last decade. Waste water treatment plant (WWTP) discharges were identified as main sources of these chemicals and related effects in the environment [1, 2]. For this reason, effluents of WWTPs were assessed to gain knowledge about discharged chemicals, their concentrations and their effects on the environment. Linking the discharged chemicals to effects or identifying the substances revealing measured effects is the main challenge for the assessment of WWTP effluents.

To meet this challenge in the present study an integrated biological and chemical approach is applied to identify genotoxic and mutagenic compounds in an effluent of a WWTP treating industrial and municipal wastewater as well as contaminated groundwater. The approach combines extensive target and suspect screening with biological effect assessment in vitro by Ames fluctuation assay on the basis of a series of grab samples taken over several weeks and in vivo by fish caging.

For chemical characterization of WWTP effluent, a target screening on 300 environmental relevant chemicals and a suspect screening on 1800 chemicals applied or produced at the industrial site was performed. Six site specific suspects were identified. Concentration trends of identified targets and suspects have been recorded, focusing particularly on indicator compounds for industrial and domestic waste water, as well as for groundwater co-treated in this WWTP. In the biological assessment, nine of eleven methanol extracts and all BR extracts exhibited mutagenicity in the Ames fluctuation assay. Based on this data, relationships between target and suspect substances and mutagenicity of samples will be presented.

[1] Fent K, Weston AA, Caminada D. 2006. Ecotoxicology of human pharmaceuticals. *Aquat Toxicol* 76:122-159.

[2] Claxton LD, Houk VS, Hughes TJ. 1998. Genotoxicity of industrial wastes and effluents. *Mutat Res* 410:237-243.

EP05-4

Identification of biotransformation products (BTPs) formed in freshwater crustaceans

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Freshwater crustaceans, *Daphnia magna* and *Gammarus pulex* are widely distributed in watersheds which are contaminated by anthropogenic compounds. After uptake of organic contaminants the organisms possibly form biotransformation products (BTPs) by means of enzymes involved in detoxification. Little is known on BTPs in crustaceans and their relevance to explain chemical fate and toxicity. In the present study, *D. magna* and *G. pulex* were exposed to selected organic contaminants and then their BTPs were identified through the suspected/non target screening methodology by using high resolution LC-tandem mass spectrometry. The structure elucidation of BTP was performed through MS/MS spectra interpretation with a fragment prediction tool. In addition, the BTP prediction tools currently available were evaluated in terms of the feasibility of application to crustaceans. As the results, various reaction mechanisms such as N-dealkylation, O-dealkylation, N-oxidation, hydroxylation, epoxidation and glycine conjugation lead to the BTPs for irgarol, terbutryn, tramadol, or venlafaxine. Irgarol and terbutryn which both have triazine moiety showed similar biotransformation pathways. No BTPs were identified for valsartan and clarithromycin so far. This may be caused in general by the lower bioaccumulation of the relatively polar and ionized compounds. More BTPs were identified for *G. pulex* compared to *D. magna*. A number of dealkylation and oxidation products were successfully predicted by the prediction tools. However, the manual prediction based on biochemical knowledge was most successful.

EP05-5

Input and fate of contaminants in surface waters observed by suspect and nontarget screening with LC-Q-TOF-MS

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The increasing availability and sensitivity of LC-MS systems has widely opened the analytical window for polar analytes, the so-called new emerging contaminants. These are increasing numbers of compounds from pharmaceuticals and personal care products, herbicides, fungicides and industrial chemicals. LC with high resolution mass spectrometry enables to perform screening approaches to detect a considerable number of known and unknown analytes in complex samples.

In this work we will show the application of target and nontarget screening with LC-electrospray ionization-quadrupole-time-of-flight-mass spectrometry (LC-ESI-Q-TOF-MS) combined with computerbased data evaluation based on statistical analysis and on tools of computational mass spectrometry to elucidate the input and fate of contaminants in surface water. The software tool MetFrag has been used to retrieve chemical structures of PubChem and to match the measured with in silico fragmentation patterns. A variety of compounds from pesticides, PPCPs and their metabolites could be found. The number of compounds showed an increasing trend from the source of the creek Ammer to the mouth which could be attributed to mainly to the input of wastewater treatment plant effluents. In the source region the herbicide metabolite desethyl atrazine indicates a historical background of atrazine pollution. Further intermittent findings of amidotriazole acid in the source region could be traced back to the influence of storm water overflow. Samples taken downstream of a wastewater treatment plant showed more than 10 000 mass peaks retrieved from the chromatogram by a deconvolution software. Typical wastewater indicators have been found like the X-ray contrast media amidotriazole acid, iomeprol and iopromide, or further pharmaceuticals like carbamazepine and diclofenac, but also the artificial sweeteners acesulfame and sucralose. Further candidates were retrieved from the huge mass list by PCA, e.g. to select relevant candidates for wastewater input. Several compounds could be identified by the application of PubChem search and mass fragmentation match using MetFrag. However, there is still a large number of candidates to be identified due to inconsistent data base and fragment match and due to a high number of possible isomers. In conclusion, there remains a more urgent call for the availability of mass spectral libraries with sufficient numbers of entries.

EP05-6

Can bioanalytical tools help us ensure that our water is safe?

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Chemical monitoring provides a quantitative assessment of single organic contaminants in a water sample but cannot account for the presence of non-target compounds such as unidentified transformation products and interactions between chemicals. Bioanalytical monitoring is complementary to chemical analysis and provides information on all bioactive micropollutants in a sample according to potency, i.e., chemicals of higher toxicity will be weighted higher than less toxic chemicals. Cell-based bioassays provide measures of the cumulative effects of chemicals that exhibit the same mode of toxic action, for which the selected bioassays are indicative, and they can give a measure of the cytotoxicity of all chemicals acting together in a water sample. Improved detection of the presence of chemicals in water enhances risk assessment and informs water management options, among them water recycling from impaired sources such as sewage, or stormwater harvesting and reuse. In this presentation the design of a modular battery of bioassays based on toxicological principles will be presented. This bioanalytical test battery was used for monitoring organic micropollutants across an indirect potable reuse scheme testing sites encompassing the complete water cycle from sewage to drinking water to assess the efficacy of different treatment barriers, including source control, wastewater treatment plant, microfiltration, reverse osmosis, advanced oxidation, natural environment in a reservoir and drinking water treatment plant. The results of the various studies presented here indicate that bioanalytical tools provide valuable additional information to chemical analysis and should be implemented in the future as a monitoring tool.

EP06 - Perfluorinated compounds: From emission sources to the place of impact

EP06-1

Estimating emission source strength of four poly- and perfluorinated alkyl substances (PFASs) in Zurich, Switzerland, using a measurement-and-modelling-combined technique

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Recent studies show that urban areas can be important sources of many semi-volatile organic chemicals (SVOCs), including poly- and perfluorinated alkyl substances (PFASs, e.g. fluorotelomer alcohols (FTOHs) and perfluorooctane sulfonamides (FOSAs)). These PFASs are released in urban areas during use and disposal of polymeric materials, chemicals or consumer products that contain PFAS residuals and directly from manufacturing sites. However, residuals vary considerably among consumer products and it is therefore difficult to estimate diffusive emissions based only on surveys of a limited number of consumer products. In this work, we address this problem by using a multimedia mass balance model to interpret measurements of four PFASs (8:2 FTOH, 10:2 FTOH, Me-FOSA and Et-FOSA) made in the city of Zurich (Switzerland), during a sampling campaign performed in August 2010. The model has been designed to quantitatively capture the day-night cycling of SVOCs in air considering the atmospheric boundary layer dynamics. The estimated yearly average emission source strength of the four PFASs are in the range of 0.4 to 22.5 kg/year and follow the sequence: 8:2 FTOH > 10:2 FTOH > Me-FOSA > Et-FOSA. To obtain more insight into the emission pathways of the four PFASs, the emission strengths estimated from our study were compared to literature data. In general, our estimated emission source strengths of FTOHs are in good agreement with other estimates, which are all based on emission factors along the life cycle of FTOHs. Moreover, our estimates confirm that there is still low but ongoing volatilization of MeFOSA and EtFOSA from consumer products manufactured prior to 2002, when the major producer stopped producing these substances. It may still take years until they disappear from consumer products and the environment. Note, there is no PFAS-related industry in Zurich, therefore, our study shows that diffusive emissions during the use and disposal phase alone are noticeable sources of these PFASs to the environment, in addition to the direct emissions from manufacturing sites.

EP06-2

Percolation characteristics of perfluorinated compounds (PFC) in soil and carry over in plants as shown by long-term lysimeter studies

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Perfluorinated compounds (PFC) can be taken up by crop plants from the soil of contaminated agricultural lands. Soil contamination may take place through irrigation with PFC contaminated water or by fertilization with sewage sludge containing PFC (1,2).

Precipitation, e.g. rainfall may result in only a proportion of the PFC reaching the plants since highly water soluble PFC may be washed out of the soil. Discharge from a landfill containing PFC may also lead to contamination of the food chain via percolation water. A lysimeter study is therefore particularly well suited to observe the carry over in plants and also any possible displacement of the substances from the soil to percolating water. In addition, a long-term study provides information on the time-dependant uptake and leaching of PFC.

Lysimeter studies were carried out on monolithic soil columns (with a volume of 1.5m³). The lysimeter soil received a single treatment with an aqueous solution of a technical mixture of PFOA and PFOS at a concentration of 25mg/kg soil. Over a period of 5 years the harvests of wheat, rye and rape (canola) were tested for the presence of PFC. Grain and straw were measured separately. Once per month the percolation water was also tested for PFC concentration. In addition to PFOA and PFOS, PFBS, PFPeA, PFHxA and PFHpA, as impurities in technical PFOA or PFOS, were all detected in both the plant material and in the percolation water. The concentrations of PFBS (an impurity of PFOS) were as high in plant material as those of PFOA and PFOS in the first year of testing (2007). This indicates that short-chain PFC are taken up by plants much more quickly than those of longer chain lengths. In addition, PFBS, PFPeA, PFHxA, PFHpA and PFOA enter the soil more rapidly than PFOS, the latter only becoming measurable in the percolation water after 4 years. Preliminary results from the long-term studies will be presented.

(1) Stahl T, Heyn J, Thiele H, Hüther J, Failing K, Georgii S, Brunn H (2009) Carry Over of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) from Soil to Plants. Arch Environ Contam Toxicol 57(2):289-298

(2) Lechner M, Knapp H (2011) Carryover of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) from Soil to Plant and Distribution to the Different Plant Compartments Studied in Cultures of Carrots (*Daucus carota* ssp. *Sativus*), Potatoes (*Solanum tuberosum*), and Cucumbers (*Cucumis Sativus*). J Agric Food Chem 59(20):11011-11018

EP06-3

Uptake of Perfluoroalkyl acids by hydroponically and field grown Lettuce (*Lactuca sativa*) and Radish (*Raphanus sativus*)

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Perfluorinated alkyl acids (PFAAs) are bioaccumulative persistent, organic pollutants (POPs), which can be detected ubiquitously in the environment. PFAAs pose a risk to human health due to accumulation in the food chain. The occurrence of PFAAs in animals, such as fish, birds and mammals including humans is fairly well documented, but little can be found in the literature about crops or plants in general. Also, most studies focus just on the two main compounds perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA).

Humans are possibly exposed to PFAAs through consumption of vegetables and other plant-related food items. The objective of this study is to understand the accumulation process of PFAAs in crops.

In a greenhouse experiment lettuce (*Lactuca sativa*, var. *attraction*) was grown hydroponically with a spiked nutrient solution to avoid sorption to soil and to make sure the offered PFAAs are completely bioavailable. The lettuces were exposed to a set of 10 perfluorinated carboxylic acids (PFCAs) and 3 perfluorinated sulphonates (PFSAs) in four different concentrations to assess the difference in behavior between PFAAs and concentration dependencies.

In a field experiment lettuce and radish (*Raphanus sativus*) were grown in lysimeters in 4 different concentrations of spiked soil to have a comparison to the greenhouse experiment and a comparison of a leafy vegetable to a root/bulb vegetable.

The results of the concentrations in the different parts of the plants show a different pattern than in the greenhouse experiment with higher concentrations in the foliage part for most of the compounds.

EP06-4

Perfluoroalkyl substances in raw and processed vegetables and fruits collected in four European countries; PERFOOD

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The human diet is considered a general source contributing to the overall PFAS burden of the human population. Possible exposure pathways include beverages, food in general and migration from food packing or cookware 7,8. In the EU project PERFOOD, standardized selection of food items, sampling procedures and analytical methods as well as evaluation strategies were applied, enabling a unique assessment of the occurrence of PFASs in European food as well as the identification of major sources of PFAS exposure via food. During the sampling campaign more than 800 raw food items were purchased, homogenized and after pooling analysed in selected laboratories. This presentation will cover the analytical results for vegetables and fruits acquired in Norway, Belgium, Czech Republic and Italy in perspective to other food items consumed regularly.

In general the PFAS levels found were very low, and mostly short chained PFASs up to C8-chains were detected. In general, the most PFASs were detected in samples from Belgium and Norway, followed by samples from Italy and Czech Republic. Mainly PFCAs were detected and occasionally some PFSs, in few samples.

In general, vegetables and fruit seem not to be a main contributor to the human exposure of PFASs via food if not harvested close to point sources. However, data from Belgium show that plants consumed as vegetables and fruit in human diet are able to take up a number of PFASs in the edible parts when exposed to them. Results for the processed vegetables and fruit items will be presented in the presentation.

From the investigated vegetables, spinach, lettuce, asparagus, fennel and potatoes, showed highest content of PFAS and will be followed up in the second sampling campaign. Courgettes, cucumbers, aubergines, and mushrooms showed lowest levels of PFAS. After grouping the single vegetable species we found the following order of decreasing PFAS content: Leafy vegetables > potatoes > pulses & legumes > stem vegetables.

EP06-5

Perfluoroalkyl acids in blood serum from first time mothers from Uppsala, Sweden: temporal trends 1996-2010 and serial samples during pregnancy and nursing

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Concentrations of 13 perfluoroalkyl acids (PFAAs) were determined between 1996 and 2010 in pooled blood serum samples from nursing primiparous women living in Uppsala County, Sweden. The aim was to investigate possible effects on human exposure following risk management measures to reduce production, emissions and use of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). PFOS levels declined on average 9% per year from over 20 ng/g serum in 1996 to below 10 ng/g

serum in 2010. PFOA levels declined with 3% per year, from around 3 ng/g serum to about 2 ng/g serum. Thus, certain sources of human exposure to these two compounds have been eliminated. On the other hand, levels of perfluorinated sulfonic acids with 4 (PFBS) and 6 (PFHxS) carbons increased on average 14 and 8.3% per year, respectively. To our best knowledge, this is the first report of statistically significant upward trends for PFBS and PFHxS in human serum after the year 2000. In 2010 the serum levels of PFHxS and PFOS were comparable. Levels of perfluoroalkyl carboxylic acids with 9 (PFNA) and 10 (PFDA) carbons increased 4.6 and 3.8% per year, respectively. Consequently, exposure to PFBS, PFHxS, PFNA and PFDA and/or to their precursor compounds has increased after the phase-out of the C8-chemistry by the 3M Company in 2002. Moreover, serial maternal serum samples during pregnancy and nursing as well as corresponding cord blood were analysed to evaluate if PFAA levels during the nursing period are representative for the fetal development period. PFOS, PFOA and PFNA levels decreased during pregnancy. PFNA declined twice as much as PFOA, suggesting compound specific differences in the blood dynamics. Strong correlations were found between PFAA levels during pregnancy, the nursing period and the cord blood. Thus, maternal PFAA levels determined after delivery can be used as a good estimate of fetal exposure. Mean serum/whole blood concentration ratios of PFOA, PFNA and PFOS were also determined and found to be close to 2, reflecting a volume displacement by red blood cells in whole blood. However, large inter-individual differences were observed with some individuals displaying a ratio close to 1, suggesting a distribution of PFAAs also to the blood cellular fraction.

EP06-6

Does exposure to 8:2 FTOH affect lung function?

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Early life exposures to perfluorinated chemicals (PFCs) have been shown to alter lung and immune function in animal models. We exposed pregnant BALBc dams (GD16) to 100mg of 8:2 FTOH that was coated onto a cardboard enrichment huts which were replaced weekly. Dams continued to be exposed to FTOH in this way and pups were born in the same environment. Upon weaning, 4 female pups were placed into each cage where they continued to be exposed to FTOH in the same way. Control dams and pups received untreated cardboard enrichment huts. One half of the females of each litter received ovalbumin (OVA) IP at days -21 and -4 and intranasal OVA day -4, -3 and -2. Airway resistance (Raw) was measured by flexiVent at 10 weeks of age. Methacholine (MCh) was nebulized and administered in incremental concentrations from 3-50 mg/ml. PC200, the concentration of MCh required to increase the airway resistance 3X from baseline airway resistance was calculated. The PC200 for FTOH exposed animals was significantly less compared to controls. This suggests that early life exposures to 8:2 FTOH may be playing a role in increasing airway hyperresponsiveness in the human population.

EP07 - Plastics: an emerging risk to the marine environment

EP07A-1

Microplastics in the marine environment: synthesis and next steps

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Marine debris is a global problem along coastlines as well as urban, coastal, and offshore waters. Plastic marine debris has been found in surface waters since the 1970s, and an increasing number of observations report plastic debris in the most remote areas of the global oceans. This global long-range transport is possible due to the shared physical properties of many plastic polymers which make them resistant to degradation in the environment. Weathering can slowly break large plastic products into pieces, called microplastics, but full mineralization takes much longer and microplastic particles are expected to persist in the environment for decades. Among many potential risks to the marine environment, microplastics can physically block gastrointestinal tracts of organisms that accidentally ingest these particles, and can serve as vectors of chemicals into marine organisms and environments. This presentation will synthesise known environmental concentrations of microplastics using both historical reports and in-depth, original data from the Chesapeake Bay, USA; present the current state of the science regarding the impacts of microplastics; and discuss future steps for this emerging field of research.

EP07A-2

UK marine litter monitoring

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The distribution of marine litter on the UK seafloor, including different types and quantities, were mapped by collecting additional data on the back of existing research cruises. Several surveys using specific types of trawls gathered detailed information over a total time period of 19 years (1992 until 2011). The fieldwork experiences allowed us to develop a standard sampling procedure with easy to use datasheets, which facilitates further analysis and future harmonisation across surveys worldwide. The results suggest widespread distribution of marine litter on the seabed of the North Sea, dominated by plastics. The data shows detailed distribution and accumulation patterns in North European waters. There is a considerable variation in geographical abundance between stations, ranging from 0 to 3224 items of debris per km². Plastic (mainly bags and bottles, 30%) accounted for a very high percentage, more than 70%. Remarkably, the available trend data indicates that quantities of macro marine litter remained relatively stable over the past two decades. In order to investigate this further, the benthic surveys were reinforced with a one off UK case study on marine litter in the water column (2011). High quantities of microplastics were found near major river outlets (>100000 particles/km²). The results will be used to inform policy makers when designing programmes of measures. This analysis is a first valuable contribution to assess marine litter in North European waters and may eventually be used to determine Good Environmental Status (GES) as defined in the EU Marine Strategy Framework Directive, Descriptor 10.

EP07A-3

Microplastics in the North Sea region: what kind of quantities and associated ecological effects are we looking at?

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The information available on the composition and distribution of microplastics (<5 mm) in the North Sea region is rather scarce because the majority of surveys to date have focused on macro-sized plastic litter. Microplastic effects studies are still in their infancy. While society broadly agrees that plastic simply doesn't belong in the marine environment, regulators are understandably calling for answers to the question, what kind of damage are microplastics doing? Incipient studies dedicated to marine microplastics effects need more time to produce a robust body of results. Therefore in this paper, we combine the current scientific knowledge about the amounts and distribution of microplastics in the North Sea region sediments, water and biota with particle toxicity knowledge of these materials from other fields of study in order to estimate the risk of microplastics in the North Sea to the individual organisms and humans exposed to them. By combining data from diverse fields of study (oceanography, ecotoxicology, drug delivery science, marine biology, etc.) we conclude that field and laboratory evidence for internal exposure in biota gives us an early warning that microplastics are likely to be regarded as bioaccumulative materials and prime biofilm substrates. Considering nano and microparticle toxicity and drug delivery data, we deduce that a variety of effects can be expected depending on the size category of the microplastics in question, since the propensity of particles to be sorbed or eliminated via various routes by various tissues is inherently size-dependent. Inflammatory responses (immune system), cellular damage and other possible physiological, ecological or behavioural responses can be expected after the introduction of foreign materials into biological tissues via the gastrointestinal or respiratory systems etc. The bouquet of evidence presented in this paper supports the position that synthetic plastic polymers are fundamentally incompatible materials for input into biological cycles.

EP07A-4

Plastic loads on coastal shorelines: where do the micro-plastics come from?

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Plastics have been entering the world oceans for over half a century. Beaches and shorelines are commonly sinks for such debris. However, despite growing concerns over debris impacts and increasing actions to remedy these problems, an understanding of the role micro-plastics play in this and the potential impacts these micro-plastics are having, is still in its infancy. With this in mind coastal and harbour beaches with different known macro-plastic loads were sampled within Central Queensland, Australia to determine the proportion of debris in different size categories.

Debris was collected using strandline transects and randomised quadrats (0.5m²). All surface debris was collected down to a depth of 5cm. Samples were then sieved into different size classes and further categorised (eg. Pellet, fragment).

Results indicate that areas with high macro-plastic loads also have high micro-plastic loads. Conversely, low impact sites had proportionately less micro-plastics than the corresponding macro-plastic loads. The nature of the shoreline (eg. Degree of wind and wave energy and current patterns) appear to influence the composition of plastics on these shorelines with high energy environments contributing to the localised breakdown of debris rather than these micro-plastics originating from outside the local area. Further data trends will be presented and the potential impacts to biota and implications for management discussed.

EP07A-5

Sorption and desorption of persistent organic pollutants from microplastics in the marine environment

Microplastics are small fragments of marine debris. Such fragments now appear to be widespread in the marine environment and have been reported at the sea surface, on shorelines and on the sea bed. Microplastics have been defined as particles less than 5 mm in diameter, but fragments much smaller (< 20 microns) than this are widely reported including pieces of nylon, polystyrene, polyethylene and PVC. It has been suggested that microplastics present potential mechanisms for the transport of persistent organic pollutants (POPs) and the release of chemical additives from plastics, to organisms. Particles of unplasticised PVC (uPVC) and ultra high molecular weight polyethylene (UHMW PE), in the size range 200 to 250 µm diameter, were investigated for their potential to sorb and desorb 4,4'-DDT and phenanthrene in seawater and using sodium taurocholate in order to simulate conditions in the gut of marine organisms. Sorption capacity and binding strength was found to be pollutant and polymer specific. Equilibrium distribution coefficients (K_d) for the sorption of phenanthrene onto PE were higher than for PVC while the K_d values for the sorption of DDT were higher for PVC than PE. The desorption of phenanthrene and DDT from plastic was faster in sodium taurocholate than in seawater, but is slower than desorption of organic contaminants from natural sediments. The results suggest that DDT and phenanthrene will desorb from plastics faster as a consequence of digestion by marine organisms than in seawater alone.

EP07A-6

Simultaneous analysis of microplastics and associated plastic additives in sediments

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Microplastics of a size of 5 mm and less in length accumulate in sedimentary marine habitats. They contain organic additives, which could have possibly toxic or endocrine effects when ingested by marine organisms. A universally applicable method was developed to analyse microplastics and associated organic plastic additives (OPAs) in sediments simultaneously.

Six samples of dry sediments with a mass of 1 kg were spiked each with 10 pieces of polypropylene (PP), polyethylene (PE), polycarbonate (PC), polyvinylchloride (PVC), polyurethane (PU), polyamide (PA), polyethylene terephthalate (PET) and acrylonitrile butadiene styrene (ABS). The sizes of the synthetic polymer pieces were about 1 mm in length. Microplastics were sorted out using an air-induced-overflow method and a sodium chloride (NaCl) solution. After this procedure, weights between 50 and 150g were left. Subsequently the polymers were separated from the sediments by flotation in a glass volumetric flask using a sodium iodide solution (NaI) (60%, w/w) which enabled the separation of polymers with a density < 1.804 g/cm³ at 20°C. The flask was closed with a glass plug and shaken for 30 s. After 15 min the surface of the NaI solution was carefully decanted into a glass beaker while decantation of any sediment was strictly avoided. This procedure was repeated five times. The solution was filtered using a vacuum filtration unit equipped with a 0.45 µm cellulose acetate-nitrate filter (Mili, China). Then the filter was washed with H₂O₂ (30% v/v) and the filter residue was stored in the H₂O₂ for one week to remove the biogenic organic material. The solution was filtered again using a glass fibre filter (Whatman, USA) with a pore size 2.7 µm. Recoveries of the whole procedure were between 70 and 100 %.

Polymer particles were analyzed for OPAs and polymer types by thermal desorption-gaschromatography (TD-GC) and pyrolysis-GC (Pyr-GC) in independent runs. The GC was equipped with a cooled injection system (CIS). OPAs and pyrolysis products were identified running a mass spectrometer (MS) in selected ion mode and full scan mode, respectively. Several organic polymer additives e.g. polycyclic aromatic hydrocarbons were identified. For all polymers typical pyrograms were obtained.

EP07B-1

Leaching of halogen free and brominated flame retardants from plastics under different conditions

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Information about the production, distribution and consumption of flame retardants in electric and electronic (E&E) equipment (plastics) is well described. However, there is a knowledge gap in the amount of flame retardants leaching from plastics of electric and electronic (E&E) equipment to the environment. In the EU funded project ENFIRO, halogen free flame retardants (HFFR) are studied that are viable alternatives to specific commercial brominated flame retardants (BFR). Leaching studies of BFRs from different types of plastics have been described in literature; however, limited information on leaching of HFFRs is available. ENFIRO studies 15 HFFRs of which 6 are metal-based. Metal-based flame retardants are stable in plastic (polymer) products, but can leach, dissociate and enter the environment. Monitoring of the fate of metal-based HFFRs in the environment is difficult as metals can have various sources of emission. Leaching tests of HFFRs from plastics is an alternative method that may contribute to the exposure and risks assessment and understanding of the fate of HFFRs in the environment. The current study shows leaching properties of different HFFRs from polymers in comparison with BFRs. Thereby, the influences of pellets vs. moulded plates and pH on the leaching properties are studied. Two types of leaching protocols were tested. The TLCP protocol, from the US EPA, use worst-case leaching conditions (low pH) to simulate a municipal waste landfill, and studies if waste has toxic characteristics and is hazardous. The second protocol (DIN 38414-S4) determines leaching by water (neutral pH), and has been widely used for regulatory purposes in Europe. We show that no differences in leaching properties between the DIN and TLCP methods for two metal-based PBT pellets were found. However, higher leaching rate coefficients of HFFRs from PBT pellets than PBT moulded plates were found, which is probably a results of the differences in surface:volume ratio and the porosity of the materials. Also the influences of nanoclay on the leaching behaviour of the HFFRs have been studied. Additional experiments were performed with marine and freshwater to study the influence of leaching conditions (salinity, humic acids, pH) to simulate different environmental conditions.

EP07B-2

Use of immunofluorescence technique in cultured fibroblasts from cetaceans as new 'in vitro' tool to investigate effects of microplastic

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Each year in the world more than 240 million tonnes of plastic are used and discarded 'end-of-life' plastics accumulate particularly in marine habitats, where contamination stretches from shorelines to the open-ocean and deep-sea. Degradation into smaller pieces means particles <5 mm, usually defined as microplastic, which are now considered a new priority in marine environment contamination. In fact, ingestion of microplastics, that can be taken up and stored by tissues and cells, provides a potential pathway for the transfer of hydrophobic organic contaminants, monomers, and plastic-additives to organisms with uncertain consequences for their health. Contaminants such as phthalates, bisphenol A (BPA) and polycyclic aromatic hydrocarbons (PAHs) are some of the principal constituents of plastic. The aim of the present study is to propose immunofluorescence technique in cetaceans cultured fibroblasts as a new "in vitro" tool to explore the susceptibility of these marine mammals to different pollutants related to marine microplastic contamination. The cell lines were cultured from biopsies of free-ranging cetaceans and skin tissue from stranded cetaceans (dead within 2-12 h). Using an indirect immunofluorescence assay, we detected endogenous proteins induced by different contaminants. Here we present the method used for qualitative and quantitative evaluation of two isoforms of Cytochrome P450 (CYP1A1 and CYP2B) induced by BPA and PAHs. Cells were treated for 48 h with contaminants in sterile culture plates with wells having individual sterile covers. After fixing and extraction with methanol and acetone at -20°C, we conducted a first reaction with primary antibodies: anti rabbit cytochrome P450 1A1/1A2 and anti 2B4 cytochrome P-450 diluted 1:500 for 1A1/1A2 and 1:100 for 2B4, for 2 h. Cells were then treated with the respective secondary antibodies (goat anti-rabbit) labelled with a fluorochrome, diluted 1:400, in the dark. Fluorochrome was detected using a solution containing 40% CITIFLUOR and 60% PBS, whereas DAPI was used as marker of chromatin for cell count. The reaction was read using a fluorescence microscope (Olympus mod. BX41). Immunofluorescence was quantified with a specially designed Olympus macro, *DetectIntZ*. A major result was the possibility of using this "in vitro" assay to evaluate the susceptibility of different cetacean species to microplastic components.

EP07B-3

Relative bioavailability of PCBs associated with microplastic

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Microplastics are present in shoreline sediments worldwide, are known to accumulate hydrophobic organic compounds like polychlorinated biphenyls (PCBs) from water and many different organisms have been observed to ingest small plastic particles; therefore, plastic debris may pose a potential harm to aquatic ecosystems from the transport and exposure of toxic chemicals. The objective of this work was to measure the bioavailability of microplastic-associated PCBs to benthic invertebrates in comparison to other environmentally-relevant particle types (all <63 µm diameter) in two ways: bioaccumulation in an oligochaete worm *L. variegatus* in 7-d exposure tests of sediments amended with PCB-laden particles, and digestive solubilization of contaminants from particles in a gut fluid mimic of the polychaete *A. marina*. The percent solubilization of PCBs by gut fluid was greatest for wood, followed by biochar, polypropylene (PP), and then coal. PCBs associated with PP were expected to be more bioavailable than the black carbons, coal and biochar, based on the lower solid-water distribution coefficients for PP. The observation of relatively lower digestive bioavailability from PP than biochar may indicate that solubilization was kinetically limited by diffusion from the interior of the plastic. Kinetic release of contaminants depends in part on the surface area to volume ratio of particles, which was very low for PP compared to the black carbons. In contrast to gut fluid solubilization, results of the bioaccumulation tests indicate that PCBs associated with PP are more bioavailable than with biochar. Total PCB concentrations in lipid measured in worms exposed to sediments with loaded PP and biochar were 76% and 97% less than spiked sediment, respectively. In these 7 d exposures, PCBs associated with plastic had more time to repartition with pore water and worm

lipids than the gut fluids in the 4 hour in vitro digestion. At the PCB homolog level, biouptake of less-chlorinated PCBs was greater for exposures with PP, indicating release to sediment pore water followed by dermal contact may be an important route of exposure. Thus, plastic debris in the environment appears unlikely to contribute significantly to digestive uptake of PCBs to organisms, that is perhaps, until weathered plastic particles are small enough so that kinetic release during the time-frame of digestion is not limiting, or if plastic particles are retained in the digestive tract.

EP07B-4

Occurrence of microplastics in mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) collected along the Belgian-French-Dutch coast

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Laboratory experiments have shown that various marine organisms ingest microscopic particulates. Microplastics smaller than 10 µm were even shown to translocate from the gut to the circulatory system of mussels. However, in all these experiments the exposure concentrations (range 1000 - 50,000 mg.kg⁻¹) were much higher than any field concentration (range 50 - 200 mg.kg⁻¹). As such it is difficult to assess the relevance (risks of adverse effects) of these laboratory observations for organisms living in natural marine environments. The aim of this project was to study the presence, and if present, the concentrations of microplastics in two marine species collected in the field: (1) the blue mussel *Mytilus edulis* and (2) the lugworm *Arenicola marina*.

Biota (mussels and lugworms), seawater and sediment from the intertidal zone were collected at 6 beaches along the French-Belgian-Dutch coastline, during September-October 2011. Microplastics were extracted from the sediment using elutriation followed by a sodium iodide (NaI) extraction, in order to separate the lighter plastic particles from the heavier particles. Seawater was filtered and a NaI extraction was performed on the settled particles. Organisms underwent an acid (HNO₃) digestion procedure. Quantification of the extracted particulates showed that - at all sampling locations - all tissue and faeces samples contained these particulates. Tissue concentrations for mussels was around 2 particles.gram⁻¹ of tissue. Lugworms contained only slightly higher concentrations of particulates: 3 particles.gram⁻¹ of tissue. As expected, these tissue concentrations are low compared to the concentrations present in the environment: water contained about 80 particles.L⁻¹ and sediment 40 particles.kg⁻¹ sediment. Although the units are clearly different, these data can be used to illustrate the difference between the environmental and tissue concentrations. Sediment also seemed to have a lower concentration of particulates than seawater. This can be explained by the fact that the sampled upper 5 cm of the intertidal zone is a very dynamic system with high perturbation levels, making it difficult for the suspended particles to settle.

The use of these organisms allowed, for the first time, the assessment of the transfer of microplastics from both the water as well as the sediment matrix to marine life.

EP07B-5

Detection of polybrominated diphenyl ethers (PBDEs) in tissue of seabirds ingesting plastics

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Polybrominated diphenyl ethers (PBDEs) were measured in the abdominal adipose tissue of short-tailed shearwaters, *Puffinus tenuirostris*, that were accidentally caught during experimental fishing in the North Pacific Ocean. Their ingestion of plastics have been frequently observed. All the examined seabirds (i.e., 7 individuals) contained plastics in their stomachs with 0.04 g to 0.41 g per individual. PBDEs were detected from all the seabird samples with concentration range of total PBDEs from 0.5 to 5.4 ng/g-wet tissue. In one individual, BDE#209 was significantly detected. BDE#209 was normally not detected in marine fish and, therefore, exposure of BDE#209 to seabird through food web is unlikely. On the other hand, BDE#209 was detected in marine plastics. The detection of BDE#209 in the seabird may be ascribed to transfer of BDE#209 from ingested plastics to the seabird tissue. In addition to this individual, two individuals showed sporadic higher concentrations of PBDEs in their tissue. They were dominated by BDE#153 and BDE#154 and their congener profiles were different from the other individuals. No significant correlation was observed between tissue PBDE concentrations and the amounts of plastics in their stomachs. The large variabilities in tissue PBDE concentrations may be explained by large variability in PBDE concentrations in plastic fragments. Our measurement of PBDEs in marine plastics showed large fragment-to-fragment variation among the plastics collected from the same sites. These variabilities can be explained by heterogeneity of plastic fragments in terms of contents of additives and residence time in seawater. Heterogenic nature of the ingested fragments may explain the variation of PBDE concentrations among the seabirds and no significant correlation between tissue PBDE concentrations and plastic ingestion. Analyses of PBDEs in ingested plastics and natural prey are necessary. More number of the seabirds with different amounts of ingested plastics, including individuals with no plastic ingestion, should be analyzed to further examine the transfer of PBDEs from ingested plastics to seabird tissue.

EP07B-6

Preliminary results on the potential assumption of microplastics by Mediterranean Fin whale: the use of phthalates as a tracer

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Micro debris floating on the Mediterranean Sea have reached 115,000 particles per km² with a maximum of 892,000 particles. Impacts of microplastics on organisms and the environment are largely unknown. More than 180 species have been documented to absorb plastic debris including planktophagous species. Until now no data are reported on the potential assumption and effects of microplastics on baleen whales. In this paper we explore for the first time the assumption and potential impact of microplastics in the mysticete species Fin whale (*Balaenoptera physalus*), suggesting the use of phthalates as a potential tracer of microplastics assumption by Fin whale through micro litter and plankton ingestion. The Fin whale, the only resident mysticete in the Mediterranean Sea (concentrated during the summer in the Marine Protected Area (MPA) Pelagos Sanctuary), feeds largely on the planktonic euphausiacean species, with each mouthful can trap about 70,000 liters of water (including the surface feeding activities), could potentially undergo to the potential risk of the ingestion and degradation of microplastics. The project is implemented through three main steps: Phase I - collection and count of microplastics in superficial plankton samples in Pelagos Sanctuary; Phase II - ecotoxicological investigation of phthalate content in superficial plankton samples of Pelagos Sanctuary; Phase III - ecotoxicological investigation of phthalate content in stranded Fin whale specimens collected on the Italian coasts. Among the 23 superficial plankton samples, 13 have shown the presence of plastic particles. The highest "microplastic density" (9.67 debris/m³) was found in the sample collected close to the Portofino MPA (Ligurian Sea). High concentration of phthalate MEHP and DEHP have been detected in superficial plankton samples collected in the Pelagos Sanctuary areas, with values approximately four time higher in the samples of the Ligurian Sea than the samples of Sardinian Sea. Regarding chemical harm to Mediterranean Fin whales, related to the potential assumption of plastic derivatives, the preliminary data of this paper underline for the first time the presence in the blubber of four stranded Fin whales relevant concentration of MEHP. This data suggest the use of phthalates as a potential tracer of microplastics assumption by Fin whale by micro litter and plankton ingestion.

EP08 - What is the current state of the science on the fate, exposure and effects of pharmaceuticals in the environment?

EP08A-1

Environmental progestin concentrations disrupt oogenesis in amphibians

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Progestins (synthetic progesterone) are extensively used in human and veterinary medicine in e.g. contraceptives and in other hormonal therapies. Recent research shows that progestins pose a threat to egg laying in wild fish. Information on the susceptibility of frogs to impacts from environmental progestin concentrations is lacking. The present study aimed to 1) characterize progestagenic effects on the full cycle of oogenesis (egg development) in frogs, and 2) determine female amphibians' susceptibility to reproductive impacts from an environmental progestin. Levonorgestrel is a commonly used progestin found in sewage treatment plant effluents at concentrations up to 30 ng/L. Sexually mature female *Xenopus tropicalis* were exposed to levonorgestrel via the surrounding water for 28 days (0, 1.3, 18, 160 or 1240 ng/L). Their ovaries were analyzed histologically with respect to frequencies of immature (in early meiotic prophase I), previtellogenic, vitellogenic, mature, and atretic oocytes. Levonorgestrel exposure caused reduced proportions of oocytes at immature, vitellogenic and mature stages and increased proportions of previtellogenic oocytes in the ovaries, compared with the controls. The lowest tested concentration, 1.3 ng/L, increased the proportions of previtellogenic oocytes and reduced the proportions of vitellogenic oocytes, indicating inhibited vitellogenesis. The present study shows that progestin concentrations found in the aquatic environment impaired oogenesis in adult frogs. Our results indicate that progestagenic effects on oocyte development include interrupted germ cell progression into meiosis and inhibited vitellogenesis. Considering the crucial role of oogenesis in female fertility our results indicate that progestins in the environment may pose a threat to reproduction in wild amphibian populations at contaminated sites.

EP08A-2

Identification of active synthetic steroid compounds in impacted river downstream from pharmaceutical industry

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The environmental occurrence of emerging pollutants able to disrupt endocrine signalling pathways other than those mediated by estrogen receptors (e.g. corticosteroids, androgens or progestagens) is of recent concern. Recent evidences have suggested that effluents from pharmaceutical industry release drugs into rivers and trigger adverse effect on wildlife. By using in vitro bioassays combined to passive sampling (i.e. Polar organic compound integrative sampler, POCIS), we previously reported the occurrence of estrogenic, glucorticoidic, anti-mineralocorticoidic, progestative compounds and pregnane X receptor (PXR) ligands downstream a pharmaceutical industry release where strong reproductive alteration have been reported in fish. In this study, we report the use of effect directed approach to identify the compounds responsible for these

activities. We first assessed mass balance calculation through chemical analyses directed by toxicity profile. Chemical analyses in POCIS crude extract showed the occurrence of high concentrations of dexamethasone, spironolactone, 6-methyl-prednisolone (up to 100 µg/g of sorbent) that well explained GR and anti-MR activities (up to 100%). Conversely, other biological activities (i.e. estrogenic, PXR-like) were poorly explained by the detected chemicals. Then, sample RP-HPLC fractionation was carried out to unravel the different activities. It allowed the isolation of estrogenic and PXR-like activities from GR, PR and anti-MR. It also revealed the occurrence of MR agonists that were masked by the strong anti-MR activity in the crude extract. In addition, HPLC calibration showed a good fitting between retention times of detected chemicals and several active fractions (e.g. 6-methyl-prednisolone in F11, dexamethasone in F12). Nevertheless, some active fractions were left unexplained by calibration standards suggesting that many active chemicals remain to be identified. Chemical analyses in these fractions are under investigations using LC-HRMS system and results will be presented. Our study demonstrates (1) the usefulness of MBA approach using pre-directed chemical analyses based on toxicity profile (2) the strength of the fractionation to unravel complex mixture effect and to finely isolate active chemicals. Overall, our results underscore the need to increase knowledge on the effects of corticosteroids and progestogens on aquatic organisms for better risk assessment.

EP08A-3

Mechanism of action of human pharmaceuticals in fish: the 5α-reductase inhibitor dutasteride as case study

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In recent years, a growing number of human pharmaceuticals have been detected in the aquatic environment, generally at low concentrations (sub-ng/L to low µg/L). These compounds are characterised by highly specific mechanisms of action (MoA), high potency, and prolonged activity in order to minimise dosing requirements and potential toxicity in patients. The functional and evolutionary degree of conservation of the drug target in non-target species, together with a cross-species extrapolation of pharmacological and toxicological information generated in mammals during the drug development phase should be considered as key factors in the environmental risk assessment process. Our research addressed the question of whether or not dutasteride, a human pharmaceutical mainly used to treat benign prostatic hyperplasia, may cause adverse effects in the teleost fathead minnow (*Pimephales promelas*) by inhibiting the activity of both isoforms of 5α-reductase (5αR), the enzyme that converts testosterone into dihydrotestosterone (DHT), despite the general assumption, in fish endocrinology, that DHT is not synthesized in teleosts, or if it is, it has modest or no physiological relevance. To our knowledge, this class of pharmaceuticals (5αR-inhibitors) has never before been tested on any fish, or any other aquatic species. The experimental work presented here was divided into two phases: Phase I, focused on the target, and Phase II focused on the effects of the drug in fish. The results of the Phase I showed that 5αRs are evolutionary conserved in the fathead minnow, both 5αR1 and 5αR2 genes were expressed in the testis, and DHT was detected in fish plasma at concentrations comparable to the human ones. These results strongly suggested that DHT has a physiological role in the fathead minnow, and constituted the rationale for testing the effects of dutasteride in this species. Dutasteride caused significant adverse effects in all the *in vivo* studies performed during the Phase II in order to evaluate its potential toxicity on fish, including early life stage and short term reproduction studies, and all the tested life stages were sensitive to the inhibition of 5αRs activity; however, none of the observed adverse effects occurred at concentrations of exposure lower than 32 µg/L indicating that, at present, the potential presence of dutasteride in the environment (PEC=0.03 ng/L) does not represent a risk to wild fish populations.

EP08A-4

Sub-lethal effects induced by the main cocaine metabolite, the benzoylecgonine, on the freshwater bivalve *Dreissena polymorpha*

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The increase in global consumption of illicit drugs has caused both social and medical problems, but also the onset of a potential new environmental hazard. It has been established that after human consumption, drugs and/or their metabolites end up in surface waters, after being carried through the sewage system, posing a potential risk for aquatic biocoenosis. However, even if many studies have showed the presence of several drugs and metabolites in freshwater in the high ng/L to low µg/L range worldwide, at present any information on their potentially harmful effects on non-target organisms is available. The aim of the present study was to investigate the cyto-genotoxic effects induced by the main metabolite of cocaine, the benzoylecgonine (BE), on a classical biological model as the freshwater bivalve *Dreissena polymorpha*. Our goal was reached through the application of a biomarker battery on zebra mussel hemocytes. The raise of genotoxic effects was investigated by the Single Cell Gel Electrophoresis (SCGE) assay, which evaluated primary DNA lesions, and by the DNA diffusion assay and the micronucleus test (MN test), which investigated fixed genetic damage. The Neutral Red Retention Assay (NRR), by evaluating the lysosome membrane stability, was used to assess benzoylecgonine cytotoxicity. In addition, the activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and the phase II detoxifying enzyme glutathione S-transferase (GST) was measured in the cytosolic fraction extracted from a pool of entire bivalves in order to reveal a possible oxidative status unbalance of treated-specimens. 14 days exposure to two increasing nominal concentrations of benzoylecgonine (0.5 µg/L and 1 µg/L), comparable to those currently measured in surface and sewage water respectively, were performed under semi-static conditions. Our results highlighted that benzoylecgonine exposure induced significant ($p < 0.05$) increases of both primary and fixed DNA damage at both the administered concentrations. In addition, since BE significantly ($p < 0.05$) decreased the stability of lysosome membranes, our data also highlighted its cytotoxicity and the possible implications of oxidative stress for the observed genotoxic effects. Lastly, BE seemed able to induce moderate effects on the activity of antioxidant and detoxifying enzymes, as shown by the notable oxidative status unbalance of treated bivalves.

EP08A-5

Chronic effects of diclofenac on fish and mussels measured using human diagnostic techniques

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The non-steroidal anti-inflammatory drug diclofenac is thought to be one of the most toxic pharmaceuticals found in the environment and has been highlighted by the European Environment Agency as being of particular environmental concern. In the current study we have adapted human diagnostic testing techniques for use with environmental samples. Mussels (*Mytilus* spp.) and rainbow trout (*Oncorhynchus mykiss*) were exposed to 1 µg/L and 1000 µg/L diclofenac under semi-static conditions, with homogenised digestive gland supernatant (15,000g) sampled from the mussels after 7 and 14 day exposure and fish blood plasma sampled after 96h. Rainbow trout exposed to the environmentally relevant concentration of 1 µg/L diclofenac for 96h showed a significant increase in alanine aminotransferase (ALT) levels. ALT is commonly clinically measured as a part of a diagnostic evaluation of hepatocellular injury to determine liver health. Exposure to 1000 µg/L significantly decreased Alkaline phosphatase (ALP) levels, used in humans to indicated liver, bone and intestinal diseases. In the mussels diclofenac exposure showed a significant decrease in digestive gland ALT expression at both 1 and 1000 µg/L after 7 days. Aspartate aminotransferase (AST) also showed a significant decrease in expression after a 7 day 1000 µg/L exposure. All molluscs assayed to date have AST and ALT and both were previously found to be significantly inhibited by metal exposure in the clam *Ruditapes philippinarum*, where it was suggested as a useful biomarker for sublethal stress. Other endpoints measured include glucose, blood urea nitrogen, total bilirubin, and gamma glutamyltransferase. Many of these endpoints have been previously observed in fish and molluscs, but we are proposing their use to evaluate the effects of pharmaceuticals in the environment in a fast, efficient and standardised way using human diagnostic techniques and machines.

EP08A-6

Effects of environmental relevant concentration of pharmaceuticals on the immune system of *Lymnaea stagnalis*

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Municipal effluent is the most important contributor of pharmaceuticals to the environment; these pharmaceuticals do not occur as single contaminants, but as complex mixtures. The immune system could represent a target system of exposure to this class of toxicants, and *Lymnaea stagnalis* is likely to be exposed to these compounds. Thus, the major prevailing therapeutic classes (neurological, anti-hypertensive, antibiotic, hypolipemic drugs) were selected, representing compounds of critical concern for the aquatic environment. Their toxicity was tested as a mixture of environmental relevant concentration of each therapeutic class, as a global mixture of all selected pharmaceuticals and compared to the global toxicity of wastewaters coming from the effluent discharge of Montreal.

L. stagnalis were exposed in semistatic conditions to each mixture in triplicates (3 snails per replicate) during 3 days, and to surface water from the St Lawrence River at upstream and downstream of the effluent discharge point. Hemolymph was collected and immunological parameters measured. Hemocyte count and viability was monitored, as well as phagocytosis activity, ROS and thiol production. Gene expression of genes involved in the immune response (AIF, TLR4, MDM, SOD, Catalase, SeGPx, GSR, NOS and NOS bis) was also measured in real-time quantitative PCR.

Both the environmental concentration of pharmaceuticals mixtures, and the effluent-tainted surface waters modulated the immune response at expression and effect levels. All mixtures decreased hemocyte viability and count. Phagocytosis and ROS production was decreased except with the antibiotic mixture which increased them. All mixture increased thiols production, suggesting an increase of phase II biotransformation by pharmaceuticals. Except for TLR4, gene expression was generally decreased by the mixtures. Environmental relevant concentration of pharmaceutical mixtures modulate the immune response of *L. stagnalis*, the neurological mixture being the most potent, and the antibiotic one having opposite effects compared to the global mixture, but similar effects to the effluent.

The effluent decreased cell viability, but increased cell count. Phagocytosis, ROS and thiols production were increased. Gene expression was slightly increased and particularly TLR4. The effects of the effluent on the immune system of *L. stagnalis* can partly be only explained by the presence of some pharmaceuticals, but also by other factors as the bacterial load.

EP08B-1

Does the environmental risk assessment within the marketing authorization procedure ensure the environmental safety of Human Pharmaceuticals?

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The authorization of medicinal products consists of two phases: a pre-market and a post-market surveillance. For all new marketing applications of human medicinal products the European legislation requires an assessment of potential risks to the environment in the pre-approval phase. The respective European guideline on environmental risk assessment of human medicinal products came into effect in 2006 (EMA/CHMP/SWP/4447/00) and an amending question and answers document in 2010.

The environmental risk assessment according to the EMA-guideline is a tiered process in which Phase I is an action limit approach only considering environmental exposure. If the predicted environmental concentration in surface waters (PEC_{sw}) exceeds the action limit of 0.01 µg/L, an in depth ERA based on studies on environmental fate and effects has to be performed in a Phase II. For estimating a potential environmental risk, the predicted environmental concentration (PEC) is compared to the predicted no effect concentration (PNEC). A PEC/PNEC ratio ≥ 1 indicate an environmental risk.

Since 2006 the Federal Environment Agency (UBA) assessed more than 700 marketing authorization applications. The major therapeutic groups assessed so far are anti-infectives, analgesics, psychotropics, cytostatics and hormones. Based on predicted and measured environmental concentrations a potential risk can be identified e.g. for some hormonal and psychotropic substances.

The presentation will illustrate the assumptions and uncertainties of the environmental risk assessment according to EMA guideline. Furthermore the legal provisions and the environmental needs are compared and discussed. To ensure a high standard for the environmental safety of medicinal products for human use a monograph system on active drug substances should be established. Only a pre-marketing monograph system on fate and effects data of drug substances in conjunction with an effective monitoring e.g. of the occurrence of active substances in the environment within the post-market surveillance will be able to ensure the environmental safety of human and veterinary medicinal products in use.

EP08B-2

Prioritizing cytotoxic drugs present in aquatic systems and their occurrence in the environment

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Cytotoxic drugs are routinely used in chemotherapy to fight cancer; they work by disrupting critical cellular processes, often through a toxic mode of action. The cytostatics are discharged with wastewaters, largely through the excretion of the unmetabolized drug. This study considers the occurrence and behaviour of these chemicals in aquatic systems via prioritization of old and new anti-cancer agents based on their consumption, excretion and environmental fate. Commonly used cytotoxic drugs were listed based on accurate consumption data from a detailed hospital survey of the NW England, urinary excretion rates from clinical studies, their likely fate and persistence in aquatic media as well as their reported presence in environmental wastewater. In addition to this, we report concentrations from WWTP influent, effluent and receiving waters across England. Samples were filtered and extracted using a combination of Strata-X and Florisil SPE cartridges in a LC-MS/MS analytical method. Cyclophosphamide, a commonly used cytotoxic drug, was detected at a maximum concentration of 22.7 ng/L in wastewater effluent, with the limit of detection ranging between 0.03-0.12 ng/L. This shortlist of cytotoxic drugs can be used for water screening programmes and ongoing work will involve the development of a multi-compound analytical method for the shortlisted chemicals based on a current published method for cyclophosphamide. The analytical method will then be applied in the development of a novel passive water sampler for these chemicals, which generally exhibit high aqueous solubilities and low KOW values. To date, only limited field studies have reported cytotoxic drugs in the aquatic environment and more extensive surveys are required to assess the distribution of these chemicals and the risk posed to aquatic biota and humans, especially in those areas where water is abstracted for potable water supply.

EP08B-3

Ecotoxicity from antimicrobials and analgesics during an influenza pandemic

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The global public health community has closely monitored the unfolding of the 2009-10 influenza pandemic to best mitigate its impact on society. However, little attention has been given to the impact that our response to a pandemic might have on the environment. Antiviral, antimicrobial and analgesic drugs prescribed to treat influenza and influenza-associated complications can be excreted into wastewater in a biologically-active form. Here we use a global spatially structured epidemic model to simulate the quantities of drugs used during an influenza pandemic. We couple this model to a wastewater and river flow model, to project drug concentrations in wastewater treatment plants (WWTPs) and receiving rivers in the Thames basin in Southern England. Ecotoxicologic modeling of species sensitivity distributions (SSD) for antibiotics (based on distributions of minimum inhibitory concentrations of antibiotics in pathogens), coupled to an assessment of their mixture toxicity, shows that projected concentrations of antibiotics in WWTPs and receiving rivers would not exceed toxicity thresholds in the case of a mild pandemic, as observed with the current H1N1 pandemic. However, at the peak of a moderate or severe pandemic, the mean antibiotic usage could increase by 13% and 252% as compared to inter-pandemic periods, respectively. Nearly one-third of the microbial community in 70% of the WWTPs in the Thames basin are projected to be growth-inhibited during a severe pandemic. A more severe pandemic might thus result in reduced WWTP efficacy, resulting in the release of partly untreated sewage into receiving rivers, leading to eutrophication, fish kill, and contamination of drinking water abstraction points. SSD modeling of the toxicity of analgesics to aquatic organisms in the Thames catchment shows that pandemic use is not likely to cause acute toxicity, however, a moderate and severe pandemic might lead to analgesic concentrations exceeding chronic toxicity thresholds for ibuprofen.

EP08B-4

Emission of human antibiotics and antineoplastics into the environment: identification of high risk exposure scenarios in Europe

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A screening tool has been developed for the identification of high risk exposure scenarios for humans and ecosystems due to the emission of human antibiotics and antineoplastics in Europe. The tool is based on country-specific consumption data, and its use results in a relative ranking of exposure scenarios. We used a spatially explicit approach. A data search was conducted on country- and substance-specific characteristics. These were combined with spatial data on the distribution of agglomerations and sewage treatment plants in Europe, and nation specific emissions were estimated. Calculations of the environmental fate of the antibiotics and antineoplastics were performed with the use of European spatial characteristics on a 100*100km grid scale, a spreadsheet-based multimedia fate model, and bioconcentration/biotransfer factors. In these fate calculations, special attention was given to the ionizing properties of the substances, especially those which are zwitterionic, i.e. fluoroquinolones and tetracyclines. Behavioral characteristics of four age-based target groups, i.e. infants, children, adults and elderly, were linked with the concentrations in exposure media derived from the fate calculations. Furthermore, we included specific consumption and activity patterns that could potentially cause high risks, e.g. the consumption of locally grown crops. Acute toxicity data from literature and publicly available databases were used for the ranking of the exposure scenarios for both aquatic ecosystems and humans. The study resulted in a set of rankings of aquatic and human exposure scenarios to antibiotics and antineoplastics on a European scale, and the scenarios most likely to cause risks for the aquatic environment or human health were identified. The rankings were incorporated in a set of GIS-maps to visualize spatial variations and can be used to identify worst-case exposure scenarios and to pinpoint those situations that should be given priority in further, more in-depth, risk assessment studies. When data were (partially) lacking, worst-case assumptions were made. Consequently, scenarios for which data are lacking are likely to be assigned a higher rank. The outcome therefore gives an indication of the urgency of the existing gaps of knowledge in the assessment of these exposure scenarios.

EP08B-5

Antibiotic-induced change of bacterial communities associated with the copepod *Nitocra spinipes*

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Environmental pressures, such as physical factors, diet and contaminants may affect interactions between microbial symbionts and their multicellular hosts. Despite obvious relevance, effects of antimicrobial contaminants on host-symbiont relations in non-target aquatic organisms are largely unknown. We show that exposure to antibiotics had negative effects on survival and juvenile development of the copepod *Nitocra spinipes* and caused significant alterations in copepod-associated bacterial communities. Moreover, there were significant positive correlations between indices of copepod development and bacterial diversity, indicating that disruption of the microflora was likely to be a major factor behind retarded juvenile development in the experimental animals. As evidenced by ribotype distribution in the bacterial clone libraries, the exposure to antibiotics caused a shift in dominance from Betaproteobacteria to Cardinium bacteria; the latter have been shown to cause reproductive manipulations in various terrestrial arthropods. Thus, in addition to providing evidence that the antibiotic-induced perturbation of the microbial community associates with reductions in growth-related traits of the host, this study is the first record of a copepod serving as a host for endosymbiotic Cardinium. Taken together, our results suggest that (1) antimicrobial substances and possibly other stressors can affect symbiont-mediated interactions in copepods and other hosts, and (2) similarly to other arthropod species, reproductive biology of copepods may be affected by Cardinium bacteria.

EP08B-6

Ciprofloxacin toxicity in five marine species

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Recent publications and research indicate that input of pharmaceuticals to the environment is set to increase. Pharmaceutical products are particularly likely to cause effects on biological systems because that is what they are designed for. Most research so far has concentrated on freshwater interactions with very little marine data for use in risk assessments. Ciprofloxacin, a fluoroquinolone antibiotic, is found in many wastewater treatment effluents and could therefore conceivably make its way into the coastal marine environment.

This research has investigated the acute toxicity of Ciprofloxacin to five marine species: 7 day growth in the red algae *Ceramium*; 96 hour growth in the brown algae, *Fucus*; 72 hour growth in the green algae *Acetochrysis*, 72 hour growth in the diatom, *Skeletonema* and 24 hour embryo development in the bivalve mollusc, *Crassostrea*. Tests were carried out according to standard OECD or ISO protocols where available, or from peer reviewed research papers. Results showed that for the most part Ciprofloxacin was not likely to be a source of concern in the marine environment with little or no toxicity recorded for most of the species. Most sensitive was *Ceramium*, with an EC50 of 10mg/l, with *Skeletonema* second. The oyster embryo assay showed little sensitivity.

The sensitivity of *Ceramium* and *Skeletonema* may be due to mode of action, or availability in the water column. A log P of 2.3 suggests weak but not exclusively lipophilic behaviour. Physiological differences between the algae, and the mollusc, may explain the difference in toxicity. Using this sort of actual marine data will make the creation of Marine Risk Assessments a more meaningful task.

EP08C-1

Geochronology of pharmaceuticals in coastal marine sediments

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Aquatic systems are subjected to the influence of wastewater discharges, which may contain significant amounts of organic contaminants. In spite of their low concentration in the water column, these chemicals may cause damage to the ecosystem in the long term due to chronic exposure. Among them, pharmaceutically active compounds (PhACs) have been the focus of many researchers within the last years. Most of these studies deal with their identification and distribution in water bodies. The present study shows the distribution and fate of PhACs in the sedimentary column, which offers a new insight that allows reconstructing the history of the contamination of an aquatic system by these compounds. A sediment core was sampled in 2008 in the deepest area of Jamaica Bay, a sewage impacted marine system heavily impacted by NYC wastewater treatment plants (WWTPs). The sediment core was sectioned at 2 cm intervals and dated by measuring ¹³⁷Cs and ⁷Be. 70 different pharmaceuticals were analyzed in these samples, as well as linear alkylbenzene sulfonate (LAS), a widely used anionic surfactant. Anoxic conditions prevailing in the sediments favor preservation of organic contaminants. The profile of LAS concentration remains fairly stable following 1978, so any changes in the concentration of PhACs are interpreted as resulting primarily from changing inputs to the WWTPs surrounding Jamaica Bay. Only 16 of the 70 PhACs that were analyzed could be detected and their concentrations were much lower (< 50 ng/g) than those for LAS (up to 250 µg/g) in the same samples, mainly as a consequence of their lower production and consumption. Their sediment records are consistent with first use dates for pharmaceuticals. Thus, ibuprofen was first sold in 1974, and it has been very popular since then, so its presence could be observed in sediments from that date until today, showing an average concentration of 10 ng/g. Concentration of propranolol increased to 3 ng/g from 1977 to 1985, and then it decreased towards the surface of the core as it was substituted by metoprolol, which has improved its sales recently so its concentration rises from 5 to 35 ng/g over the last decade. Psychiatric drugs, such as carbamazepine or fluoxetine, could be also detected in spite of their relatively high solubility. Overall, the approach shown here allows monitoring the exposure of aquatic systems to PhACs during several decades and any changes that may occur in their use by nearby populations.

EP08C-2

Sorption and accumulation of pharmaceuticals in wastewater-irrigated fields in the Mezquital Valley, Mexico

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Due to population growth and urbanization, arable fields are increasingly irrigated with untreated wastewater worldwide. Environmental and health risks, such as proliferation and formation of antibiotic resistances, caused by pharmaceutical residues are profoundly affected by the compounds' binding and accumulation in soils. The Mezquital Valley north of Mexico City is the largest wastewater irrigation area facing these risks worldwide. We investigated the accumulation and sorption of the antibiotics ciprofloxacin, sulfamethoxazole, trimethoprim, clarithromycin, and the anticonvulsant carbamazepine in soils with different duration of wastewater irrigation (0-100 years). In contrast to our expectation, no accumulation of extractable ciprofloxacin and clarithromycin was detected. However, sulfamethoxazole and carbamazepine concentrations in the soils increased until a steady state of about 40 µg kg⁻¹ soil (sulfamethoxazole) and 55 µg kg⁻¹ (carbamazepine) was reached after approximately 15 years of irrigation. We hypothesized that this increase in concentrations with irrigation time was at least partly caused by a more efficient sorption of these compounds as a consequence of the accumulation of organic matter in irrigated soils. Sorption of sulfamethoxazole to soils irrigated for different periods of time was, however, weaker than sorption to non-irrigated soil. (De)sorption experiments indicate that a saturation of the soils sorption sites explains reduced sorption in irrigated soils compared to non-irrigated soil. Sorption of ciprofloxacin was always strong irrespective of the soil organic matter content and the duration of wastewater irrigation. Strong sorption counteracts an accumulation of extractable ciprofloxacin in soil. Intermediate sorption leads to an accumulation of extractable sulfamethoxazole and carbamazepine in soil until an equilibrium between input with wastewater and dissipation is reached. Historical records of inputs allow an estimation of dissipation rates under field conditions. The saturation of sorption sites for sulfamethoxazole and carbamazepine during wastewater irrigation and interactions of these substances with other compounds contained in the wastewater increase their leaching to groundwater.

EP08C-3

Sorption and leaching behaviour of four emerging pollutants pharmaceuticals in agricultural soil

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Mobility of pharmaceuticals in soils is little known. These pollutants can be introduced in the soil by the wastewater irrigation practice that represents increasing interest nowadays. Traditional approach to study the mobility of organic pollutants taking into account the Kow parameter would not be appropriated for many pharmaceuticals because they are polar or ionic compounds, i.e. charged at wastewater and environmental pH for which an ionic interaction mechanism could be responsible for their retention by soils. Cationic exchange capacity (CEC) of the soil and other soil factors such as pH, organic matter content (OM), or presence of surfactants modified by the wastewater irrigation could influence the mobility of pharmaceuticals in soil. The aim of this study was to investigate the mobility of some frequently detected wastewater derived pharmaceuticals in soils under laboratory conditions. Four pharmaceuticals were chosen: carbamazepine (CBZ), venlafaxine (VEN) and their respective major human metabolites, 10,11-dihydro-trans-10,11-dihydroxyl carbamazepine (DIOL) and O-desmethyl venlafaxine (ODV). They are frequently detected in wastewater at concentrations higher than 1 µg/L and they have different Kow and pKa values. The main results were: 1) batch sorption/desorption study showed that VEN and ODV were largely sorbed by two soils with low OM content and their desorption was dependent on the soil cationic exchange capacity. CBZ and DIOL were little sorbed by both soils and their desorption was not due to cationic exchange. The soil with greater CEC showed more affinity for all compounds. 2) leaching study on soil columns confirmed batch experiment with CBZ and DIOL leaching much more rapidly than VEN and ODV. VEN and ODV were only leached with 100 mM Ca²⁺ solution. No influence was observed with cationic and anionic surfactant solutions. Retardation of leaching was more important for the soil with greater CEC. This work showed a clear influence of soil CEC in sorption/desorption mechanism of ionic pharmaceuticals VEN and ODV. The Kow value by itself would not be able to predict correctly mobility of ionic compounds in soil. Other parameters related with CEC should be taken into account.

EP08C-4

Uptake of Pharmaceuticals into the Terrestrial Environment

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Increasing amounts of pharmaceuticals are being detected in soils. This is primarily due to sewage sludge being applied to fields as well as the use of reclaimed wastewater for irrigation, both of which contain high levels of pharmaceuticals which are then transferred to the soil. This study investigated the uptake and depuration of pharmaceuticals into earthworms (*Eisenia fetida*) with the ultimate aim being to develop models to improve the assessment of the risks posed by pharmaceuticals in the terrestrial environment. The studies were based on the OECD Guideline 317 and involved a 21 day uptake phase followed by a 21 day depuration phase. Studies were performed using radiolabelled compounds to allow for a lower limit of detection thus enabling environmentally relevant concentrations to be used. *E. fetida* and soil sampled throughout the experiment were extracted and analysed by liquid scintillation counting. Selected samples were also analysed by radioHPLC to assess whether any metabolism had occurred. The results from the study were modelled using OpenModel (v 1.2 Nottingham University) and based on a one compartment toxicokinetic model.

For both fluoxetine and diclofenac there was uptake within the first 6 hours of the experiment. The modelled uptake rate constants (K_{in}) show that fluoxetine had a higher overall uptake rate than diclofenac at 0.7428 L/kg d⁻¹ and 0.1484 L/kg d⁻¹ respectively. When *E. fetida* were transferred to clean soil both compounds were seen to be

immediately eliminated from the organism, however modelled depuration rates were considerably slower than uptake rates for both pharmaceuticals. By the end of the depuration phase there were still traces of both pharmaceuticals in the earthworms. The modelling results were then used to evaluate existing quantitative structure-activity relationships used in environmental risk assessment.

Bioconcentration factors were estimated from the uptake and depuration rates. The diclofenac BCF was 167 and the BCF for fluoxetine was 133. Both of these values are comparable to BCF values estimated from equations used in risk assessment, such as the Technical Guidance Document. This research shows earthworms, *E. fetida*, can accumulate pharmaceuticals if they are present in soils at environmentally relevant concentrations. Work is ongoing to assess the uptake of additional pharmaceuticals into earthworms, as well as to investigate how uptake can be influenced by soil parameters such as change in pH.

EP08C-5

The anti-arrhythmic drug flecainide: environmental detection and conserved mode of action in fish

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Flecainide is sodium channel blocker used to treat cardiac arrhythmic disorders in humans. We hypothesized that flecainide could cause adverse environmental effects, based on its sales and usage statistics, logP (4.0) and a presumably conserved mode of action in fish. We have therefore quantified levels of flecainide in environmental samples, and studied effects of the drug in rainbow trout (*O. mykiss*) in vivo and in vitro, using endpoints reflecting its known mode of action in humans. Using a triple stage quadrupole MS/MS TSQ Quantum Ultra EMR system, we could detect flecainide in treated sewage effluents (123 ± 17 ng/L), in surface water upstream (2.7 ± 1.2 ng/L) and downstream (36.8 ± 14.4 ng/L) from sewage treatment plants and in muscle of wild fish (0.16 ± 0.03 µg/kg). Rainbow trout were cannulated via the buccal cavity, and once a stable baseline was attained, stepwise increments (10x at each step) of flecainide were injected. Flecainide injections, equivalent to a human dose adjusted for body weight (2mg/kg), resulted in an acute significant decrease in heart rate and a tendency for an increase in arterial blood pressure. Mean flecainide level in plasma from injected fish was 0.9µg/ml, i.e. rather similar to human therapeutic plasma concentrations (0.4µg/ml). Preliminary results from in vitro studies on twitch force and rate of contraction in rainbow trout ventricular and gastric strip preparations suggest decreases of both endpoints at levels close to human therapeutic concentrations. Taken together, this indicates at least a partly conserved mode of action between human and fish as well as a roughly similar potency, and the possibility of effects in non-target tissues. Waterborn dose-response studies, controlled bioconcentration studies and analyses of plasma levels in wild fish will allow for further assessment of environmental risks with flecainide.

EP08C-6

Accumulated antidepressants in wild-caught fish - a relaxed wildlife?

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Numerous recent studies have revealed pharmaceutical residues in the influent and effluent of waste water treatment plants and all kinds of surface water. In these studies mainly water samples have been collected which were concentrated by solid phase extraction prior chemical analysis.

On the other hand to deduce mechanisms of toxic action or to evaluate the toxicity of mixtures, and to interpret field data on bioaccumulated toxicants the tissue residue approach has been taken into account most recently. Therefore, we have focused in our study on a multi-residue analysis of polar to lipophilic pharmaceuticals from different trophic levels of the aquatic food chain (fish prey, fish, and fish eating birds).

Determined pharmaceuticals were the frequently detected ibuprofen (an analgetic), diclofenac (an analgetic), mefenamic acid (an anti-inflammatory drug), sulfamethoxazole (a bacteriostatic antibiotic), atenolol (a beta blocker), diphenhydramine (an antihistamine), diltiazem (a calcium channel blocker), carbamazepine (an anticonvulsant), fluoxetine (an antidepressant) and its main metabolite norfluoxetine.

Since our preliminary results have revealed exclusively residues of norfluoxetine, fluoxetine, diphenhydramine and carbamazepine in fish and fish prey the list of analytes were extended to other antidepressants (sertraline and citalopram) but also to more lipophilic pharmaceuticals like gemfibrozil, fenofibrate and naproxen or lipophilic metabolites of diclofenac (2-[2-(chlorophenyl)amino]benzaldehyde) and ibuprofen (2-[4-(2-hydroxy-2-methylpropyl)phenyl]propionic acid), which have been in parts detected in surface water.

Trace analysis of compounds was performed in a multi-residue method based on HPLC coupled to mass spectrometry (LC-MS) whereby identification and quantification of pharmaceuticals in biota were arranged in MS/MS mode. MRM transitions were classified in a different elution time window to increase measurement sensitivity.

The identified concentrations of analytes were ordinary higher in liver than in muscle tissue.

ET01 - A systems biology approach to predictive ecotoxicology

ET01-1

Network inference tools in ecotoxicology: application to *Daphnia Magna*

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Environmental protection agencies worldwide are presented with a huge challenge as a result of human innovation. Large numbers of chemicals are being released into the environment through a number of sources including chemical spills, sewage, industrial waste or agricultural run offs. Many of the underlying mechanisms of toxicologically relevant chemicals are currently unknown or incompletely characterized. "Omics" technologies have provided a high-throughput unbiased approach to address this issue. Statistical modelling approaches, identifying groups of features predictive of toxicity outcome have shown to provide informative results, especially when pathway knowledge is incorporated. This approach, however, is limited to the gene to gene interactions represented by the functional annotation. In this context, several reverse engineering methodologies have been developed to provide means of inferring the underlying regulatory network without prior knowledge [1-3]. The resulting interactions can then be subjected to further methodologies to identify highly interconnected sub-networks or functional modules which can provide knowledge towards identifying novel adverse outcome pathways [4]. To demonstrate the effectiveness of these techniques we applied these methodologies to an mRNA expression dataset derived from *Daphnia Magna* exposed to sub-lethal concentrations of a number of ecotoxicologically relevant chemicals. We apply a well validated reverse engineering methodology, ARACNE [2], and identify functional modules which may provide knowledge towards understanding the response of this species to chemical exposure.

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ET01-2

Integrative monitoring of wetlands WWTP effluent remediation in the WIPE project

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Waterharmonica is a Dutch concept of natural filtration in constructed wetlands, applied to wastewater treatment plant (WWTP) effluent. Particles and nutrients are removed by aquatic organisms and reed beds, and the oxygen balance is restored, leading to positive effects on biodiversity. The WIPE project (Waterharmonica Improving Purification Effectiveness) was an extensive study of wetlands artificially constructed to remediate effluent from three wastewater treatment plants in The Netherlands. The main objectives of this project were to study the fate and toxicity of emerging organic pollutants from the WWTP effluents in order to assess if the discharged water meets the criteria of the Water Framework Directive (WFD) for good quality surface water. An extensive integrated monitoring program was applied at four positions each of three constructed Dutch wetlands, from raw effluent to discharged water. This program consisted of passive sampling, chemical analyses, bioassays and stickleback (*Gasterosteus aculeatus*) gene expression responses and fish ecology.

Several sampling campaigns were carried out to assess the chemical quality with a wide range of organic chemical analyses and a suite of in vivo and in vitro bioassays. Simultaneously, the ecology of the stickleback (survival, growth and reproduction) was studied by exposing the fish in mesocosms to water from four positions of the wetlands. Large variations in WWTP effluent quality were observed over time. At one of the wetlands an increased fish mortality was observed after exposure to the effluent, while a clear growth inhibition was observed in fish exposed in another wetland. No clear effects on reproduction success were observed in the surviving fish. Chemical analyses revealed that bioavailable levels of most organic micropollutants were not reduced, but a decrease in chemical toxic equivalents was observed after passage of the different wetlands compartments. Certain toxic responses (e.g. estrogenic endocrine disruption) also seemed to decrease in the wetlands. The in vitro ER-Luc measurements of XAD extracts seemed to correlate well with gene expression responses in stickleback. Less clear relationships between in vivo biomarkers and in vitro bioassays were observed for other parameters, such as cytochrome P450 1A (CYP1A) induction, thyroid induction and multi xenobiotic resistance (MXR) inhibition. The WIPE dataset is used to design a smarter WFD monitoring strategy for chemical water quality.

ET01-3

Transcriptomic responses of three-spined stickleback (*Gasterosteus aculeatus*) to wetland effluent remediation in the WIPE project

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The WIPE project was an extensive study of wetlands artificially constructed to remediate effluent from three wastewater treatment plants in The Netherlands. As part of this project, that also included microbiology, analytical chemistry and in vivo and in vitro bioassays, sticklebacks were exposed to water from the wetland systems in flow-through mesocosms, or maintained in a control mesocosm. Fish were hatched in the mesocosms and sampled after 1 year, with livers preserved for transcriptomics.

Based upon the stickleback genome sequence and results of optimization experiments, a 15,000 probe oligonucleotide microarray was designed for stickleback liver. Hepatic gene expression profiles were determined for 106 female and 75 male sticklebacks from 12 mesocosms. In males 3493 and in females 4290 transcripts were significantly differentially expressed (FDR<0.05), when comparing control and all exposed fish. The majority of genes differentially expressed between males and females were located on chromosome XIX, previously identified as the stickleback sex chromosome.

When assessing a large dataset reflecting responses to complex mixed stimuli, such as WWTP effluents, individual biomarkers can be examined but more systematic approaches to data interpretation using prior knowledge can be valuable. As an example of biomarker response, at one sampling site expression of the three vitellogenin genes and estrogen receptor alpha were elevated in males. Extracts from passive samplers at this position elicited the greatest response in the ER-luc bioassay. Differentially expressed genes were characterised with respect to prior knowledge of their functions and their chemical regulators and disease associations (Comparative Toxicogenomics Database), resulting in hypothetical predicted profiles of chemicals and biological responses at each site. A number of changes correlated with liver weight, including repression of mitotic genes and associations with cholesterol and fatty acids. Responses were detected that are consistent with exposure to pollutants, such as PAHs, that reflected chemical data showing that some PAHs, including pyrene, exceeded PNEC values. Additionally, responses consistent with PCBs, oxidative stressors, endocrine disrupters and pharmaceuticals and personal care products were highlighted. Benefits and limitations of these approaches will be discussed.

ET01-4

Effects of metals and pesticides in *Enchytraeus albidus* - transcription, cellular energy allocation, oxidative stress biomarkers and reproduction: a systems biology approach

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An important challenge in terrestrial ecotoxicology is to unravel the modes of action of toxicants which are known to have a negative impact on ecosystems. *Enchytraeus albidus* is an important standard species in terrestrial risk assessment. This species is a typical inhabitant of a wide variety of soil types and fulfils important functions. Effects of chemicals in *E. albidus* have been addressed mainly assessing effects on their survival and reproduction. The present work comprises results of experiments using *E. albidus* where effects of the metals cadmium and zinc, and the pesticides dimethoate, atrazine and carbendazim were tested. Different concentrations and exposure durations were used as well as various endpoints from different levels of biological organization - gene expression, cellular energy allocation, oxidative stress markers, survival, reproduction and avoidance behaviour. The main goal was a systems toxicology approach where effects should be integrated. In concentrations known to cause a reduction on reproduction, all chemicals induced significant changes in the antioxidant enzyme activities and substrate levels and, in some of the conditions, oxidative damage was observed. In general, cellular energy allocation (CEA) results indicated reduced amount of energy reserves and disturbing of the mitochondrial electron transport system (ETS) by increasing the cellular respiration. This increase in the ETS activity corroborated the transcriptional evidences, where there was an induction of genes coding for proteins involved in the mitochondrial electron transport system. Gene expression analysis further enabled the identification of common mechanisms of toxicity and also key biological processes affected by each compound. The information gathered with gene expression analysis, along with the reduction of CEA and the verified oxidative damage on the membrane cells, can help to explain the decrease on the reproductive output at a later stage. It further indicated evidences of conserved mechanisms across-species. This presentation includes a combination of several studies that build along years and demonstrates the usefulness of integrating endpoints to better understand and predict the mechanisms of action of chemicals in a broader context.

ET01-5

High level dynamical models in *Mytilus edulis*

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Introduction. High-throughput omics including transcriptomics, proteomics and metabolomics could help in understanding an organism's response to various stresses. The acquisition and processing of such data for non-model species from their natural habitat is still a challenge, but would enable better comprehension of their response to pollution or seasonal changes.

Mussels, filter feeding sessile organisms living in the sea have been used for environmental monitoring and sessility makes them particularly suitable for studying the effects of chemicals in coastal regions.

Omics technologies alone provide large amounts of information that might prove difficult to interpret. Gene Ontology enrichment analysis can identify pathways that could be involved. Building static similarity networks helps further in finding groups of co-regulated genes possibly important in particular response. The strength of dynamic models relies in their ability to represent temporal changes, whether during natural cycles or in response to chemicals.

Approach. Here we present a systems biology approach of developing high level dynamical models of the annual cycle in blue mussel *Mytilus edulis* using 1H-NMR metabolomics data. Metabolites with similar temporal patterns were clustered and ordinary differential equation (ODE) based dynamical models were built between cluster profiles, using NIMOO [1]. This reveals potential time-dependant relationships between the clusters of metabolites.

Results. A dynamical model representing *Mytilus edulis* metabolite seasonal variation revealed differences between males and females. Perturbations of metabolite levels in Southampton identified clusters of metabolites that show feminising temporal patterns, suggesting the presence of feminising chemicals.

Importance. This work applies dynamic modelling approach to high-throughput data for understanding temporal changes in non-model species living in the wild environment. References.

[1] R. Gupta, A. Stincone, P. Antczak, S. Durant, R. Bicknell, A. Bikfalvi, and F. Falciani. A computational framework for gene regulatory network inference that combines multiple methods and datasets. BMC Systems Biology, 5(1):52, 2011.

ET02 - Advanced statistical methods in quantitative ecotoxicology

ET02-1

Statistical ecotoxicology - bright lines and dark alleyways

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Statistical modelling and inference plays a crucial role in ecotoxicology. Indeed, it could be argued that without the guiding principles of proper statistical design and analysis, ecotoxicology would be so severely handicapped as to be rendered ineffective. Of course there is a lot more to ecotoxicology than concentration-response (C-R) experiments and the identification of 'safe' concentrations of contaminants in an ecosystem using statistical concepts such as the Species Sensitivity Distribution (SSD). However, given the prominence and importance of statistics as a critical element of the scientific process in ecotoxicological practice it is both timely and necessary to ask "how well are we doing?"

This paper attempts to answer this question by providing an overview of the 'State of Statistics' (SoS) in ecotoxicology. In it, we briefly trace the history of involvement of statistics and statisticians and examine the development and population of the ecotoxicologists' statistical 'toolkit'. We reflect on what has worked well, what has let us down, and opportunities for future development.

ET02-2

Well past time to stop using NOELs and LOELs

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In the popular song, Hotel California, by the band the Eagles, "they just can't kill the beast". That is exactly our situation - we (and many others) have not yet been able to kill the "beast" that comprises No Observed Effect Levels (NOELs) and Lowest Observed Effect Levels (LOELs). Given that the concentration-response curve is one of

the founding paradigms of environmental toxicology it is absurd that hypothesis testing continues to be used to report toxicity. We submit that NOEL/LOELs do not meet the criteria of reporting concentration/dose response relationships. The only scientifically defensible measure of toxicity is the description of the concentration/dose response “curve”. If toxicity tests are going to be research tools, then the data analyses must accurately describe toxicity. Since the 1970s the “curve” has been demonstrated to provide detailed information including the slope, the ECx values and the error term. Software is available that makes the computation straightforward. Bayesian curve fitting has been developed that allows estimation of a No Effect Concentration. The Bayesian curve fitting also provides credibility limits for the range of the curve. Since it is clear that the use of the “curve” is the only rational approach, we propose 5 recommendations: 1. Papers that rely on NOEL/LOELs should not be considered for publication in peer-reviewed scientific journals. 2. Curve fitting is the preferred model for concentration/ dose response relationships, ideally including confidence intervals and with supporting data. 3. NOEL/LOELs and similar data derivations should not be used as descriptions of concentration/dose response. 4. Papers that rely on historic NOEL/LOELs should only do so after every attempt has been made to replace these data derivations with concentration/dose response data. 5. Papers that treat NOEL/LOELs as data for further analysis such as the derivation of SSDs should receive extraordinary scrutiny. Regulatory agencies across the world must also begin a transition to the use of curve fitting to describe exposure-effect relationships. Although it is understood that for many chemicals NOELs/LOELs are the only results from some toxicity tests, the uncertainties in the accurate representation of the concentration-response curves result in unacceptable uncertainty, obviating informed decision-making.

ET02-3

Interpretation of toxicity data requires appropriate models for process and noise

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Quantitative interpretation of data requires models of some sort. In general, it requires a model for the process that has produced the observed data, as well as a model for the “noise” (the inevitable deviations between the process model and the data). Useful data interpretation requires a “mechanistic” process model, combined with a noise model that is appropriate for the observations. In (eco)toxicology, toxicity testing of compounds plays a central role. To analyse the data from toxicity tests, curve fitting is very popular. In many cases, this represents a situation with a poor model for both the process and the noise. The process model is just a description of the data that helps to interpolate to an ECx. For fitting the process model to experimental data, least-squares analysis is by far the most popular approach in this context. However, least squares represents a noise model, which comes with its own assumptions, which are almost always violated in practice. Toxicokinetic-toxicodynamic (TKTD) models are the most useful type of process model for analysing toxicity data. However, because these models fit the effect patterns over time, they require dedicated noise models. For quantal data such as survival, an appropriate model exists (the multinomial distribution). For continuous data such as body size and reproduction, more research is needed. In this contribution I want to raise the discussion about appropriate process and noise models in ecotoxicology, with an emphasis on the latter.

ET02-4

Estimation of critical effect concentrations: when choosing the right error model matters

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Ecotoxicology is in essence multidisciplinary and mathematical modelling is undoubtedly one of its main components. At a time when there is widespread recognition of the drawbacks of NOEC/LOEC values, statistical modelling approaches are particularly suited to estimating critical effect concentrations such as ECx or NEC values.

Generally, we can write:

$$Y=f(X; \theta) + \varepsilon$$

where Y represents the data; $f(X; \theta)$ is the deterministic part of the model (linear or non linear), with X the independent variables and θ the parameter vector; ε is the stochastic part, or the error model, chosen according to the data to describe randomness in realizations.

A review of modelling approaches used in ecotoxicology, or on routines dedicated to the estimation of critical effect concentrations (like the R-package drc), suggests that considerably more time and effort is devoted to the elucidation of the deterministic part of the model, and relatively little consideration given to defining an appropriate error structure. Nevertheless, the error model cannot be ignored for inference, model checking or prediction. Furthermore, based on our own publication experiences in the ecotoxicological literature has demonstrated that to the difficulty in convincing reviewers of the importance of the error model. The following correspondence from anonymous referees illustrates the point:

“All the effort you put into deriving a sophisticated error structure of your model seems wasted”.

“How important - in a practical sense - is it to get the error model right and the likelihood correct? How big is the difference if you would do the parameter estimation using a mathematically very correct approach (like you probably would do) or a slightly wrong, easy to use approach as I have done? How much would the parameters differ? I don't know how much, but I would like to know”.

This is in fact the question we want to address in this paper: how influential is the choice of the error model in the quality of conclusions drawn about hazardous concentrations? With three examples extracted from the literature, we will illustrate the consequences of an inappropriate choice on point estimate of critical effect concentrations as well as on their confidence interval.

ET02-5

Variability in ecotoxicology: deliberate ignorance or just not getting it

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In the analysis of ecotoxicological datasets we tend to ignore the variability in the parameters being measured, fail to report known variances, fail to manage overall experiment-wise error rates and ignore the intrinsic value of variability in providing insight into biological responses to stressors. This is probably mostly a reflection of the relative youth of our field of science, a determination to find fundamental or simple answers to puzzling and complex issues and a desire to provide conservative numerical contaminant thresholds that satisfy the requirements of practitioners and regulators. Nonetheless, it does affect the practice of ecotoxicology in real-world situations and influences real world costs of environmental management.

In this paper we will examine the general lack of control of experiment-wise Type I error rates in multi-species and multi-sample assessments of toxicity, arguably the most common practical application of ecotoxicology in environmental assessment. While this may be a conservative trade-off that increases the statistical power to detect toxicity by an increase in the Type I error rate, actual measurement of statistical power or Type II error rates are rare in ecotoxicology, as is measurement of experiment-wise Type I error, so the value of this trade-off is rarely known. Modern SSD approaches compound this problem because endpoints from many studies are combined without consideration of the effect of compounded Type I errors on an overall model; a model from which, at least in the ANZECC/ARMCANZ framework, specific values of protective concentrations or hazardous concentrations are derived with, again, no overt reporting of error of the overall error rate. Furthermore, the test endpoints used as input to the SSD are commonly “standardised” by the use of application factors and acute to chronic ratios that are either arbitrary or derived from a limited number of tests without consideration of their variances.

The net result is that environmental management targets are specified that are based on layers of conservatism, and that have error rates that could be measured and reported, but generally are not. How much this costs society and operators in terms of compliance with overly-conservative guidelines is not known and incalculable. We believe that it is possible to better report and measure these hidden error rates, and that better insight into them may well provide for more cost-effective management.

ET02-6

Statistical evaluation of experimental designs for modelling of concentration-response functions in ecotoxicology

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Modelling concentration-response curves is now a classical way of analyzing ecotoxicological bioassays, which often replaces the severely criticized NOEC approach. However, most ecotoxicological bioassays are conducted according to designs appropriate for hypothesis testing but not for regression analysis implied in a modelling approach. The adaptation of experimental designs to regression analysis was rarely discussed in ecotoxicology. The use of D-optimal criterion to develop cost-effective designs for ecotoxicological bioassays was recently proposed. But the application of such local optimal designs requires a prior choice of the model, and a good prior knowledge of the values of the model parameters. This is a strong limitation on their applicability for defining standard protocols for ecotoxicological bioassays. In fact the prior knowledge of parameters is often approximate and a local optimal design based on wrong parameter values may be bad. Moreover, calculated optimal values for concentrations may be difficult to experimentally reach, or too precisely defined in regard to measurement precision of the concentration of the studied compound.

While evaluating the optimality of an experimental design, instead of using fixed values for model parameters, one could use a prior distribution for model parameters, summarizing the possible values the parameters may take, according to the prior knowledge of the ecotoxicologists. We performed simulations based on such prior distributions in order to evaluate standard and modified designs for ecotoxicology, taking the example of 21-day Daphnia magna survival bioassays. In such bioassays, tested

concentrations are generally defined following a geometric sequence, often of common ratio 2, centered on the expected LC50 value guessed from prior knowledge. The same number of individuals is generally tested for each concentration and for the control group. We quantified the impact of the total number of tested organisms and of the common ratio of the geometric sequence (for a fixed total number of organisms) on the precision of LC50 and LC5 estimations. Results may help ecotoxicologists to choose the more appropriate design and fix the total number of tested organisms, depending both on their prior knowledge about the LC50 value and on their expectation about precision of estimates.

ET03 - Animal alternatives: methods, endpoints and testing strategies

ET03A-1

An updated assessment of the relationship between the Zebrafish embryo toxicity test and the OECD 203 fish toxicity test - what can we say now?

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An integral aspect to the development of the Zebrafish Embryo Toxicity Test (ZFET) is the determination of its relationship to the OECD Acute Fish Toxicity Test Guideline (TG 203). The most recent update (Lammer et al. 2009) included comparisons of ZFET to the five most commonly tested species in the OECD 203. Since 2009, a large increase in the literature has occurred and a major validation study been conducted (20 chemicals performed in triplicate in 3-7 different laboratories). An updated assessment is timely. We review the data as of 2009, additional information available today, and provide new assessments. ZFET studies have increased to >500 on approximately 200 chemicals. OECD 203 data are available for approximately 150 of these compounds representing about 1250 individual toxicity tests. Fifteen major chemical categories are found in the database including industrial chemicals, agrochemicals, pharmaceuticals, feed stuffs, biocides, polymers, inorganics, and metals that spanned 9 orders of magnitude. Orthogonal regression was used to compare ZFET-OECD 203 overall and the resulting regression had a slope of 0.99, intercept of -0.03, and a correlation >0.9. We used class-specific QSARs to compare predictions for chemicals tested in ZFET and the OECD 203 (this provides perspective for chemicals that lack matching fish data). Fish QSARs provided equivalent predictions for fish embryos (e.g., for neutral organics, ZFET and fish predictions had equal levels of departure from the standard QSAR published in USEPA ECOSAR. Overall, the addition of new data to the ZFET-Fish database continued to improve the regression relationship, markedly expanded the domain of tested chemicals, and provided evidence that future QSAR developments will be useful.

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"Disclaimer: The opinions expressed and the arguments employed herein are those of the authors and do not necessarily reflect the official views of the OECD or of the governments of its member countries, or the European Commission"

ET03A-2

Determination of internal chemical concentration in zebrafish (*Danio rerio*) embryos - a toxicokinetic approach

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The zebrafish (*Danio rerio*) embryo is an established animal replacement model organism in toxicology. A broad range of toxicological applications of zebrafish embryos is described in literature. In these applications the observed effects are usually linked to the ambient exposure concentration. But the internal concentration might reflect the biological effective dose and therefore provide access to describe biological potency of a chemical.

Several studies using radioactively labelled compounds for the determination of the internal chemical concentration in zebrafish embryos are published. For a broader application an approach without the use of radioactively labelled compounds could be useful. Therefore an approach for the determination of the internal chemical concentration in zebrafish embryos without the use of radioactively labelled compounds is introduced here.

The time dependent internal concentration of compounds with different $\log K_{ow}$ was measured at different exposure concentrations in zebrafish embryos. A one compartment first-order toxicokinetic model was used in order to estimate uptake and elimination rate constants. Data of fluoranthene as model substance are exemplarily shown here. In this study, zebrafish embryos were exposed to an aqueous solution of one test substance in a static but agitated system under controlled conditions. Samples of exposed embryos were taken at 8 time points between 3 to 72 hours of exposure. Fluoranthene was extracted from whole embryos and analysed with HPLC-FLD/DAD. The concentration-time profiles were assessed.

Uptake rate constants (k_1) and elimination rate constants (k_2) were estimated by fitting the time course of internal concentrations with a one compartment first-order toxicokinetic model. There was no concentration dependence of the bioconcentration factor observed. This agrees with the assumption of a simple first-order uptake and depuration model.

In conclusion, the determination of the internal chemical concentration in zebrafish embryos without the use of radioactively labelled compounds was realized successfully and is recommended for a broader application. Using the one compartment first-order toxicokinetic model for this study, the suitability of the model performance and the experimental data is demonstrated.

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ET03A-3

Single-compound exposure versus co-exposure approaches in the in vivo EROD assay with the zebrafish (*Danio rerio*)

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This study is part of the joint research project "DanTox" funded by the German Federal Minister of Education and Research (BMBF) and aims at developing eukaryotic test systems to investigate ecotoxicological effects of contaminated sediments. The visualization of specific activation of the Ah-receptor directly in the zebrafish (*Danio rerio*) embryo via live imaging is one of these bioassays. The measurement of ethoxyresorufin-O-deethylase (EROD) activity is regarded as a highly sensitive indicator of specific planar polycyclic aromatic hydrocarbons (PAHs) and structurally related compounds. CYP-induced fluorescence signals result from the ability of CYP1A to convert 7-ethoxyresorufin (7-ER) to auto-fluorescent resorufin. The transparency of the zebrafish embryo allows direct detection of EROD induction via epi-fluorescence microscopy. The co-occurrence of agonists and inhibitors of CYP1A is typical of complex environmental mixtures and required modifications of the in vivo EROD assay. Therefore, zebrafish embryos were used in order to evaluate the EROD-inducing potential of common single-compound exposures in parallel with binary mixtures of each compound (or sediment extract) and the PAH-type AhR agonist β -naphthoflavone. For chemical testing, the following substances were selected: methyl mercury(II)chloride, chlorpyrifos, Aroclor 1254, bisphenol A, 2,3-benzofuran, quinoline and β -naphthoflavone as positive control. The sediment samples were collected from three sites: two from the Rhine River (Altrip and Ehrenbreitstein) and one from the Vering Canal in Hamburg.

Due to their molecular structure, methyl mercury(II)chloride, chlorpyrifos and bisphenol A failed to induce EROD. More precisely, chlorpyrifos turned out to be a strong CYP1A inhibitor (< EC10 value) and caused typical edema-related toxicity. Aroclor 1254 as well as the heterocyclic compounds quinoline and 2,3-benzofuran showed a particularly short temporal induction pattern and lead to inhibition of the CYP1A catalytic activity in a concentration- and time-dependent manner. In order to avoid fluctuations in CYP1A activity due to prolonged exposure with chronic effects as a consequence, the exposure time was reduced to 3 h, which resulted in a stronger, less variable and more sensitive EROD response. Pretests with the embryo toxicity test suggest the presence of AhR agonists in the sediment extracts; further testing is on-going.

ET03A-4

Improving the capacity of zebrafish embryos for predicting acute fish toxicity - analysis of neurotoxic endpoints using the photomotor response (PMR)

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Fish embryo toxicity exhibits a high correlation with acute fish toxicity. However, some neurotoxic compounds appear to represent outliers in this correlation. These compounds either do not provoke mortality at all (e.g. flucythrinate and esfenvalerate) or at levels well above those for acute toxicity in adults (e.g. azinphosmethyl or propoxur). We hypothesized that the neurotoxic mode of action is not leading to lethality in embryos but that measurement of sublethal indicators of neurotoxicity would allow predicting the effects in adults. Therefore, the photomotor response (PMR, Kokel, Nat Chem Biol 6, 231-237) was analysed as a potential indicator of neurotoxicity. The PMR is a characteristic, quantifiable movement pattern of the embryos which is triggered through an intense light stimulus. The alteration of the movement pattern by exposure to chemicals was shown to correlate with the mechanism of action. The PMR measurement was conducted using video recording and image analysis with a commercial system. After an optimization process we were able to detect a concentration dependent change in the PMR - patterns. We determined the PMR of two AChE inhibitors (azinphos-methyl and propoxur), two voltage gated sodium channel agonists (flucythrinate and esfenvalerate) and one voltage gated sodium channel antagonist (triacaine). Thereby the polar narcotic substance 3,4-dichloroaniline was used as a non-neurotoxic reference compound. By comparing a motion index (pixel changes within a 20 second analysis window), of exposed embryos (exposure from 2-33/37 hpf) to a control, increased or decreased movement was determined and visualised by a barcode

which was further used for cluster and PCA analysis.

Within a certain concentration range PMR pattern of neuroactive molecules clustered in accordance with their mode of action. At highest tested concentrations effects on the PMR was most likely caused by secondary effects rather than by direct neurotoxic effects (around LC₁₀ of embryos). Application of an effect-index for the whole measurement or a specific response phase allowed a concentration response modelling and calculation of EC₅₀ values. The EC₅₀ of neurotoxic compounds were found in the same range of concentrations as effect levels for acute toxicity in adult fish. The data indicate that including neurotoxic endpoints may improve capacity of the zebrafish embryo test for predicting acute fish toxicity.

ET03A-5

Looking for estrogen mimics using transgenic tg(cyp19a1b-GFP) zebrafish embryos

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The tg(cyp19a1b-GFP) transgenic zebrafish expresses GFP under the control of the cyp19a1b gene, encoding brain aromatase. This gene has two major characteristics: (i) it is only expressed in radial glial progenitors in the brain of fish and (ii) it is exquisitely sensitive to estrogens. Based on these properties, we demonstrate that natural or synthetic hormones (alone or in binary mixture), including androgens or progestagens, and industrial chemicals induce a concentration-dependent GFP expression in radial glial progenitors. As GFP expression can be quantified by in vivo imaging, this model represents a very powerful tool to screen and characterize compounds potentially acting as estrogens mimics either directly or after metabolism by the zebrafish embryo. This study also shows that radial glial cells that act as stem cells are direct targets for a large panel of endocrine disruptors, calling to more attention regarding the impact of environmental estrogens and/or certain pharmaceuticals on brain development. Altogether these data identify this in vivo bioassay as an interesting alternative to detect estrogen mimics in hazard and risk assessment perspective.

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ET03A-6

Refining the 48h-zebrafish embryo test - a mode of action dependent approach

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In the process of developing alternatives to animal based toxicity tests, the fish embryo toxicity test (FET) has gained significant attention in recent years. Great efforts have been made to extend the scope of application for zebrafish embryos in particular. Consequently, many genomics and post-genomics methods have found their way into fish embryo studies to address ecotoxicological questions beyond acute toxicity. As for many substances, pesticide effects on the zebrafish embryos often occur only outside the time frame of the standard ZFET to date or on submorphological levels. The recently revised European directive on the protection of animals used for scientific purposes now specifically tolerates the use of not yet independently feeding embryonic and larval stages of non-human vertebrates for testing, what will further promote the development of methods to broaden the scope of the ZFET within the regulatory framework. Here we present the assessment of pesticide toxicity on the zebrafish embryo on different developmental stages and different levels of organization. For thirteen insecticides we conducted a standard FET (48h) and an extended FET (120h). A selection of the tested substances was subjected to whole-genome expression analysis. Correlation was better between D. rerio embryo EC₅₀ values and adult O. mykiss acute toxicity values than for lethal FET effects only. Adult O. mykiss acute toxicity was more sensitive than embryo toxicity. For many chemicals tested, the first 48hpf were found not to be the most susceptible stage. Morphological effects at 48hpf often comprised minor, non-lethal effects. Exposure to the same concentrations but beyond 48h led to more severe and lethal effects. Chemicals used in microarray experiments induced differential gene expression at the EC₂₀ or below. Pesticides of different MoAs displayed different numbers of differentially expressed genes. Pesticides with functionally similar MoAs shared a higher number of differentially expressed genes than those with dissimilar ones. Regulated genes at 48h related to molecular functions, which can be linked to the morphological effects and to the given MoA. The inclusion of every observable morphological effect and the extension of test duration can render a more realistic estimation of the adult toxicity than a standard 48h ZFET. Further, different transcriptome responses may reveal, additional test data provided, MoA specific transcriptional signatures or gene markers.

ET03B-1

A novel animal replacement system for the detection of endocrine disruptive capabilities in sexual development

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Alternatives to animal testing for prediction of local toxicity and genotoxicity have been recently established. However, currently these methods are not suitable for measuring endocrine effects in developing organs such as e.g. embryonic gonads. Here we present a phenotypic anchoring of a comprehensive study on sex-specific gene expression analysis accompanied by histological analysis of endocrine disruption in chicken embryo gonads, having the potential for an animal replacing system for endocrine disruptive toxicologic and ecotoxicologic examinations of chemicals.

Chicken embryos were inoculated with different amounts of tributyltin (TBT) and bisphenol-A (BPA). Embryos were incubated and their gonads analyzed histologically 2 d prior to hatching. From identically treated embryos right and left testes and ovaries were separated and genome-wide transcription profiles generated using SuperTag Digital Gene Expression (ST-DGE, SuperSAGE) profiling.

Male and female gonadal tissues both revealed histological aberrations in response to TBT and BPA. Female gonads became masculinized in response to TBT and, vice-versa, BPA-treated male gonads underwent feminization. Moreover, in female gonads inoculated with BPA clearly visible structural aberrations occurred. Following exposure to both chemicals mortality increased especially in the most affected sex (TBT: females, BPA: males). The expression profiles of more than 60 million mRNAs revealed massive effects of both chemicals, TBT and BPA, on important cellular signaling pathways. Gene expression differences were most pronounced in the phenotypically most affected sex.

Our results demonstrate that endocrine disruptive chemicals exert their effects on several levels including but not restricted to known hormone-based pathways. Together with an ongoing study of gene expression differences in very young life stages and different chemicals these data will form the basis for a blow-by-blow analysis of sex-specific gene expression during embryonic development. The ongoing project aims at the development of an in vitro method for testing chemicals in chicken eggs for replacement of juveniles and (sub-) adult rodents using stages with no pain perception. This should allow a highly sensitive assessment of modes of action of chemicals, which might show consequences in the next generations.

ET03B-2

Assessment of the impact of an abandoned dump site on the health of adjacent soil ecosystems by in vivo and in vitro testing with Eisenia fetida

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Earthworms are considered as sentinel organisms for soil health assessment, and among them, Eisenia fetida has been selected for the development and application of standard tests due to its sensitivity and easy maintenance. E. fetida has been successfully employed for real soil assessment through biomarker measurements. Recently, an in vitro test with E. fetida coelomocytes was developed, including in vitro exposure to metals and elutriates of standard and real soils contaminated with pollutants alone or in mixtures. In the present work, biomarkers were determined in vitro in coelomocytes after both in vivo exposure (soils) of earthworms and in vitro exposure (soil elutriates) of coelomocyte explants extruded from stock healthy earthworms. The in vitro responses after in vitro and in vivo exposures were compared, aimed to replace the in vivo exposure approach in the future and contribute thus to reduce the use of animals in soil toxicity testing. For comparisons, two biomarkers (NRR and riboflavin content) were used for ecosystem health screening in soils surrounding an abandoned dumpsite. The selected biomarkers indicated a decrease in coelomocyte viability and reduction of riboflavin content after exposure to the most polluted soils around the dumpsite (in vivo exposure) and their elutriates (in vitro exposure). The high toxicity of soils was confirmed after 14 d in vivo exposure, as 100% mortality was recorded. It was concluded that in vitro testing of coelomocytes after in vivo and in vitro exposure methods provided similar information about the health of soils surrounding the dumping site. The selected cellular biomarkers were able to discriminate between polluted and unpolluted soils, even at low pollution levels. Therefore, when biomarker endpoints such as NRR capacity and riboflavin content are measured in vitro, the in vitro exposure approach is suitable to replace the in vivo exposure assays for soil health assessment.

ET03B-3

Genotoxicity of uranium in embryonic zebrafish cells

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Uranium is a heavy metal used in the nuclear industry and for military applications. Studies on mammals have shown that uranium is genotoxic but up to now the major DNA repair pathways activated by uranium remain to be analyzed. Particularly, the repair of double strand breaks (DSBs) remains to be addressed in aquatic vertebrates such as zebrafish. In this study we analyzed molecular level effects of exposure to depleted uranium in embryonic zebrafish fibroblasts (ZF4 cells). We assessed DNA damage and the activity of the major DSB-repair pathways, i.e. non-homologous end-joining (NHEJ) repair, after a 24 hours exposure to uranium concentrations ranging from 1 to 250 μ M, by immunodetection of the phosphorylated variant of histone H2A member X (γ -H2AX) and the DNA-dependent serine/threonine protein kinase (DNA-PK). DNA Single Strand Breaks (SSBs) were also detected using the alkaline comet assay. Cytotoxicity and bioaccumulation were measured for each exposure condition. Additionally, the localization and ultrastructural effects of internalized uranium within ZF4 cells was determined using transmission electronic microscope (TEM-EDX).

Exposure to uranium results in the production of DSBs a few hours after incubation. These breaks trigger the phosphorylation of H2AX proteins. We showed that the DNA-PK kinase activity, essential for NHEJ, is altered by the presence of uranium. The presence of uranium in cells disturbs but not inhibits the repair rate of DSBs. Such a result suggests an impact of uranium upon the reparability of DSBs and the potential activation of alternative DSBs repair pathway leading to the propagation of possible misrepaired DSBs. In parallel, we observed the formation of precipitates in lysosome-like vesicles for 250 μ M of uranium in the medium. The appearance of these precipitates is concomitant with the decrease of the number of DSBs per cell. This process might be a part of a defence system whose role in counteracting cytotoxicity calls for further dedicated research.

ET03B-4

Mapping ethynylestradiol (EE2) effect on gene and protein expression in *in vitro* and *in vivo* exposed rainbow trout (*Onchorhynchus mykiss*)

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Evaluation of *in vitro* methods performance compared to *in vivo* are crucial for implementation of the 3R's (reduction, refinement and replacement) in ecotoxicological testing. *In vitro* models such as continuous cell lines and primary cell cultures are well characterised high-throughput screening tools for several compounds including endocrine disruptors [1,3]. Use of different *in vitro* models has provided knowledge about the different modes of action (MoA) and the potency of chemicals that induce perturbations of natural endocrine action. Endocrine disruptors such as alkylphenols, ethynylestradiol and bisphenol A act as estrogen mimics by binding to and activating estrogen receptor (ER)-mediated cellular responses. As *in vitro* methods lack some of the organ complexity and toxicokinetics provided by whole organism models, these methods may not adequately address the multitude of cellular responses occurring after chemical exposure. The objective of the present study was to determine whether primary hepatocytes from rainbow trout (*Oncorhynchus mykiss*) can represent a feasible alternative to use of *in vivo* testing of environmental ER agonists. *In vitro* and *in vivo* cellular responses were characterized after exposure to the model estrogen ethynylestradiol (EE₂) by a combination of global gene expression and assessment of single gene and protein expression. In obtained microarray results, fish exposed *in vivo* to EE₂ had a significant up- (1674) and down (1449) regulation of totally 3123 genes where vitellogenin was significantly up-regulated (3600 times). The vitellogenin gene and protein expression was also highly induced in the EE₂ exposed primary hepatocytes, showing a concentration/time dependent relationship. Results obtained from the *in vivo* and *in vitro* model shows similarities in response on the gene and protein level of Vtg when exposed to EE₂. In conclusion, the rainbow trout primary hepatocytes have shown to be a promising tool for characterising estrogenic response *in vitro*. Due to the probable preservation of innate cellular properties, these cells may represent a versatile screening for various compounds.

ET03B-5

Predicting chemical concentrations in fish and fish cells to account for internal exposure in toxicity assessment

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Environmental regulations require comprehensive testing and risk assessment before a chemical can be approved for use. In ecological risk assessment of chemicals in water, fish play a very important role, being the only vertebrate representative of freshwater systems. Quantification of chemical toxicity is generally based on measurements of external exposure; however, in order to understand, interpret and extrapolate toxicological effects, using internal concentrations of chemicals is more suitable. For this reason we need to understand the relationship between the external and internal concentration of chemicals in fish as well as in *in vitro* systems, and to quantify internal concentration of a chemical using toxicokinetic (TK) modelling. Therefore in this study we aim to: predict chemical concentrations in fish, measure and predict chemical concentrations in fish cells, link chemical concentrations in cells to the effect on cells and link the effect on cells to the effect on fish.

To predict chemical concentrations in fish we used a Physiologically Based Toxicokinetic (PBTK) model. Based on the PBTK model, internal concentrations of organic chemicals were predicted and compared with measured internal concentrations in rainbow trout. To measure chemical concentrations in cells, we exposed a rainbow trout gill cell line (RTgill-W1) to the radiolabelled chemicals for 1, 2, 4, 8, 16 and 24 h at 19 °C. Amounts of chemicals were then measured in medium, plastic well and cells. Results demonstrated a clear relationship between predicted and measured internal concentrations in fish. Statistical analyses also confirmed a good agreement between measured and predicted values (coefficient of determination, factor₁₀ and the general distance between measured and predicted values were 0.79, 95% and 3.54, respectively).

The distribution of a chemical in a well plate differed for different compounds. For instance, the difference between Pentachlorophenol (PCP) and Malathion (Mal) can be caused by different log KOW of these compounds (PCP: 5.12, Mal: 2.36). In addition, we found that the concentration of a chemical in cells is time-dependent.

Thus, both approaches, PBTK modeling and experiments on fish cell lines, can be used to obtain internal concentrations of various chemicals which open applications in ecotoxicology and ecological risk assessment.

ET04 - Bioavailability and bioaccumulation - impact of environmental, biological and ecological variation

ET04A-1

Problems faced when evaluating the bioaccumulation potential of substances under REACH

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The identification and regulation of substances which combine persistency, bioaccumulation potential and toxicity (PBT-substances) is one central aspect of the European chemical legislation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals). PBT substances may elicit adverse long-term effects after release to the environment. The determination of a substance having PBT-properties is based on a set of distinct cut-off criteria identified in Annex XIII of the REACH regulation. Regarding the bioaccumulation potential the evaluation is focused on the substance's bioconcentration factor (BCF) as a decisive criterion. The REACH guidelines provide a selection of standardised test procedures for measuring BCF and guidance in appraising test results. Additionally, alternative test results such as bioaccumulation factors (BAF) and biomagnifications (BMF) as well as additional indications for a bioaccumulation potential such as trophic magnification (TMF) derived from field studies may be considered for the assessment using a weight-of-evidence approach. However, the assessment of bioaccumulation will mainly depend on standardised tests. The currently used test systems with aquatic exposure have been demonstrated to generate reliable results for the majority of neutral, lipophilic organic substances, which facilitate a clear decision making by means of the crucial BCF cut-off criteria of Annex XIII. However, certain substance groups such as highly hydrophobic organic substances, amphiphilic and non-lipophilic compounds are difficult to evaluate following common test strategies due to inappropriate test systems or accumulation mechanisms not based on lipophilicity. Recent scientific progress has already been made to establish alternative test systems and to refine the bioaccumulation assessment by consideration of additive accumulation mechanisms and indications. This presentation aims to give an overview on actual shortcomings in the current bioaccumulation assessment under REACH.

ET04A-2

Nestling predatory bird feathers reveal ecosystem-dependent bioaccumulation of persistent organic pollutants

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Bioaccumulation levels and patterns of persistent organic pollutants (POPs) in predatory birds are often confounded by individual biological variation, such as sex, age, diet and migration. Therefore, as food ingestion is a major POP exposure pathway, exposure assessment studies increasingly incorporate carbon (C) and nitrogen (N) stable isotope analysis (SIA) to quantify confounding variation in feeding ecology.

In this study, we hypothesised that the study of nestlings minimises confounding of certain biological variables, such as age, metabolic capacity, migration and reproductive capacity. We additionally hypothesised that POPs behave differently in marine and terrestrial ecosystems. To test these hypotheses, we analysed body feathers from nestling Northern Goshawk (NG; *Accipiter gentilis*), Golden Eagle (GE; *Aquila chrysaetos*) and White-tailed Eagle (WTE; *Haliaeetus albicilla*) to relate POP bioaccumulation to feeding ecology, i.e. carbon source ($\delta^{13}C$) and trophic level ($\delta^{15}N$), and investigate how relationships may differ between species foraging in different ecosystems.

NG, GE and WTE nestling body feathers all contained quantifiable levels of Σ PCBs (mean: 55.95, 36.36 and 73.83 ng g⁻¹), p,p'-DDE (mean: 48.56, 30.34 and 22.07 ng g⁻¹) and Σ PBDEs (mean: 3.08, 1.00 and 5.78 ng g⁻¹). Generally, the bioaccumulation profile suggests that marine ecosystems are a source for PCBs, terrestrial ones for p,p'-DDE, and that vicinity to urbanised regions influences levels of PBDEs.

$\delta^{13}C$ and $\delta^{15}N$ were positively correlated in nestling WTE feathers ($r = 0.589$; $P = 0.001$), but not in the terrestrial species ($P \geq 0.094$). This suggests differential C and N

cycling in terrestrial and marine ecosystems and is prominently reflected in correlations between SI and POPs. Firstly, while $\delta^{15}\text{N}$ and POPs correlated significantly in all species, correlations between $\delta^{13}\text{C}$ and POPs were only significant in the marine foraging WTE ($-0.449 \geq r \geq -0.388$). Secondly, in contrast to negatively correlated $\delta^{15}\text{N}$ and POPs ($-0.426 \geq r \geq -0.377$) in WTE, correlations in NG and GE were positive ($0.450 \leq r \leq 0.697$). Our results show that variable bioaccumulation patterns between marine and terrestrial foraging predatory birds are related to species-specific feeding ecology. Furthermore, although studying nestlings minimises confounding of certain biological factors, we will further investigate how sex and body mass variation may additionally explain bioaccumulation differences.

ET04A-3

Fate and bioavailability of insecticides across DOM-gradients in model ecosystems with biofilms and primary consumers

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1. Introduction

To better understand the impact of pesticide contamination on aquatic ecosystems we need fundamental information on how pesticides enter food webs and interact with organisms at different trophic levels.

2. Materials and methods

We used model ecosystems with cultivated biofilms and snails to quantify the fate and bioavailability of insecticides with different hydrophobicity (carbofuran, lindane, chlorpyrifos) across a DOM concentration gradient (low, medium, high). Pesticide pulses were added at the start of the 72-h experiments. Treatments without tiles and biofilms were run to quantify passive uptake. Snails were allowed to feed for 48 h. This set-up mimics the mechanisms at the base of food webs that act during runoff-events in agricultural streams.

3. Results and discussion

Partitioning to biofilms reduced chlorpyrifos water concentrations by up to $10 \pm 1.0\%$ at high-DOM, but was noticeably higher at low-DOM than at medium-DOM. Conversely, lindane water concentrations were $17 \pm 0.7\%$ higher in the presence of biofilms for all DOM-treatments. Pesticide BCF for biofilms were affected by both DOM and pesticide type, due to differences between lindane and chlorpyrifos treatments. BCFs for snails were only affected by the type of pesticide, and nearly 60% higher for both lindane and chlorpyrifos than for carbofuran. Bioaccumulation factors (BAF) between snails and biofilms showed differences across DOM-gradients for lindane and chlorpyrifos. The lack of concentration-dependent effects of DOM on pesticide fate and bioavailability suggests that also DOM-quality affects these end points. Although the share of pesticides recovered in biofilms was consistently less than 1%, their concentrations in biofilms were more than 75- (carbofuran) and 382-times (lindane) higher than those in water.

4. Conclusions

- The high and efficient sorption of lindane, and chlorpyrifos show that biofilms may act as a sink for pesticide peak concentrations during run-off events.

- DOM and biofilms in aquatic environments affect the fate of pesticides following runoff-events and their incorporation in aquatic food webs.

ET04A-4

Bioaccumulation and biomagnification of POPs in species from the Scheldt estuary (Belgium)

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As persistent organic pollutants (POPs) are spread worldwide over various environmental compartments, they might also be bioavailable to organisms present in the same surroundings. Their chemical properties like stability, lipophilicity and persistence, give them the ability to easily accumulate into fatty tissues. When pollutants are not excreted or metabolized in the body, they can be passed on from prey to predator, resulting in elevated POP concentrations in species at higher trophic levels. As humans are also at the higher trophic levels, travelling details about bioaccumulation and biomagnification processes are of high importance for an accurate risk assessment about POPs in edible species.

In this study, POP concentrations in aquatic species collected from the Scheldt estuary are linked with environmental concentrations and the trophic level of the species, to investigate which POPs are bioaccumulated from the environment into a certain species and which are even biomagnified through the estuarine food chain.

Therefore fish, crabs, mussels, shrimps, snails, worms, plants and sediment were collected in June 2011 from three locations (Terneuzen, Bath, Antwerpen) along the Scheldt estuary by fike fishing, trawl fishing and by hand. In all the biota samples, 33 PCB congeners, 7 PBDEs, DDXs, chlordanes, HCHs and HCB were targeted for analysis. In the sediment samples, also PBDE 209 was measured. To determine the trophic position of the collected species, carbon and nitrogen stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were measured.

ET04A-5

Food web accumulation of cyclic siloxanes in Lake Mjøsa, Norway

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The biomagnification potential of cyclic volatile methyl siloxanes (cVMS) was analysed in the Lake Mjøsa food web in Norway leading to brown trout (*Salmo trutta*). The food web biomagnification of octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexatetrasiloxane (D6) was analysed by trophic magnification factors (TMFs) and compared with TMFs of legacy contaminants such as polychlorinated biphenyls (PCB) and polybrominated diphenyl ether (PBDE). Of the cVMS, only D5 showed significant TMFs with values above 1, implying biomagnification. The food web accumulation of D5 was however, sensitive to species included at the higher trophic level, thus whole food web TMF differed from TMF when smelt or trout was excluded. For legacy POPs (e.g. PCB-153), the TMFs were insensitive to the food web composition, as was also reflected in a better model fit for PCBs than for D5. Nevertheless, the present study documents food web biomagnification of D5 in the Lake Mjøsa food web, from zooplankton (*Daphnia* and *Calanus*) and Mysis to planktivorous (vendace and smelt) and piscivorous (trout) fish.

ET04A-6

Trophic transfer of decamethylcyclopentasiloxane (D5) in aquatic food webs in comparison to polychlorinated biphenyls used as benchmark compounds

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The potential of a chemical substance to accumulate in aquatic organisms and to increase in concentration with increasing trophic level are criteria that are used to classify substances as being bioaccumulative in the environment. Bioaccumulation is the accumulation of a substance in the tissues of a living organism through any route. Biomagnification is the increase in concentration of a substance in living organisms that are separated by a single trophic level step on a food chain. Trophic magnification, which describes the increase in concentration of a substance in living organisms that occupy successively higher trophic levels within a food web, is used to assess bioaccumulation and biomagnification of chemicals in the environment.

Sediments and aquatic organisms were analyzed to evaluate trophic magnification of decamethylcyclopentasiloxane (D5; CAS No. 541-02-6) in the freshwater food web of Lake Pepin (Minnesota, USA) and in the marine food web of Oslofjord (Norway). Isotopic signatures for the stable isotope of nitrogen (^{15}N) and carbon (^{13}C) were used to identify trophic level positions occupied by the organisms and to evaluate the flow of carbon in the food webs. Additional samples from Lake Pepin were also analyzed for the polychlorinated biphenyl materials 2,2',4,4',5,5'-hexachloro-1,1'-biphenyl (PCB-153; CAS No. 35065-27-1) and 2,2',3,4,4',5,5'-heptachloro-1,1'-biphenyl (PCB-180; CAS No. 35065-29-3), which are 'legacy' chemicals that are both known to bioaccumulate in aquatic organisms and biomagnify in aquatic food webs. Concentrations of D5 in Lake Pepin and Oslofjord were greatest in the lowest trophic levels and significantly decreased up the food web, with the lowest concentrations being observed in the highest trophic levels. In contrast, concentrations of PCB-153 and PCB-180 in Lake Pepin were lowest in the lowest trophic levels and highest in the highest trophic levels. These results indicated that trophic dilution of D5, not biomagnification, was occurring across the aquatic food webs. The high level of agreement for results between Lake Pepin and Oslofjord demonstrated that trophic dilution of D5 was not related to differences in the environment (freshwater vs marine) or to exposure.

ET04B-1

Sources and mechanisms of cadmium bioaccumulation by the freshwater decapod crustacean *Macrobrachium australiense*

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Metal bioaccumulation by aquatic invertebrates in the environment can often be explained by chemical parameters such as metal concentrations within the solution and particulate phases by exposure-dose-response relationships. Simple relationships of this type are not known for invertebrates in the highly turbid Strickland River, Papua New Guinea. Here, indigenous prawns demonstrate significant differences in bioaccumulated metal concentrations (predominantly cadmium) between populations exposed to mining effluents compared to those in reference tributaries. However, metal concentrations of waters and sediments are not significantly different between sites.

This study investigated the potential sources and mechanisms of cadmium (Cd) bioaccumulation by the freshwater decapod *Macrobrachium australiense* using ^{109}Cd -labelled

water and food sources. Synthetic river water (SRW) was spiked with environmentally relevant concentrations of Cd and prawns were exposed for seven days with daily renewal of test solutions. Prawns were subsequently allowed to depurate in Cd-free SRW for fourteen days. Dietary assimilation of Cd was assessed through pulse-chase experiments where prawns were fed ^{109}Cd -labelled fine sediment, filamentous algae and carrion (represented by cephalothorax tissue of water-exposed prawns). Radio-analyses during the exposures were used to determine influx and efflux rate constants for Cd in water, and the assimilation efficiency (AE) and efflux rate (K_e) of Cd from each dietary source.

Results indicated that *M. australiensis* readily uptake Cd from solution and that uptake rate increased linearly with increasing exposure concentration. During depuration, water efflux rates were low ($0.9 \pm 5\% \text{ d}^{-1}$) and were not dependent on exposure concentration. AEs of dietary sources were comparable for sediment and algae (approx 50 %), but lower for carrion (33 %) and efflux rates were low ($0.2\text{--}2.6\% \text{ d}^{-1}$).

The results demonstrated that prawns are likely to bioaccumulate Cd readily from both water and food sources. The rapid uptake but slow efflux of bioaccumulated Cd may explain why monthly or weekly measurements of Cd in water and sediments provide inadequate information regarding exposure or dose to explain metal accumulation patterns. A biokinetic model of Cd accumulation by *M. australiensis* is presented based on the findings.

ET04B-2

Bioavailability of dissolved organic carbon-complexed ^{65}Cu -DOC

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Stable Cu isotope (^{65}Cu) was complexed with various representative dissolved organic carbon (DOC) types, including coastal seawater DOC, fulvic acid (FA), cyanobacteria spirulina (SP) DOC, histidine (His), cysteine (Cys), and lipophilic diethyldithiocarbamate (DDC) at different concentrations. The uptake of these dissolved Cu species by the coastal green mussel *Perna viridis* was quantified for the first time. Copper complexed with different DOC types were taken up in some measure by mussels, depending on the DOC types. However, complexation generally reduced Cu uptake as compared to that of inorganic Cu species, and DOC type-specific negative relationships were found between DOC levels and Cu uptake. Strong Cu binding sites (including His and organic sulfur functional groups) within DOC appeared to control the inhibitory effects of DOC on Cu uptake, possibly due to the competitive binding of Cu between the dissolved phase and biological membranes. Therefore, differences in strong Cu binding site levels may explain the differences in bioavailability of Cu complexed with different types of DOC. At the same time, the variations in Cu-DOC uptake may also be partly attributed to the absorption of Cu-DOC complexes, especially for the small Cu-DOC complexes (e.g., Cu-Cys, His or DDC). Our study highlights the importance of considering the specificity of Cu-DOC complexes when assessing biological exposure to dissolved Cu in natural waters, especially during events, such as phytoplankton bloom periods, that could modify DOC composition and concentrations.

ET04B-3

A stratified probabilistic survey of mercury in biosentinel fish and Diffusive Gradient in Thinfilm devices in San Francisco Bay, CA, USA

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Although there are many regional programs to monitor contaminants in fish, few employ probabilistic survey designs, with hypothesis testing and comparison across biosentinel types. We surveyed mercury (Hg) concentrations in forage fish to determine spatial patterns in Hg bioaccumulation across 99 sites in San Francisco Bay. We also compared these results to concentrations in field deployed Diffusive Gradient in Thinfilm devices (DGTs), and determined Hg stable isotopes in fish and sediments, to evaluate potential sources of fish Hg. Results indicated that forage fish Hg and Hg isotopes followed a broad spatial gradient in the Bay. Fish Hg concentrations and $\delta^{202}\text{Hg}$ isotopes significantly declined with distance from the Guadalupe River, which drains historic mining areas. Fish and sediment Hg isotopes were highly correlated ($r = 0.91$), suggesting sediments derived from mining and other historic sources to be the primary source of Hg in the fish. Fish Hg concentrations were reduced at sites draining waste water treatment plants, suggesting biodilution due to nutrient inputs. Fish and DGT Hg concentrations were not well correlated, with DGT samples exhibiting high variability among nearby sites, and elevated Hg concentrations near locally contaminated sediments. In combination, these findings indicate that Hg methylation and bioaccumulation in San Francisco Bay involves decoupled processes operating on separate spatial scales. Whereas DGTs reflect processes operating at local scales, the ultimate provenance of fish Hg appears to be historic sediments, driven by broader regional gradients.

ET04B-4

The role of feeding habits in controlling the Hg bioaccumulation and biomagnification in freshwater tilapia

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The bioaccumulation and biomagnification of mercury (Hg), especially methylmercury (MeHg), is commonly found in aquatic organisms. The mercury level in fish is not only related to its surrounding water conditions but also the feeding habit as well, and predatory fish always has high Hg level because of the Hg biomagnification through trophic transfer. Whether the biomagnification potential (in terms of trophic transfer factor, TTF) could be influenced by feeding habit is an interesting but unknown question. To explore this issue, we conducted a 40-days Hg (both inorganic and organic form) accumulation experiments, by feeding freshwater tilapia with three types of Hg labeled food (aquatic grass, freshwater shrimps, commercial pellets) at a certain ingestion rate. The results showed great differences between inorganic Hg and MeHg accumulation patterns. More important, it revealed for the first time that the prey type could greatly affect the accumulation patterns as well as fish growth and the Hg biomagnification potential. The biokinetic parameters (assimilation efficiency and efflux rate) were further determined by using radioisotope technique, and provided a good explanation of the underlying mechanism. Moreover, a three-month field study was designed to investigate the influences of feeding condition to Hg accumulation process in tilapia under local conditions, by comparing the growth and Hg accumulation of fish feeding on commercial food and natural food. These results again showed the important role of feeding habit in controlling the Hg bioaccumulation and biomagnification process in fish.

ET04B-5

Dietary accumulation, maternal transfer and metabolic inter-conversion of methoxylated, hydroxylated and synthetic PBDEs

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Polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants, and the structurally related hydroxylated PBDEs (OH-PBDEs) and methoxylated PBDEs (MeO-PBDEs) occur in precipitation, surface water and in wildlife and humans. The occurrence of OH-PBDEs in wildlife and humans is of considerable concern due to their greater toxicities relative to PBDEs and MeO-PBDEs. Although the precise origin of OH-PBDEs has not been determined the dominant hypothesis has been that OH-PBDEs are formed by hydroxylation of PBDEs by phase I enzymes and MeO-PBDEs are then formed by methylation of the OH-PBDEs. The aim of this study was to investigate the hypothesized biotransformation or inter-conversion of PBDEs, MeO-PBDEs and OH-PBDEs. Specifically, we used 1) an *in vitro* approach with hepatic microsomes from metabolically activated rats, chicken and rainbow trout to characterize the metabolic relationships among these compounds; and 2) an *in vivo* approach to study the dietary accumulation, maternal transfer, and tissue distribution of PBDEs, MeO-PBDEs and OH-PBDEs in sexually mature Japanese medaka (*Oryzias latipes*). Results obtained both from *in vitro* and *in vivo* experiments demonstrated significant production of OH-PBDEs from MeO-PBDEs while metabolization of PBDEs to OH-PBDEs was negligible. It is hypothesized that this previously unidentified mechanism is the main contributors of OH-PBDEs found in wildlife and humans. These results suggest that risk assessment of PBDEs and their metabolites needs reevaluation and human exposure to MeO-PBDEs that occur naturally in marine organisms should be considered.

ET04B-6

Use of fugacity ratio methodology for assessment of bioaccumulation potential: PCB Congeners versus cVMS materials D4 and D5

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Participants in a 2009 SETAC Pellston Conference concluded that the fugacity ratio approach is a practical framework for decision making in chemical management and regulation, particularly with regard to assessment of bioaccumulation potential. A fugacity ratio expresses the status of chemical distribution relative to a state of chemical equilibrium, which is thermodynamically defined as a situation where the fugacity ratio is 1. A fugacity ratio greater than 1 therefore indicates that the chemical in the organism is able to achieve a higher fugacity (or chemical activity) than that in the medium to which it is exposed. In that case, the organism is able to magnify the chemical potential in its environment. A fugacity ratio less than 1 implies that the chemical concentration in an organism is less than its thermodynamic equilibrium value in the

medium to which it is exposed; this is essentially the opposite of biomagnification and is termed 'bidilution'. The application of the fugacity ratio methodology has a number of advantages. First, the technique can make use of all laboratory data for which bioaccumulation metrics exist and it can also use data from field monitoring programs. Secondly, the fugacity ratios are able to express the alphabet soup of bioaccumulation measures (i.e., BCF, BMF, BSAF, TMF) on a similar basis, i.e., as a dimensionless ratio. Third, the fugacity ratios can be visualized in a weight-of-evidence approach, which allows all available data to be viewed together and which also increases confidence in the outcome of the assessment. Most importantly, the fugacity ratio approach provides a formal method for testing the hypothesis whether biomagnification occurs by testing whether fugacity ratios are generally greater than unity (1.0). This approach has been applied to the cyclic volatile methylsiloxane (cVMS) materials octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), along with positive controls, such as PCB congener 153 (2,2',4,4',5,5'-hexachlorobiphenyl). The laboratory fugacity ratios of PCB 153 are >>1 for fish/water (BCF), fish/food (BMF), and benthic/sediment (BSAF). Field data also show fugacity ratios for PCB 153 greater than one in biota/water, biota/sediment, and in food webs. In comparison, similar laboratory and field fugacity ratios for D4 and D5 were less than 1.0

ET04C-1

OECD TG 305 under revision: effect of different extraction procedures for the determination of aqueous analyte concentrations on the result of fish bioconcentration studies

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Bioconcentration factors (BCFs) are usually determined in fish bioconcentration studies according to OECD TG 305. The standard method for water sample preparation in BCF tests is liquid-liquid extraction (LLE) providing total analyte concentrations. Highly hydrophobic organic chemicals (HOC, with log K_{ow} > 5) readily bind to organic matter (OM) leading to a reduced bioavailability for fish which may lead to an underestimation of the true uptake when total aqueous concentrations are measured. Solid-phase microextraction (SPME) enables the determination of total and freely dissolved aqueous concentrations. However, SPME is still a rather unusual method in regulatory ecotoxicological research. A validation approach was carried out to investigate the suitability of SPME for BCF studies.

In a first laboratory approach the effect of different types and concentrations of organic matter (Aldrich humic acid (AHA), fish feed, and filter residue taken from flow-through fish tests) on the bioavailability of highly hydrophobic organic chemicals (HOCs, with log K_{ow} > 5) such as hexachlorobenzene, o-terphenyl PCB 153, PCB 209, and benzo(g,h,i)perylene was tested. Analysis of samples with SPME was compared to conventional sample preparation with LLE.

The bioconcentration potential of the selected HOCs was then investigated in two flow-through fish tests with rainbow trout according to OECD TG 305. Analyte concentrations in the water phase were measured after extraction by LLE and SPME. Based on the results obtained steady state and kinetic BCFs were estimated for all test items.

The results of the flow-through fish tests show that the analysis of total aqueous concentrations is leading to comparable results when LLE and SPME (with internal standards correction) are applied. However, total organic carbon (TOC) concentrations in the test water should be kept as low as possible in BCF tests on HOCs to avoid the underestimation of the true uptake when total aqueous concentrations are measured.

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ET04C-2

The effect of pH on the toxicity and bioaccumulation of weak organic acids and bases

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The effect of pharmaceuticals and veterinary drugs in the environment has recently become an area of focus. In fact, the toxicity and bioaccumulation of many of the most common compounds have been documented. However, most pharmaceuticals are acids and bases. Of a data set of over 500 pharmaceuticals, it was found that 77.5% had a group that could ionize within the biologically relevant range. Because ions are more polar than their corresponding neutral counterparts, the biological uptake of these compounds is dependent on the pK_a value of the compound, and on the pH of the water in which they are dissolved.

Recent work has revealed that the toxicity and bioaccumulation of acids and bases may be over 100 fold higher at pH values that induce the more neutral state. For acids, compounds are more toxic and bioaccumulative at pH values that lie below the pK_a value of the compound while bases are more toxic and bioaccumulative at pH levels above the pK_a value of the compound. It has also been found that the pH effect is higher for those compounds with intermediate K_{ow}.

For an accurate risk assessment of these compounds it is therefore necessary to take the pH of the exposure solution into account. However, testing toxicity and bioaccumulation at multiple pH levels represents a great economic cost - particularly when it is considered that up towards 33% of the industrial chemicals registered for REACH are in fact ionizing compounds.

Based on a compilation of available pH dependent toxicity tests using ionizing compounds it is stipulated that a single worst-case test may suffice. For acids this would optimally be two pH units below the pK_a of the compound, and 2 units above for bases. For some compounds this will lead to tests outside biologically relevant range, wherefore a minimum of pH 6 and maximum of pH 9 is suggested for these tests. Acids with pK_a values below 2, and bases with pK_a values above 12 do not undergo pH dependent ionization in the biologically relevant pH range, wherefore standard test procedures for these compounds can be applied. The effect of pH on the uptake of zwitterionic and amphoteric compounds has not been investigated, but it is speculated that the highest toxicity will be found at the isoelectric point.

ET04C-3

Modelling specific mechanisms of bioaccumulation: protein binding and active uptake of surfactants

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Chemicals that bioaccumulate by specific mechanisms in addition to lipid/water partitioning reveal characteristic physico-chemical properties. The range of substances showing specific (i. e. active) mechanisms of uptake in the gastro intestinal tract (GIT) or specific protein binding include, for example, poly- and perfluorinated carboxylic acids.

Data describing processes and symptoms of specific mechanisms of bioaccumulation of surfactants (target chemicals), pharmaceuticals and industrial chemicals (reference chemicals) have been retrieved from the literature. Emphasis was laid on interactions (partition coefficients, rate constants and binding affinities) related to binding to serum proteins, surface activity and active transport phenomena.

Intercorrelations of relevant properties reflect similarities and differences between mechanisms of bioaccumulation of diverse chemical classes. Multivariate statistics are applied to discriminate the rate-limiting processes causing different bioaccumulation of surfactants, particularly perfluorinated carboxylic acids. The effects of solvent polarity will be addressed for different biophases (lipids, proteins, etc.) as well as the influence of critical micelle concentration (CMC). The known mechanisms of active uptake are specified with regard to chemical classes and organism groups. Possible dependencies between particular uptake mechanisms and intrinsic features of the chemicals are analysed.

Abraham-type modelling (LSER linear solvation energy relationship) has been applied to describe mechanisms of bioaccumulation in diverse partitioning systems, e.g. lipid/water, membrane/water, protein/water. LSER models explicitly consider the relevant contributions of hydrogenbondacceptors (α) and -donors (β) as well as effects of the molar volume of the solute relative to the respective solvent (V_I/100). Calculated LSER input parameters allow for prospective modelling based on mechanistic interpretation of relevant processes.

The modelling of specific mechanisms allows to predict the bioaccumulation potential of surfactants, e.g. poly- and perfluorinated carboxylic acids, based on process-based criteria for protein binding, surface activity and active transport phenomena. LSER modelling provides for a mechanistic interpretation of relevant processes.

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ET04C-4

Partitioning of organic chemicals to structural proteins

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Bioaccumulation of organic chemicals is typically described on the basis of the assumption that the partitioning into lipid phases is the dominant accumulation mechanism. A number of partitioning/binding studies have been reported for blood proteins such as serum albumin. However, there are only limited data available for partitioning of organic chemicals to structural proteins. These proteins are not made to bind chemicals, and thus their binding may be nonspecific, and the extent of the binding may be smaller than that of proteins that serve as a transporter or a receptor. However, relative abundance of structural proteins is generally high in organisms and thus partitioning to these proteins can be significant. In this study, binding of various neutral organic chemicals to several model structural proteins is evaluated with experimental and modeling approaches. Proteins from chicken and codfish muscle were similar in terms of binding of neutral organic chemicals. In contrast, binding by collagen was generally weaker than that of the muscle proteins. Binding to bovine serum albumin (BSA) measured before was generally stronger than that of the studied structural proteins. Thus, although serum albumin is often considered as a generic protein, our comparison suggests that the use of serum albumin as a model for structural proteins can substantially over-estimate the binding capacity of the bulk protein in organisms. To establish estimation models, a polyparameter linear free energy relationship (PP-LFER) model was calibrated using the measured partitioning data. Fitting of the PP-LFER model to these muscle proteins was substantially better than that to BSA. Finally, a composition-based

mass distribution model was used to estimate tissue-water distribution coefficients. Protein-water and lipid-water partition coefficients needed for this model were estimated using the PP-LFER models. The results suggest that the protein fraction could have little contribution to the accumulation of hydrophobic compounds even in lean tissues. In contrast, a high contribution of the protein fraction is estimated to the partitioning of some polar compounds to such lean tissues. The data and estimation models presented here will be useful to understand and estimate the accumulation capacity of organisms/tissues in varying compositions.

ET04C-5

Interaction of environmental contaminants with P-glycoprotein from trout

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P-glycoprotein (P-gp) is a membrane protein acting as cellular efflux pump of a wide variety of structurally unrelated chemical compounds. It confers "multixenobiotic resistance" (MXR) of cells and has an important role as "active barrier", preventing compounds from crossing tissue-compartment interfaces. There are two aspects of chemical interaction with P-gp: 1) By preventing chemicals from crossing compartment interfaces, such as from food into the blood-stream in the gut, bioconcentration rates are lower than would be expected from physico-chemical properties of a compound; 2) Chemical interaction with P-gp can lead to disruption of its function, thus rendering the P-gp barrier for chemicals dysfunctional. This could explain why bioconcentration of certain chemicals varies when present in combination with other compounds. There is recent evidence that P-gp from fish- in analogy to the well-studied homologs in mammals - also has properties as MXR transporter. However, test systems for functional studies of P-gp from ecotoxicologically relevant fish species are missing. We have recently established a cell line derived from RTL-W1, a rainbow trout (*Oncorhynchus mykiss*) liver cell line. The cells overexpress trout P-gp and were obtained by continuous exposure of RTL-W1 cells to low levels of vinblastine, a toxic P-gp substrate. Whereas P-gp is highly overexpressed in those cells, other efflux transporters are down-regulated compared to the wild-type cell line. Thus, these cells provide the opportunity to study specific interactions of chemicals with trout P-gp. As expected, the selected cells are substantially more resistant to the toxic cancer drug, doxorubicine, than the wildtype cells, as determined in cytotoxicity tests after 3 days of exposure to different doxorubicine concentrations. The selected cells were also more resistant to the pesticides, rotenone and metazachlor, indicating that P-gp activity may result in lower bioconcentration than would be expected. Pentachlorophenol and celestolide caused increased accumulation of the fluorescent transporter substrate calcein-am in the selected cells, indicating that those compounds disrupted P-gp activity, acting as chemosensitizers. Our selected trout cell line could be a useful component of a bio-tool kit for studies of ADME (adsorption-distribution-metabolization-excretion) processes of chemicals that are important to understand factors determining bioconcentration of chemicals in fish.

ET05 - Ecotoxicology and ecosystem services: a southern perspective

ET05-1

Regional scale risk assessment of threats to the yellowfish (*Labeobarbus* spp.) and the ecosystem services they provide in the Vaal River, South Africa's hardest working river

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The Vaal River is South Africa's most economically valuable aquatic ecosystems, and one of Africa's hardest working rivers. The ecosystem services provided by this system include a unique yellowfish dependent angling industry which is considered to be one of the largest and socially most valuable services of the system. Due to the excessive use of the Vaal River, many stressors occur which may pose a threat to the yellowfish populations of the Vaal River and the socio-economic values they offer to society. The nature and extent of threats by stressors to the yellowfishes in the Vaal River is largely unknown, and the current social and economic value of the yellowfish dependent angling industry in the Vaal River has not been documented. The aim of this study is to carry out a regional scale risk assessment of threats to the sustainability of the yellowfishes in the Vaal River and associated social and economic value of the yellowfish dependent angling industry in the system. The approach adopted to reach the aim includes the application of the relative risk model to assess threats by multiple stressors to yellowfish populations in nine risk regions in the Vaal River. Thereafter a socio-economic assessment of the value of the yellowfish dependent angling in the Vaal River has been undertaken. This information has been used to assess the threats to the yellowfish in the Vaal River and the ecosystem services they provide. Findings show that yellowfish in the Vaal River are not only of ecological importance as a part of the biodiversity, but of great social and economic value to South Africans as well. The seasonal economic value of the yellowfish dependant angling industry was determined to be worth over US\$16.7 million. Thereafter, the study shows that due to the excessive use of the ecological services in the Vaal River, this value of yellowfish is at risk of being impacted on by chemical pollution, flow alterations, habitat alterations and disturbance to wildlife stressors. Although currently conditions may be acceptable, trends show that if increasing use of the ecological services of the Vaal River continue the likelihood that the biodiversity of our country would be threatened and that many South Africans would suffer social and economic losses. To avoid these losses and possibly enhance the value of yellowfish as an ecosystem service in the Vaal River existing environmental management regulations should be implemented.

ET05-2

Ecotoxicological study about the effects of Endosulfan in the viviparous fish *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes)

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Ecosystem services such as purification of air and water are being impaired by many human activities such as unsustainable agricultural practices. The use of pesticides leads to the pollution of many aquatic habitats worldwide. Viviparous fish *Jenynsia multidentata* (Anablepidae, Cyprinodontiformes) is proposed as a bioindicator because its wide distribution along the Neotropical region of South America and inhabits polluted an unpolluted sites. The aim of this work was to develop an integral analysis of pesticide effects at different levels of organization in *J. multidentata* under Endosulfan (EDS) exposure. First, the acute toxicity (LC50) of technical-grade EDS was determined and histomorphological alterations in gills and liver was described and quantified. Second, sublethal toxicity tests (0.014-1.4 µg.L⁻¹) were carried out. As biomarkers of effect, response of detoxification and antioxidant systems (GST, GPx, GR, and CAT) and the effects on Lipid Peroxidation (LPO) were measured in different organs. Neurotoxic effects of EDS were evaluated through the AChE activity in brain and muscle. At behavioral level, swimming activity was recorded. As biomarkers of exposition, accumulation of technical-grade of EDS and Endosulfan sulfate (ES) was measured.

The LC50-96 was lower in males (0.72 µg L⁻¹) than females (1.32 µg L⁻¹). The histomorphological alterations in gills such as epithelial lifting are considered as defense responses to minimize the entry of EDS into the blood stream. In liver, the histological alterations varied between reversible changes at lower concentrations to irreversible changes at the highest concentration. At biochemical level, liver and brain were the most damaged organs and presented the highest levels of LP. At behavioral level, hypoactivity was observed in relation to the exposure time. These swimming activity changes were associated with the inhibition of AChE activity in muscle modifying the normal behavior of fish. The accumulation of the metabolite ES in all tissues indicate biological transformation of EDS. Moreover, measurement of technical grade EDS as well as ES is suitable biomarkers of exposure under field conditions under field conditions. If the stressful conditions persist, the mentioned alterations would lead to changes at population levels.

ET05-3

Effects of Chlorpyrifos exposure on growth and feed utilization in Australian catfish, *tandanus tandanus*

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The native Australian catfish *Tandanus tandanus*, also called the "eel-tailed catfish" is found in most river systems throughout the country and in pre-settlement times was a major food fish for indigenous Australians. It is an edible species that is still favoured by rural communities. It is also considered to have potential for aquaculture. However several attempts to develop and aquaculture industry for this species have failed to date. Their wild catch declined dramatically from "fairly abundant" during 1970-1971 to the species being declared "fully protected" in most of the regions of the country at present. Organophosphate (OP) pesticides are widely used in Australia and are therefore a common contaminant in the aquatic environment. These chemicals may have significant impacts on non-target aquatic species, including fish which are particularly sensitive to OP toxicants and though their effects on fish have been widely studied, in Australia, no study has been conducted on the effects of pesticides on the Australian catfish. Our study evaluated the effects of a pulse exposure of chlorpyrifos followed by subsequent recovery in optimum conditions on the growth and feed utilization of Australian catfish, *T. tandanus*, simulating field pesticide exposure conditions. *T. tandanus* were exposed to a short term pulse of chlorpyrifos at 2 or 10 µg L⁻¹ and grown in optimal conditions for 6 weeks. Growth and feed utilization of catfish were significantly impaired after exposure to chlorpyrifos. The hepato-somatic index of catfish exposed to 10 µg L⁻¹ chlorpyrifos was significantly elevated. The bi-weekly growth rate of exposed fish increased with time post-exposure and was correlated with the recovery of brain Acetylcholinesterase (AChE) activity. The results revealed that the fish were able to recover from the pulse of pesticide exposure since their growth rate was found to be as high as the control during the last 2 weeks. It is therefore possible, that poor growth performance resulting from exposure to OPs would be compensated for and fish recover if favorable conditions are prolonged. This implies the necessity for the reduction of repeated spraying of OP pesticides in the field and it is recommended that more such investigations be conducted on native, iconic food fish of cultural value.

ET05-4

Screening level ecological risk assessment of rewetted acid sulfate soils in the lower Murray River System, South Australia

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The River Murray, adjacent wetlands and the Lower Lakes (Alexandrina and Albert) in South Australia have been classified as wetlands of international importance under the Ramsar Convention, due to their unique ecological and hydrological significance. Prolonged drought due to the southward movement of the subtropical ridge atmospheric pressure system as a direct result of climate change, compounded by over allocation of water upstream, has resulted in very low water flows from 2005-2010. Consequently, the Lower Murray River system was being seriously impacted by a combination of low water levels and the presence of acid sulfate soils (ASS).

If detected and managed appropriately, ASS may not pose a major risk, however, if disturbed or left unmanaged, they pose risks to humans and the environment. To better understand the risks associated with rewetting of these ASS, and to provide recommendations for appropriate management options, a screening level ecological risk assessment was undertaken. The aim was to protect ecosystem services from the risks of ASS that may alter a balanced community of aquatic biota.

Risks to aquatic biota from stressors including metals, acidity, major ions and nutrients released from Murray River system dried soils that had been rewetted via rainwater or river water were determined. Predicted environmental concentrations, with correction for background water quality data, were calculated from metal release data for the soils in laboratory leachate experiments for current water levels (about -0.5 AHD) for four geographical areas using a range of exposure scenarios. These were compared to predicted no effect concentrations derived from hardness-corrected water quality guidelines, both acute and chronic. An overall risk ranking for each stressor at each location was determined, based on the likelihood and consequence of effects. This SERA provided the first semi-quantitative assessment of risks to aquatic biota associated with rewetting of ASS following climate change-induced drought.

ET05-5

Anthropogenic impacts on ecosystem services of a subtropical lake in southern Africa

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Lake Pongolapoort (LP), situated in the sub-tropical northern KwaZulu-Natal, South Africa (SA), is the recreation angling hotspots of SA and one of the few places in SA to catch Africa's most sought after freshwater angling species, the tigerfish, *Hydrocynus vittatus*. Due to the popularity of angling, tigerfish have a direct positive impact on the socio-economic wellbeing of the local community. As tigerfish in SA is listed as a threatened species, legislation requires that captured fish are released alive, (catch-and-release angling, C&R), however, the ultimate success of C&R angling depends on high release survival rates. The potential thus exist that, although the angling industry is a main ecosystem service of the LP, it might also be a major threat to the tigerfish population. Furthermore, the surrounding area of the LP is an endemic malaria area. The application of DDT in this area as a malaria-control measure has been ongoing since 1946. As relative high levels of DDT has been detected in LP tigerfish muscle, this might further impact on the health of LP tigerfish. The aim of this study was thus to quantify two of the most threatening anthropogenic impacts (recreational angling stress and DDT) on the tigerfish population of LP. A total of 109 *H. vittatus* were sampled in February 2009 (n=62), July 2009 (n=19) and April 2010 (n=28). Fish were collected by standard recreational angling and time to land and handle the fish were recorded. Fish were then anaesthetised in a 32 mg⁻¹ concentration of clove oil, 2m³ blood was drawn from the caudal vein and analysis for plasma glucose, lactate and cortisol concentrations. Axial muscle samples of 30 (February 2009) and 15 (July 2009) tigerfish were removed and analysed for DDT. A one-way ANOVA revealed that the plasma cortisol and plasma glucose concentrations were not significantly influenced by angling time (cortisol $P = 0.109$; glucose $P = 0.887$). However, there was a strong positive correlation ($r^2 = 0.749$) with an increase in total time causing an increase in lactate concentrations. Levels of DDT and metabolites were found in all the tigerfish muscle samples. A high DDE/DDT ratio indicated historical input of DDT into the system. The data presented here as well as additional data on tigerfish age and histopathology show that the tigerfish population of LP is under stress due to anthropogenic stressors, such as recreational angling stress and historic DDT application as a malarial control maganism.

ET05-6

The impact of gold mining on mercury pollution in the Witwatersrand Basin, South Africa

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Gold mining in the Witwatersrand basin, SA contains the biggest and richest mines in the world. Mercury, which occurs in some gold-bearing ores, was also used for gold recoveries till 1915 and is still used in artisanal mining. Presently some old gold tailings dumps are reprocessed. Consequences of these activities are the release of Hg to the environment.

Risk assessment of Hg pollution in SA is based on total concentrations which is not sufficient to understand its transport and fate. It is necessary to evaluate Hg speciation, and characterize potential sources, pathways, receptors and sinks in order to implement mitigation strategies and minimize risk.

The purpose of this work was to carry out an assessment of Hg pollution in the Wesr Rand areas with long history of gold mining and to investigate the Hg distribution, transport and fate.

Water, soil and sediment samples were collected during the late dry season in mining site located on the West Rand and through the game reserve to the Cradle of Humankind. Samples were prepared using Rpodriguez Martin-Doimeadios et al. method [2], and Hg species, inorganic (IHg) and methylmercury (MHg), were determined by Isotope Dilution GC-ICP-MS validated with certified reference material (IAEA405).

High Hg concentrations were found in surface water (0.01 - 220.63 ng IHg L⁻¹ and 0.04 - 2.12 ng MHg L⁻¹) and sediments (46 - 2090 ng IHg g⁻¹ and 2 - 5 ng MHg g⁻¹) (Fig.1). Mercury enrichment was observed in bulk sediment reaching 3900 ng IHg g⁻¹ and 83 ng MHg g⁻¹ at 20 cm depth.

Field parameters measurements in water samples have demonstrated a typical case of acid mine drainage with high redox potential, high electrical conductivity and the pH dropping as low as 3.

High Hg concentrations found in this study suggest that post-gold-mining operations may be important contributors to the pollution of stream draining the mining site and affect the nearby game reserve and precious Cradle of Humankind. Mercury enrichment in bulk sediments also confirms the long term pollution of the site. Organic matter and sulfur contents together with redox potential and pH appear to be important contributing factors to the methylation process. Methylation of Hg is occurring in the water system and allow MHg to enter the food web.

ET06 - Ecotoxicology of amphibian and reptiles. Novel approaches for linking contaminant effects with population declines

ET06A-1

Changes in morphology, behaviour and energy reserves of the African Toad *Bufo regularis* exposed to lethal and sublethal concentrations of Diazinon

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The lethal and sublethal toxicity of the insecticide diazinon on the African toad, *Bufo regularis* were evaluated to assess changes in morphology, behaviour and energy reserves. The median lethal concentration of the insecticide was found to be 0.44mg/l indicating the high toxicity of the insecticide. Uptake of the pesticide by the toads caused dose-dependent deformities and behavioural abnormalities. More pronounced poisoning symptoms were observed at higher concentrations. The pesticide caused differential increase in glucose levels in the blood with a concomitant reduction in liver glycogen. The increase in glucose levels and the reduction in glycogen levels indicate disorders in carbohydrate metabolism due to pesticide induced stress. The findings revealed that changes in morphology, behaviour, glucose and glycogen levels could be considered suitable biomarkers in evaluating the effect of diazinon.

ET06A-2

Combined effect of malathion and nitrate on survivability of tadpoles of Indian cricket frog *fejervarya limnocharis*

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We have examined individual and combined effect of realistic concentration of Malathion and nitrate on the survivability of Indian Cricket Frog tadpoles. In this fully factorial experiment, tadpoles were subjected to individual and combinations of 0, 500, 1000 and 2000 µg malathion/L and 0, 4 and 8 mg NO₃-N/L. When tested alone, malathion found to be detrimental to tadpoles and reduces the survivability, while nitrate increase the survivability. The response exhibited at the combined treatment does not follow the trend recorded at different concentration of each of the tested chemical. Presence of nitrate enhances the effect of Malathion at lower concentration, while at combination of higher concentrations of nitrate and Malathion, the trend of survivability was determined by Malathion alone. Since the breeding season and tadpole stage of this frog coincide with application of pesticide and nitrate, there is every possibility that these combinations could affect frog population in agro-ecosystems.

ET06A-3

Amphibians at risk ! Susceptibility of terrestrial amphibian life stages to pesticides

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The 'global amphibian decline' is manifested with more than 30% of all amphibian species on some level of threat according to the IUCN. Pesticides are considered one factor for the reduction of species and observed population declines. Pesticides reach aquatic habitats by drift and runoff whereas they are applied at high field rates in crops where they adhere to soil particles and plants. Although amphibians are depending on water bodies for their development the majority of their adult life cycle is spent in the terrestrial habitat. So far no specific risk assessment is carried out for the terrestrial life stage of amphibians since it is assumed that they are covered by the procedures in place for birds and mammals.

We present data for presence of amphibians in two different agricultural landscapes in Germany and discuss the difference in skin properties and uptake between amphibians and mammals. An extensive review (Brühl et al. 2011) revealed that only few toxicological data exist for amphibians that were conducted to mimic an overspray scenario and dermal exposure of amphibians.

This research gap was filled by conducting rate-response test with different pesticides and juvenile Common Frogs (*Rana temporaria*) using a spraying chamber and realistic field rate application scenarios. Observed mortalities at field rates were situated between 20 and 100%. There was no difference between fungicides, insecticides and herbicides. Furthermore it remains unclear if the observed toxicity is based on the active substances or the solvents in the formulations or a combination of both.

We conclude that terrestrial life stages of amphibians are present in agricultural landscapes and crops during application of pesticides. Passage of pesticides through a permeable skin is much higher than for mammals and therefore the dermal uptake route is the most likely exposure route for amphibians. So far this is not considered in bird and mammalian risk assessment where the focus lies on oral uptake.

Our data set for the toxicity of a few registered pesticides reveals alarming mortality levels at the field rate. Therefore current risk assessment procedures are not protecting amphibians sufficiently. Hence carefully designed studies are urgently needed and it is imperative to develop a specific risk assessment procedure for amphibians to avoid further impacts on amphibian populations associated with agricultural areas.

ET06A-4

Ranking ecological risk of multiple stressors on amphibians

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In this study we rank the potential threats to amphibians in their actual and potential habitats in the Netherlands. The study combines laboratory toxic effect data (acute median Lethal Concentrations, LC50) for anuran species with environmental monitoring data for different stressors and actual survey data of anurans in the Dutch freshwater bodies. Combining all the data together, the ecological risk (ER) was calculated for ammonium, nitrate, cadmium, copper, pesticides (18 different compounds), and acidification (pH) as a probability that anuran species are exposed in the field to stressor concentrations exceeding their LC50s. The total ER at the LC50 level, as calculated for Dutch freshwaters was found to be 2.73%. We ranked the stressors in the decreasing order of their ER: pH, copper, diazinon, ammonium, and endosulfan. Taking into account the bioavailability of copper, the ER for it might be reduced. Therefore, the main threats affecting anurans populations arise from acidification of their habitats, ammonium, and certain pesticides exposures. These results are valuable for conservation management of amphibians when prioritization for mitigation of stressors is needed. The method of deriving the ER is a useful tool for location-specific risk assessment of multiple stressors for selected amphibian species and can be applied in other locations for species of concern.

ET06A-5

Are standard avian risk assessments appropriate tools addressing the risk to reptiles?

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According to the new data requirements under the current EU regulation 1107/2009, 'available and relevant data, including data from the open literature for the active substance of concern, regarding the potential effects of an active substance to birds, mammals, reptiles and amphibians should be presented and taken into account in the risk assessment.'

Whereas for birds, mammals and amphibians specific data requirements are available, nothing is stipulated for reptiles. As stated in the Guidance of EFSA - Risk Assessment for Birds and Mammals (2010): 'information should be provided to enable an assessment of the direct impact on birds and mammals likely to be exposed to the active substance, plant protection product and/or its metabolites. In order to determine the risk, toxicity data are taken, along with an estimate of the likely exposure concentrations'. The guidance provides a tiered approach to assess both, direct acute and reproductive risk to birds. Therefore one approach for reptile risk assessment may be the most recent state of the art risk assessment applied for birds, taxonomically related to reptiles. The necessary backgrounds for such an approach, however, are reliable data on the exposure and toxicity of PPP. For reptiles such data and generic data recorded in agricultural landscapes are nearly completely missing. For that reason publically available literature was searched in order to obtain information on the ecology of reptiles in agricultural areas in North and Central Europe, relevant exposure routes, and any toxicity data of PPP may affect reptiles. The results were used to evaluate and discuss the viability of the avian risk assessment approach for reptiles. Furthermore it shows potential data gaps that need to be fulfilled to successfully implement reptile risk assessments.

ET06A-6

Composition of the herpetofauna in intensively managed monocultures in Spain

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According to the new data requirements under the EU regulation 1107/2009 reptiles and amphibians have to be addressed in risk assessments of plant protection products (PPP). But little information is available about the herpetofauna in agricultural landscapes, especially in intensively managed monocultures. However, it is important to know which reptile species occur in relevant densities inside agricultural habitats. During 2007-2009 orchards, field crops and vineyards in different parts of Spain were visited from early spring- to mid summer, and reptiles and amphibians were recorded. Overall 4 amphibians and 14 reptile species were detected. Mentionable differences were observed between the different survey areas and crops (from 6 species in fruit orchards up to 13 species in citrus orchards). The species found were classified in four different diet guilds (herbivorous, omnivorous, insectivorous and carnivorous). None of the detected amphibian and reptile species is exclusively herbivorous. All of the detected amphibians and the most lizards can be assigned to the insectivorous guild. Only the Mediterranean turtle (*Mauremys leprosa*) and the ocellated lizard, *Timon lepidus*, have to be treated as omnivorous. All of the snake species feed on vertebrates. Available habitat data given in the IUCN Red List (IUCN 2011) and by Fryday & Thompson (2009) was compared to our findings per species. The grass snake, *Natrix natrix*, was the only species that might be at risk of exposure by PPPs according to Fryday & Thompson (2009), and found during our surveys. Because of the obvious differences between published data and our observations further field studies are needed to obtain quantitative data on species compositions of the herpetofauna and densities of possible focal species for risk assessments for PPP in agricultural land in Spain.

ET06B-1

Thermal tolerance of amphibians and their invasive predator in a polluted environment

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It is clear that many threats such as habitat loss, chemical pollution and global warming are contributing to both local and global amphibian declines. Yet aside from a few examples, we still know very little about the role and magnitude of synergistic effects among these stressors. Demonstrated in marine ectotherms, thermal tolerance is limited by the incapability of an organism's circulatory and ventilation systems to supply sufficient oxygen to match with its oxygen demand (i.e. aerobic scope). Beyond certain critical temperatures, survival of aquatic ectotherms becomes passive and time-limited, relying heavily on anaerobic metabolism and molecular protection (e.g., induction of heat shock proteins). Detoxification is known to be an energy-demanding process. It is thus likely that an organism under thermal stress will suffer further decrease in aerobic scope and loss of performance in a contaminated environment, which may in turn threaten their survival. Our study thus focused on the relationships between pollution and thermal stress on three lowland wetland amphibian larvae in South China, namely Asian common toad (*Bufo* sp.: *Duttaphrynus melanostictus*), brown tree frog (*Rhacophoridae*: *Polypedates megacephalus*) and marbled pigmy frog (*Microhylidae*: *Microhyla pulchra*), and the relative performance of the invasive fish predator *Gambusia affinis* as opposed to local larval amphibians. This study determined and compared lethal concentrations of two commonly used agricultural pesticides, glyphosate and methomyl, on the target amphibian and fish species, as well as lethal temperatures of these animals. The investigation on the synergistic effects of thermal stress and pesticides is currently being conducted using integrated measurements of physiological end-points and biomarkers including growth rate, oxygen consumption rate, and expression of heat shock protein and lactate dehydrogenase. Our preliminary results showed that pesticide tolerance varied highly between the three amphibian species, with *D. melanostictus* being the most tolerant, followed by *P. megacephalus* and *M. pulchra*, and that the invasive predator *G. affinis* had a wider thermal tolerance window (37.16°C) than *P. megacephalus* (33.97°C) and *M. pulchra* (34.36°C). Further information generated from this study on the synergy between pollution and thermal stress will aid in developing conservation measures to better protect amphibian populations against these rising threats in South China region.

ET06B-2

Effects of a mixture of agricultural pesticides and estrogenic compounds on developing tadpoles

Acute and chronic toxicity tests with the Australian striped marsh frog (*Limnodynastes peronii*) and the cane toad (*Rhinella marina*) were undertaken to determine if the combination of common agricultural pesticides (glyphosate, atrazine, endosulfan) and estrogenic compounds (4-nonylphenol and estradiol) at environmentally realistic concentrations effect tadpole development and survival. There is a growing prevalence of these combinations occurring in agricultural landscapes through the application of biosolids as fertilizer. Findings indicated that complex mixtures reduced survival, and impaired growth and development compared to the individual chemical exposures. The presence of estradiol appeared to have little influence on these endpoints. Endosulfan was found to be the most toxic compound in our mixtures, causing erratic swimming behaviour and darkening of the skin following exposure to this compound alone and in mixture. However, the acute toxicity was only enhanced with the presence of sub-lethal concentrations of the other compounds. Further research will help identify specific mixtures and chemical ratios causing effects, but our results support the hypothesis that complex mixtures at sub-lethal concentrations cause detrimental effects on developing tadpoles.

ET06B-3

Effect of the acute exposure (96 h) to xenoestrogens on 'Lithobates catesbeianus' tadpoles

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The aim of this study was to evaluate if a concentration of 17-alpha-ethinylestradiol (EE2) normally found in Brazilian industrial effluents can exert some impact on the cardiac function of bullfrog tadpoles (25 Gosner stage), *Lithobates catesbeianus*. To this end, animals were exposed for 96 h to 10 nM of EE2 and their responses were compared to those obtained to controls. During exposure, the animals' activity level (AL - % of active individuals) was monitored twice a day. Immediately after exposure, the in loco heart rate (fH - bpm) was determined. Afterwards, animals were sacrificed and the ventricles were removed for the preparation of ventricular strips to record isometric contractions (Fc - mN. mm⁻²). The results indicated that EE2 did not affect tadpoles' AL (P > 0.05), although it resulted in a tachycardia in animals exposed to EE2 (fH = 66 bpm) when compared to their controls (fH = 52 bpm). These findings suggest that EE2 may act directly on the cardiac muscle of bullfrog tadpoles, rather than its effects being a result of an increased cardiac demand due to a higher activity level in order to the aversive stimulus of the exposure to the xenoestrogen (i.e., avoidance response). Additionally, the comparison between the values of Fc between the two experimental groups showed that the xenoestrogen exerted a positive inotropic response. Thus, it can be suggested that the increase on cardiac performance induced by the exposure of bullfrog tadpoles to this xenoestrogen elevates considerably the animal energy expenditure, diverting a large amount of energy that tadpoles could use for their growth and development. These alterations make the animals more susceptible to predators and also reduce the likelihood that they will reach reproductive stage.

ET06B-4

Novel methods to assess health of amphibian populations: preliminary results

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It is now widely accepted that amphibians are undergoing precipitous decline on a worldwide scale. In some cases reasons for decline are known (e.g. habitat loss, chytridiomycosis infection, invasive species), however, specific causes in the majority of cases are not well understood. An important consideration in the study of populations is to focus on those that are in decline in order to assess causative factors. However, it is also unethical to harm individuals from these populations, and therefore there is a need to develop non-destructive methods to assess population health. We identified two sites with differing levels of pesticides (NP = non-polluted; P = polluted; characterised by analytical chemistry), and measured various endpoints related to "health" and "reproduction" of the common toad, *Bufo bufo*. We also sacrificed a subsample of individuals in order to correlate non-destructive biomarkers with more traditional markers of effect (blood hormone levels and gonadal histopathology). We found that weight and snout-vent length were lower in individuals from the polluted site (n = 32, t-test p < 0.001). Interestingly, corticosterone levels were also higher in the P group (n = 20, t-test p = 0.07), and a positive correlation of weight and corticosterone levels was observed in the P (n = 10, r² = 0.43, p = 0.04) but not the NP (n = 10, r² = 0.06, p = 0.5) site. In addition, the fat body weight (FBW) was decreased in the P (p = 0.09), however, weight was not correlated with FBW (n = 20, r² = 0.01, p = 0.15). Neither chytridiomycosis nor Ranavirus were detected in any individuals. Contrary to a previous report in *Bufo marinus*, there was no difference in the sexually differentiated forelimb width nor nuptial pad size according to pollutant input. There was also no difference in relative testis size, however, relative Bidders' organ size was larger in individuals from the P site (n = 20, p = 0.06). Analysis of the testicular histopathology is ongoing. In addition, no difference in sperm parameters or testosterone levels were observed, which were also not related to any morphological androgen sensitive endpoints (forelimb width, nuptial pad size, testis size). Preliminary results indicate that survival parameters, such as size and "stress" may be affected by agricultural input, however, minimal effects on reproductive parameters were observed. The increased Bidders' organ size may be due to the higher levels of estrogens at the P compared to the NP site.

ET06B-5

Screening breeding ponds of the Common toad (*Bufo bufo*) in the UK for evidence of endocrine disrupting activityB. Pickford¹, N. Mitsui², T. Oka³, O. Tooti⁴¹Brunel University, Uxbridge, United Kingdom²Institute of Environmental Ecology, IDEA Consultants, Inc., Shizuoka, Japan³National Institute for Environmental Studies, Tsukuba, Japan⁴Japan Environmental Management Association for Industry, Tokyo, Japan

To date, there has been no attempt to assess the potential impacts of endocrine disrupting contaminants of the aquatic environment on wild populations of native amphibians in the British Isles, though this group has suffered declines in the agricultural landscape. We selected nine breeding sites of the Common toad (*Bufo bufo*) in the UK on the basis of predicted surface concentrations of agrochemicals and past population trajectories based on counts of adult toads in the breeding migration. Samples of spawn were enclosed in mesh cages, and hatching, survival, growth and development were monitored through larval development. Larvae and toadlets were sampled at several timepoints for gonadal histopathology at five of the study sites. We also deployed semi-permeable membrane devices (SPMD) and Polar Organic Chemical Integrated Samplers (POCIS) at all sites for 4 weeks and screened extracts for endocrine disrupting activity in recombinant Yeast Estrogen Screen (YES) and Yeast Androgen Screen (YAS), and in primary monolayer cultures of *Xenopus* hepatocytes for estrogen-dependent induction of vitellogenin. None of the SPMD extracts exhibited estrogen receptor or androgen receptor agonist activity. SPMD extracts from many study sites displayed weak anti-androgenic and anti-estrogenic activity in the YAS and YES, respectively, though similar activity was observed in control membranes. Among POCIS extracts, two sites exhibited concentration-dependent androgenic activity in the YAS, while three other sites (two in areas of intense arable cropping) exhibited significant estrogenic activity in the YES. Extracts from the same three sites also exhibited estrogenic activity in the *Xenopus* hepatocyte assay. Hatching rate varied among sites, but could not be fully explained by predicted surface water concentrations of pesticides, conductivity, pH or endocrine activity of the extracts. Overall survival/persistence of *Bufo* larvae in the cages was poor, which could reflect poor ability to feed, predation, or escape from the mesh cages. The sites with lowest larval survival had medium-to-low total predicted surface water concentrations of agrochemicals. Most of the sampled larvae had undifferentiated gonads, as did many of the toadlets. There were no significant differences in sex ratio among the study sites sampled for histopathology, at any of the sampling points, and there was no evidence of regressed or matured testicular oocytes in any of the sampled toadlets.

ET06B-6

Investigating the threat from pollution to the endangered species *Caretta caretta* (Linnaeus, 1758) in the Mediterranean: the use of novel non-invasive biomarkersS. Casini¹, I. Caliani¹, M. Giannetti¹, S. Maltese¹, D. Coppola¹, N. Bianchi¹, T. Campani¹, S. Ancora², L. Marsili¹, M.C. Fossi¹¹University of Siena, Siena, Italy²Siena University, Environmental Sciences Department, Siena, Italy

The aim of this study was to develop and apply a non-invasive protocol, integrating biomarker responses and a contaminant analysis, in order to investigate the threat from pollution to *C. caretta* of the Mediterranean. For the study, forty-five specimens of this endangered species were sampled in a non-invasive way in Italian Italian marine rescue centers or free-ranging along the Spanish coasts (Murcia region). The specific goals were to: a) develop a non-destructive sampling methodology of various biological materials; b) develop and apply a set of ecotoxicological biomarkers in order to explore different levels of interaction between contaminants and the organism, from biomarkers of exposure (induction of CYP1A in skin biopsies, accumulation and excretion of porphyrin) to biomarkers of indirect and direct effects by investigating neurotoxic (esterases inhibition in plasma) and estrogenic (vitellogenin in plasma) effects, oxidative stress (lipid peroxidation in plasma), genotoxicity (Comet, diffusion and ENA assays in blood) and liver damage (ALT, AST and γ -GT in plasma); c) investigate levels of contaminants mostly present in the Mediterranean (OCs and PAHs in blood; Pb, Cd, Hg in carapax); and finally, d) to elaborate biomarker and contaminant responses taking into consideration age and sampling site. The non-invasive sampling methodology was successfully developed and applied. Appropriate quantities of blood, skin biopsy, carapax and excreta were collected, for the successful development and application of biomarker and contaminant detection methods. A statistically significant correlation was found between cadmium and γ -GT levels. Induction of skin CYP1A, as well as high comet assay response and total PAH levels were found in two young specimens sampled off-shore of the Calabrian coasts (Brancalione) after an oil spill. Analysis of the results by classes of age showed that levels of PAHs and Hg tend to increase with age. The same trend was found for lipid peroxidation and ENA assay responses. The analysis of results by sampling areas showed that BChE had significantly lower levels of activity in specimen samples along the Murcia region coasts, an area of intense agricultural activities. We believe that the findings of this study contribute significantly to developing non-invasive protocols to study the threat to *C. caretta* from

ET07 - Environmental OMICs: a global answer to environmental questions

ET07A-1

Environmental proteomics to predict the effects of global warming on aquatic organisms exposed to pollutants

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Beyond shifts in species distribution, most organisms are forced to acclimate or adapt to long-term changes in temperature and frequently exhibit temperature compensation mechanisms. Moreover, aquatic species are also subjected to polluted environments. It is consequently necessary to assess the combined effects of heat stress and xenobiotic exposure for predicting the impacts of global warming on wildlife. We report here the results from two studies on the proteomic responses in fish species under combined heat stress and pollutant exposure. The first study aimed at identifying proteins whose expression has been modified by temperature and selenium (Se) exposure in larval green (GS) and white sturgeons (WS). The aim of the second study was to investigate whether acclimation to 3 temperatures may lower or enhance the ability of European bullhead to tolerate subsequent exposure to cadmium (Cd). In GS and WS, gel replicates were first grouped according to heat treatment. Proteins involved in protein folding, protein synthesis, protein degradation, ATP supply and structural proteins changed in abundance in response to heat and/or Se. In bullhead, thermal acclimation was the first parameter affecting the protein expression profile, while fish's ability to respond to Cd was clearly affected during acclimation to 21°C. The identified differentially expressed proteins were associated for instance with protein turnover, folding and chaperoning, transmembrane transport, metabolic regulation, cell signalling, and cytoskeletal reorganization. This work provides insights into the interactive effects between heat acclimation and a subsequent exposure to Cd, and suggests that further studies on the identified proteins could offer essential information to better understand the mechanisms of action shared by these two environmental stresses. In conclusion, we showed using two distinct experiments that proteomics is a suitable tool to investigate the effects of heat stress on the cellular phenotype. A specific proteome could be defined for each stress condition, and likely represents the cellular responses to specific mode of action. When cross-tolerance is observed, those proteomic signatures can bring clues to understand shared mode of action. The opportunity to focus on this cellular phenotype acquired during acclimation is discussed in the perspective of evolution theories and should be taken into account in order to predict the effects of global warming on wildlife.

ET07A-2

The Concentration-Response Concept in Proteomics: effects of a narcotic (Phenanthrene) and a specific acting Substance (Gemfibrozil) to the Proteome of Zebrafish Embryos (*Danio rerio*)

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Proteomics studies in ecotoxicology have the potential for the detection and quantification of proteins related to chemical stress. These proteins might serve as novel biomarkers and provide insights into mode of action or mechanisms of toxicity. As studies based on 2-DE are comparatively elaborate, time-consuming and expensive, the finding of robust biomarkers is limited by the numbers of replicates, statistical methods and also by the experimental design. For these reasons, many published proteomics studies in the area of (eco-) toxicology are confined to one or two tested concentrations. As reactions at the molecular level are expected to be a function of exposure concentration, the analysis of few concentrations might direct the experimenter to fragmentary or incomplete conclusions. In the present study an experimental design for a proteomics study was tested for detection of induced protein regulation. This design included the analysis of concentration dependence without increasing the replicate number of 12 parallel 2D-gels. The tests were done using the zebrafish embryo as a vertebrate model of teratogenicity. To test whether the proteome analysis is able to discriminate between substances with different mode of action (MoA), the substances Phenanthrene (narcotic MoA) and Gemfibrozil (specific MoA) were selected for the proteomic tests. For the 2D-Gelelectrophoresis experiments, *Danio rerio* embryos were exposed to six different concentrations ranging from 1% of LC50 to the LC20 (Gündel et al. 2011). The selected concentrations were based upon a teratogenicity assay with zebrafish embryos accomplished in closed glass vessels. Proteomics analyses were done by 2D-DIGE technique (Marouga et al. 2005) with minimal CyDye labeling. Protein abundance profiles of around 700 signals were studied in the Phenanthrene exposure tests. About one third of the protein spots could be detected to show reactions correlating with stressor concentration. From this group about 70 protein spots already showed changes beginning at low and very low effect concentrations. In this study it could be shown that increasing the number of measured concentrations instead of replicate numbers in 2D-Gelelectrophoresis experiments, the detection of robust stress markers was possible. From these proteins many may be already detectable at very low lethal effect concentrations such as the LC01. In this way the possible output of proteomics experiments may be increased considerably.

ET07A-3

Integrative environmental genomics of cod (*Gadus morhua*) reveal the mechanisms underlying MeHg- and PCB 153 induced toxicity

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Atlantic cod (*Gadus morhua*) is an important species in both North-Atlantic fisheries and aquaculture. However, coastal cod populations are highly stationary, and are therefore particularly susceptible to environmental insults. Coastal- and petroleum industry are expanding into cod habitats, and limited information exist on the effects on cod to both acute- and long-term exposure to several environmental contaminants. Importantly, in order to monitor and maintain sustainable coastal cod populations, it is necessary to understand how such contaminants may affect growth, reproduction, and health of this species. Methylmercury (MeHg) and PCB 153 have several properties in common. Both compounds act as neurotoxins, they are persistent environmental pollutants, and ubiquitous contaminants that are biomagnified in aquatic food chains. To develop a deeper understanding of transcriptional and translational responses of the cod genome to MeHg and PCB 153, we have initiated a toxicogenomic approach combining transcriptomics, proteomics, and bioinformatics, as an attempt to integrate these responses into mechanistic insights. The liver proteomes of MeHg- and PCB 153 exposed cod have been resolved with both gel-based and LC-MS/MS based methodologies, while the corresponding liver transcriptome from these samples have been analyzed with microarray analysis using a cod-specific array. We have simultaneously established an in-vitro exposure system using cod liver slices for accompanying the in-vivo exposure experiments. Moreover, this interdisciplinary study has provided detailed information regarding the modes of action of both MeHg and PCB 153, including responses in several genes previously not known to be affected by these compounds. The differentially regulated proteins are also candidates for new and more sensitive biomarkers for MeHg- and PCB 153-exposure for use in environmental monitoring and risk assessment.

The project iCod: integrative environmental genomics of cod (*Gadus morhua*) is funded by the Norwegian Research Council (project 192441/I30). Thanks to the Genofisk Consortium and the Cod Genome Sequencing Project Team for sharing data in advance of the public release of the cod genome data (www.codgenome.no).<br type='moz' />

ET07A-4

In vitro exposure of nanoparticles in mouse: an integrated proteomic and lipidomic study

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"Omics" is a rapidly expanding technology in both industry and academia. Its development can provide a powerful tool to promptly detect complex biological problems caused by a multitude of stressors, and contribute to the comprehension of eventual unknown mechanisms of toxicity.

Nanoparticles (NPs) are emergent compounds characterized by fast-growing research due to their potential applications in a myriad of areas as diverse as electronics, cosmetics, pharmaceuticals, environmental devices.

Numerous products NP-based are already available commercially although contradictories effects have been shown from the recent scientific studies.

The aim of this project was to elucidate the possible biological effects on primary cultures of mouse hepatocytes exposed to inorganic (TiO₂, CuO, ZnO and Ag) NPs.

Briefly, the nanoparticles were characterized by scanning electron microscopy (SEM) and Z-potential analyses. The cell cultures of mouse hepatocytes were exposed to the selected NPs at 1 and 5ppm concentrations for 48 hours.

Reactive oxygen species (ROS) production was measured by cell-free dichlorofluorescein diacetate (DCFH) assay and the treated/untreated hepatocytes were analysed and compared by proteomics and lipidomics.

The cytosolic proteome was analysed by two-dimensional fluorescence difference gel electrophoresis (2D-DIGE) and Coomassie staining. The differentially expressed proteins were characterized by image analysis, and identified by mass spectrometry.

Lipids were separated by one-dimensional thin layer chromatography and the major phospholipids and neutral lipids were quantified by optical densitometry.

The combining of proteomics and lipidomics presented in our study is a novel approach for the detection of the impact of NPs in biological system. Although proteomics would inform about the protein expression, biological processes such as signal transduction pathway, receptor recognition, membrane protein translocation would be seriously affected by modification in the lipid composition.

ET07A-5

Altered protein expression in stickleback (*Gasterosteus aculeatus*) gills after silver nanoparticle exposure

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Engineered nanoparticles (ENPs) are rapidly becoming an important part of new technology and are today used in a variety of products, from computers to pharmaceuticals and body care products. Silver nanoparticles (AgNP) are the most widely used nanoparticle, especially due to its antimicrobial properties. AgNP may be introduced into the aquatic environments during production processes and from hundreds of products already present in the market. There are numerous potential environmental risks of AgNP that needs to be elucidated including its toxicity. Silver ions are toxic to fish due to their ability to bind to thiol groups inhibiting enzymes such as Na⁺/K⁺ ATPases that are extremely important to the gills osmoregulatory function. In order to study the effects of AgNP in fish, sticklebacks (*Gasterosteus aculeatus*) were exposed to AgNP (0,1 mg/L) and silver ions (0,01 mg/L) in a freshwater semi-static system for seven days. Gills samples were taken for proteomic studies, transmission electron microscopy (TEM) and Na⁺/K⁺ ATPase activity measurements. The behavioral (e.g. agglomeration rate) of the AgNP in the test water and levels of ionic and total silver were monitored throughout the experiment. Amine-reactive TMT Isobaric Mass Tagging was used to quantitatively label proteins extracted from gills for identification and analysis by mass spectrometry (TMT sixplexTM label reagent set, Thermo Scientific). Proteins were identified with the help of a Stickleback specific proteome database. Ionic silver exposure resulted in more regulated proteins compared to the AgNP exposure. 116 proteins were regulated in the Ag⁺ exposed gills (53 down and 62 up) while only 43 were regulated in the AgNP exposed (22 down and 21 up). Among the regulated proteins only nine proteins were equally affected by both treatments meaning that 34 of the observed protein regulations were AgNP specific. Several of the regulated proteins in the AgNP exposed fish have a function in cell structure and cytoskeleton. Also, a mucus forming glycoprotein (mucin 2) was up-regulated in the AgNP exposed fish gills suggesting that the gill epithelial cells were stressed. The presence of AgNP in gills cells was detected using TEM. This study was supported by FORMAS and proteomic analysis performed in collaboration with the proteomics core facility at Gothenburg University.

ET07A-6

Transcriptomic responses in Japanese medaka (*Oryzias latipes*) exposed to individuals or mixtures of polycyclic aromatic hydrocarbons: mixture toxicities or fasting effects?

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In the present study, we assessed the effects of single or mixtures of polycyclic aromatic hydrocarbons (PAHs) by transcriptome analysis. Four-week-old Japanese medaka (*Oryzias latipes*) were exposed to phenanthrene (5, 50 ppb), pyrene (3, 30 ppb), or their mixtures for 96 hours. Fish were sampled at 0, 24, or 96 hours after the exposure test started. Total RNA extracted from whole body of medaka was subjected to microarray experiment. Principal component analysis of transcriptome data at 24-hr exposure showed that three groups were formed and were separated from each other, indicating that PC1 (32.1%) and PC2 (12.3%) reflected the effects of mixtures and single PAHs, respectively. The profiles of altered gene expressions at 24-hr exposure were similar between phenanthrene- or pyrene-exposed groups; however, the effects of their mixture were completely different from those of individual compounds. Exposures to phenanthrene or pyrene induced the genes related to *immune response* (GO:0006955) and *induction of apoptosis* (GO:0006917) in a dose-dependent manner, which seemed to be an acute response to PAH exposures. Meanwhile, exposures to mixtures of phenanthrene and pyrene dramatically suppressed the expression levels of genes associated with *collagen catabolic process* (GO:0030574) and *glycolysis* (GO:0006096), although each individual chemical showed no effect on the expressions of these genes. On the other hand, the PCA result of 96-hr exposed groups did not show the similar trend, and the gene expression patterns exhibited neither dose-response nor PAH mixture-specific effects. The effects of fasting, detected as significant changes in gene expression profiles of control fish among time points. Data analyses using GO terms revealed that 96-hr fasting significantly suppressed *collagen catabolic process* and *glucose homeostasis* (GO:0042593), which were also observed in fish exposed to mixtures of PAHs. The effects of fasting seemed larger than those of PAH exposures at 96-hr, which may be a major factor of interference with the detection of biological responses to PAH exposures. Thus, our analysis can detect mixture toxicities of PAHs, as well as the effects of individual PAHs. However, some effects of PAHs were overlapping with the effects of fasting. Therefore, for precise evaluation of chemical effects by microarray experiment, fasting should be considered as a factor to affect the gene expression profiles in experimental animals.

ET07B-1

Transcriptomics analysis of intersex condition in rainbow darter (*Etheostoma caeruleum*) exposed to sewage effluents in Grand River, Ontario, Canada

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Rainbow darter (*Etheostoma caeruleum*) is a small benthic fish found in North America. Rainbow darters are very abundant and spatially distributed throughout the Grand River system (Ontario, Canada) which is a model river for the study of the sewage effluent effects on fish populations because of high municipal sewage input. Rainbow darters are sensitive to sewage effluents in this environment, showing the presence of intersex condition in males. The primary objective of this research was to identify molecular pathways that are associated with intersex and to explore the impact of sewage effluent on higher level biological endpoints. In May 2011, we collected female and male rainbow darter at 9 sites in Grand River in a gradient of sewage effluent pollution. There were no significant changes in condition factor, hepatosomatic index (LSI), or gonadosomatic index (GSI), in fish in polluted environments compared with the reference site, but variability was increased downstream. Histology revealed that males had a high incidence of intersex in polluted sites as characterized by the presence of follicles within the testis. Male fish had less 11-Keto-testosterone and testosterone production at the downstream sites. To better understand mechanisms underlying intersex in the rainbow darter, known genes involved in sexual differentiation (*sox9b*, *foxl2* and *dmrt1*) and reproduction (*esr1*, *erb*, *ar*, *vtg*, *aromatase* and *cyp11a*) were cloned and evaluated with real-time PCR, comparing males, females and intersex fish. In addition, a rainbow darter microarray was developed using 454 pyrosequencing to characterize molecular pathways that are involved in intersex. For example, *Sox9b* and *foxl2* were significantly up-regulated in the intersex condition while *dmrt1* keep express on intersex. *Vtg* expression was significantly increase in intersex in comparison with males from reference and polluted sites. Cluster analysis of microarray data shows individuals that have a more advance intersex condition (i.e. vitellogenin oocytes) have a very unique gene expression pattern compared to males and females. The microarray analysis between males from reference sites, males from polluted sites and intersex males demonstrate that biological processes that included oogenesis were different in intersex compared to males. Finally, this study identifies novel genes and cell pathways underlying intersex condition in teleosts.

ET07B-2

Sequencing the *Chironomus riparius* transcriptome to compare the sensitivity of gene expression and life-cycle endpoints to toxicant exposure

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The non-biting midge *Chironomus riparius* (Insecta, Diptera) is routinely used in sediment toxicity tests, where the toxicity of compounds is assessed by observing effects on life-cycle endpoints, such as emergence, survival and growth. This approach has proven to be effective, with currently four OECD guidelines being available for acute and chronic sediment toxicity tests with chironomids. With the rapid developments in sequencing technology, a steady increase is seen in the number of ecotoxicological studies where endpoints on a lower level of biological organization, i.e. gene expression, are used to assess the effects of toxic compounds. The aim of the present study was to develop a *C. riparius* gene expression microarray and compare the sensitivity of gene expression endpoints with life-cycle endpoints in sediment dwelling *C. riparius* larvae. Therefore, we performed 14-day sediment toxicity tests with four compounds, i.e. the essential metal copper, the non-essential metal cadmium, the organometal tributyltin and the polycyclic aromatic compound phenanthrene, and measured the effects on growth and survival. EC50 and LC50 values were obtained for all compounds, except for phenanthrene where the surviving larvae were not impaired in their growth, thus no EC50 value could be calculated. To obtain the *C. riparius* transcriptome, a portion of the exposed larvae was pooled with midges of different developmental stages and used to construct a normalized cDNA library for pyrosequencing. This yielded 1549146 sequence reads which were assembled into 23709 isotigs and 135082 singletons. BlastX search and functional annotation with Blast2GO showed that 83% of the isotigs that had a blast result, had the highest homology with dipterans, while 92% matched best with insects in general. Prior to developing the 135K *C. riparius* gene expression microarray, we validated the sequence reads using a comparative genome hybridization and gene expression analysis on a 1000K *C. riparius* microarray. This resulted in a confirmed transcriptome assembly of 20,481 isotigs and 19,021 singletons, and allowed the selection of 135000 reliable probes for the final microarray. This gene expression microarray was used for gene expression studies with the toxicant exposed larvae and will allow us to compare the sensitivity of the molecular and life-cycle toxicity endpoints.

ET07B-3

A novel method for cross-species gene expression analysis - applications for ecotoxicology

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Cross-species comparisons and extrapolations is a cornerstone of ecotoxicological risk assessment. A generalized method that could be applied to the growing number of ecotoxicological relevant transcriptomic studies would therefore be highly valuable in the identification of evolutionary conserved modes of action as well as novel biomarkers of exposure or effect. Indeed, comparing gene expression profiles from different species is a powerful way to identify evolutionary conserved transcriptional responses. However, due to evolutionary events such as gene duplication, there is no one-to-one correspondence between genes in different species and comparison of their gene expression profiles is therefore complex. Here, we have developed a new method for cross-species meta-analysis of transcriptomic data. In contrast to other procedures, our method takes the homology structure between compared species into account and can therefore compare expression data from genes with any number of orthologs and paralogs. A simulation study shows that the proposed method results in a substantial increase in power compared to other previously suggested procedures. As a proof of concept, we analyzed microarray data from heat shock experiments performed in eight species where we identified several well-known evolutionary conserved transcriptional

responses. The method was also applied to five different gene expression studies where fish had been exposed to estrogenic substances. Well-known biomarkers of estrogenic exposure and mechanisms of action were identified together with more novel findings.

ET07B-4

Metabolomics approach to evaluate the effects of polybrominated diphenyl ethers (PBDEs) on the central nervous system and the hypothalamic-pituitary-gonadal axis of marine medaka (*Oryzias melastigma*)

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Owing to the extensive applications of polybrominated diphenylethers (PBDEs) as flame-retardants, residues of PBDEs can be easily found in the aquatic environment and biota. Resulting from their lipophilic characteristics, PBDEs can be bio-accumulated along the food chain. Although great efforts have been devoted to the study of the environmental and toxicological impacts of PBDEs on the coastal marine ecosystem, our knowledge on their general health effects on marine biota still remains obscure. Because of their ability to pass the blood-brain barrier and to accumulate in the brain, they have adverse effects in the central nervous system in the exposed organisms.

Because of the intertwined nonlinear and dynamic interactions among large numbers of cellular components, such as genes, proteins and metabolites, organisms often respond to external stresses and stimuli in complex and unpredictable ways (Nicholson & Lindon, 2008). In order to understand the systemic responses and behaviors of a biological entity, an integrative approach to study and model the pathways and networks involved in the overall functioning of the entity is needed (Henry et al., 2003). Such a system biological approach usually involves the perturbation of the biological system and monitors the resulting impacts at the various “omics” levels, such as its genomics, proteomics and metabolomics.

In this study, we used marine medaka (*Oryzias melastigma*) as a marine vertebrate model organism to study the changes induced by the exposure to BDE-47, a predominant PBDE residue in the marine ecosystem, on the profiles of selected neuro-transmitters and their metabolites. By using a novel LC-ESI-MS/MS metabolomics platform, we were able to evaluate the multi-parametric metabolic responses on central nervous system (CNS) and hypothalamic-pituitary-gonadal (HPG) axis of a living system to PBDEs exposure. Our results indicate that food-borne exposure to BDE-47 is able to induce abnormal expression of a number of neuro-transmitters in the central nervous system of the model organism.

ET07B-5

Metabolomic approach to study the mechanisms of toxicity of a non-ionic surfactant in fish

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Non-ionic surfactants account for 50% of the total surfactant production and they are the second highest synthetic surfactant in terms of worldwide consumption.

Between them alcohol polyethoxylates (AEOs) have the highest production volume in EU today, and they have replaced alkylphenol polyethoxylates (APEOs) due to the estrogenic properties of some of their metabolites. After their use, AEOs are released either directly or via wastewater treatment plants into aquatic ecosystems assuming minimal risk for the health of aquatic life. Although AEOs don't present estrogenic properties as APEOs, there is little information on the effect of exposure to sub-lethal concentrations and the possible disruption on biochemical pathways of organisms that could adversely affect their health. In order to investigate this topic, a metabolomic profiling approach was used to characterise changes in tissues and biofluid of *Solea senegalensis* exposed to the non-ionic surfactant hexaethylene glycol monododecylether (C12EO6). Fish were exposed to two different concentrations of the chemical in a flow-through system for 120 h, followed by a 72 h of depuration period. Blood, liver and gills were extracted with methanol and the metabolites present in the extracts were profiles using LC-TOF-MS. Analysis of extracts revealed a high number of metabolites produced from the phase I and phase II biotransformation of the parent compound by the organism. Metabolomics analyses also revealed that the biochemistry of internal molecules was affected by the surfactant exposure. Increased concentrations of cortolone-3-glucuronide, and bile acids were measured in plasma and identified as potential endogenous markers of surfactant exposure. The concentrations of taurocholic acid, hydroxytaurocholic acid, cyprinol sulphate and scymmol sulphate increased in plasma by up to 10⁸ times higher after exposure indicating that hepatotoxicity may have resulted from cholestasis and impeded flow of bile from liver to the intestine. Plasma levels of L-palmitoylcarnitine decreased by up to 10⁻³ fold as a result of surfactant exposure indicating effects on the metabolism of fatty acids. After the depuration the levels of L-palmitoylcarnitine were re-established indicating that the normal metabolism of fatty acids was recovered but the concentrations of bile acids kept increasing which showed that the damage caused to the organism was still present.

ET07B-6

Metabolism of and adaptation to arsenic in the earthworm, *Lumbricus rubellus*

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As contamination in the terrestrial environment can have deleterious effects upon the flora and fauna exposed. The persistent nature of the metalliod provides a strong selective pressure that may lead to the occurrence of adapted populations. While certain organisms demonstrate tolerance, others are able to acquire genetically based resistance, whereby survival is ensured following exposure to concentrations that are normally lethal. Such resistance may come at a price, with reduction in genetic variability following speciation events potentially increasing the sensitivity of adapted species to future environmental stress, while diverting resources limiting overall fitness. It is therefore important to determine the sensitivity of populations and potential ecological implications of resistance. Our investigations compare exposure sensitivity to metals among naïve and tolerant populations of the earthworm *Lumbricus rubellus* and evaluate the capacity for gene flow within contaminated environments via integration with phylogenetic data. Furthermore, we are employing a systems biology analysis (metabolomics and transcriptomics) to clearly resolve As metabolism within naïve *L. rubellus* that will supplement investigations for the demonstration of genetic mechanisms adopted by adapted individuals using Restriction Associated DNA sequencing (RADseq).

ET08 - Extrapolation within wildlife toxicology

ET08-1

Increasing the reliability of the chemical effect assessment for wildlife with interspecies correlation estimations

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The median hazardous dose of a chemical for warm-blooded species (HD50) is one of the key characteristics in the relative ranking of toxic chemicals. Generally, experimental information is available for a limited number of warm-blooded species only, which causes statistical uncertainty. Furthermore, when small datasets contain an unrepresentative sample of species, they can cause systematic uncertainty in chemicals' median lethal doses. The number of species can be enlarged with interspecies correlation estimation (ICE) models, but these are uncertain themselves. The goal of this study is to quantify the possible gain in reliability of the HD50 values for warm-blooded wildlife species after enlargement of the sample size with ICE predictions. For 1137 chemicals, we compared potential systematic uncertainty and statistical uncertainty between HD50s based on experimental data (HD50Ex) and datasets combining experimental data and ICE predictions (HD50Co). HD50Ex values ranged between 0.10 and 9,500 mg/kgwwt-1, and HD50Co between 1.1 and 6,100 mg/kgwwt-1. For over 97 percent of the chemicals, HD50Ex values exceeded HD50Co values, with a systematic uncertainty (i.e. the ratio of HD50Ex/HD50Co) of typically 3.5. This finding suggests that the limited availability of mammalian, experimental toxicity data results in a systematic underestimation of the wildlife toxicity of a chemical. Statistical uncertainty factors (i.e. the ratio of the 95th/5th percentile) ranged between 4.8-100 and 1.1-102 for the combined dataset, but between 1.0-100 and 2.5-1022 for the experimental dataset. For thirty percent of the chemicals, enhancement of the toxicity with ICE predictions gave a reduction in statistical uncertainty of up to twenty orders of magnitude, for the remaining seventy percent a combined dataset resulted in statistical uncertainty of maximally two orders of magnitude. The supplementation of experimental toxicity data with ICE predictions makes it possible to eliminate large outliers of statistical uncertainty. We conclude that both systematic uncertainty in chemicals' HD50 values and statistical uncertainty can be reduced by supplementation of experimental data with ICE model predictions, particularly in cases of limited toxicity data ($n \leq 4$) for mammals only.

ET08-2

Contribution of wintering area to levels of organochlorines and polybrominated diphenyls in plasma of an avian top predator in the North Atlantic

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Top marine predators, including seabirds, are often sampled to monitor levels of persistent organic pollutants (POPs) in the marine environment. Despite the restrictions on use and production of many POPs and declines in some legacy POPs in the environment, concern remains about levels of these chemicals present in marine biota due to their potential toxicity. Many species of seabird are migratory, but there is a lack of studies investigating the possible contribution of time spent in their wintering area to their POP burden. Advances in tracking technology now mean it is possible to link the POP levels in an individual bird to their wintering area, recorded using geolocators. The great skua (*Stercorarius skua*) was chosen as the study species as it is a top predator and has breeding populations that contain individuals which winter in geographically discrete locations over a large geographic area. Geolocators were deployed in three breeding colonies in 2008, in Scotland (Foula; $n=16$), in Southeast Iceland (Öræfi; $n=40$) and in

Svalbard (Bjørnøya; n=24) and 20 were recovered in 2009. Carbon isotope signatures differed between birds wintering in different areas. The $\delta^{13}\text{C}$ signature of the eighth primary feather sampled from each logger bird from a known wintering area was used to classify the wintering area of a wider population of breeding individuals from the three colonies. We assessed the influence of wintering area on POP levels in plasma of birds sampled during the breeding season. All Scottish birds wintered in the eastern Atlantic. Icelandic great skuas wintering in the western Atlantic had significantly higher levels of organochlorines (OCs) and Polybrominated diphenyls (PBDEs) than those wintering in the eastern Atlantic. In the Bjørnøya population there was no difference in OC or PBDE levels between birds wintering in the east and west Atlantic. This may be due to differences in diet between the Icelandic and Bjørnøya breeding populations, the latter birds feed at a higher trophic level during the breeding season than the other two colonies and having significantly higher POP levels than Icelandic and Scottish great skuas. Although breeding colony significantly affects POP levels, wintering area also contributes to explaining POP levels in birds and should not be neglected.

ET08-3

Responses of wild small mammals to arsenic polluted soils on a partially remediated mining site in Southern France

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Arsenic (As) is a chemical element naturally occurring in soils but industrial and mining activities have long been releasing high As levels into the environment. These elevated concentrations might affect directly exposed organisms and also organisms that feed on them along the food chain. Despite this, little is known about As behaviour in the environment. Chemical analyses of soils provide information on contaminant concentration but not on bioavailability, transfer, and toxicity to wildlife, all being critical points for ecological risk assessment (ERA). In As case, transfers highly depend on environmental matrices, thus a local estimation of As bioaccumulation is needed for ERA. On a partially remediated site, we measured (i) As concentrations in soils to estimate the efficiency of remediation works and (ii) As bioaccumulation in small mammals to improve knowledge of As transfers within food web and of associated risks.

The study site is a partially remediated former gold mine in southern France (Salsigne, Aude, France). In 45 soil samples, As concentrations and soil characteristics were measured. Small mammals were captured with baited snap-traps, and animals were identified at species level, sexed, weighed and measured, and age of rodents was estimated from the eyes lens weight. As concentrations in organs were quantified by graphite furnace atomic absorption spectrophotometry.

Exploitation of the rich ores in the study area led to high As soil concentrations (median NZ4 = 17,900 ppm). The two kinds of remediation works (by clean soil addition or by As immobilisation and phytoremediation) resulted in different soil As levels between zones: some were similar to the control site and the other were similar to non remediated areas (with the exception of an extremely contaminated zone, NZ4). Small mammals living there showed As accumulation in liver, kidneys and lungs among the highest recorded in the literature (e.g. As median in kidneys wood mouse on contaminated area = 5.3 ppm with a maximum of 51 ppm). Discrepancies in As concentrations spatial patterns between soils and organs may be a reflect of the foraging activity of animals. The analysis of small mammal's diet, currently in progress, should help to test this hypothesis. Finally, the high internal As concentration call for an analysis of the effects of As on small mammals, both at the individual and population levels, which are also in progress and will be soon available.

ET08-4

Assessing the exposure and effects of persistent contaminants in river otters (*Lontra canadensis*) in Victoria Harbour, Vancouver Island, British Columbia

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Monitoring contamination in wild otter populations has proven challenging due to their elusive nature and the invasive sampling techniques traditionally involved (e.g. trapping and tissue sampling).

Elliott et al. (2008) recently reported that scats collected from river otter latrines in Victoria Harbour, BC, Canada contained levels of PCBs that exceeded 9 mg/kg lipid, a published criteria for reproductive impairment in mustelids. Guertin et al. (2010) applied an individual based approach by combining fecal DNA genotyping with contaminant and diet analysis of individual river otter scat samples from the Victoria Harbour population. This approach revealed variation in individual contaminant exposure across the landscape along a gradient from industrialized to natural near shore environments. Genetic data also indicated small scale population structuring, suggesting that only certain otters (one subpopulation) were being exposed to contaminants.

This study combined live animal sampling and radio-telemetry with non-invasive scat sampling. The objective was to evaluate the reliability of individual, population and contaminant data derived from river otter scat. This approach was also well suited for defining individual home ranges and characterizing contaminant exposure in this population. Individual river otters were radio-tracked to investigate home range, distribution and movement patterns. Telemetry data reveals that individual river otters inhabit home ranges that span approximately 5kms of coastline. Movement patterns indicate that there is limited if any mixing between the proposed subpopulations and that certain otters inhabit the contaminated areas year round. Understanding the movement patterns and ranges of these individuals will help to further investigate bioaccumulation of contaminants from prey to predator as well as any physiological effects from this exposure. The data derived from feces will be compared to data derived from river otter blood and tissue to investigate bioaccumulation of contaminants from prey to predator as well as any physiological effects from this exposure.

ET08-5

Anticoagulant rodenticides in New Zealand birds - so what?

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The focus for monitoring residual concentrations of anticoagulant rodenticides in wildlife has largely been on birds of prey and mammalian predators. Here we report the results of such monitoring undertaken in New Zealand (NZ) during 2009-2010, predominately of harrier hawks (*Circus approximans*) but including other bird species. Concentrations of multiple coumarin anticoagulants (brodifacoum, bromadiolone, flocoumafen coumatetralyl and warfarin) were measured in liver tissue using a HPLC method. Residues of at least one anticoagulant were detected in 22 out of 27 harrier hawks. Three hawks had one anticoagulant only, and about half (13 of 27) had two anticoagulants present, most commonly brodifacoum and flocoumafen. Three anticoagulants were present in four of the 27 hawks, and four anticoagulants present in another two hawks. Overall, brodifacoum (n=18) and flocoumafen (n=17) were the most frequently detected.

These data add to the growing international literature describing the prevalence of anticoagulant residues in non-target wildlife, and also highlight the recurrent question of 'so what?' We propose that a laboratory trial using a model bird species and repeated dietary exposure to anticoagulants would be an appropriate first tier step in addressing this question. The aim would be to characterise levels of harm, describe how liver concentrations correspond to risk of mortality and determine the repeated exposure patterns that could ultimately produce a mortality 'tipping point' for individuals.

ET08-6

Incidence of poisoning by anticoagulant rodenticides in non-target wildlife and domestic animals in Spain

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Anticoagulant rodenticides (ARs) levels were studied in liver of 401 found dead wild and domestic animals in Spain with evidences of AR poisoning, including 2 species of reptiles (n=2), 42 species of birds (n=271) and 18 species of mammals (n=128). Baits (n=32) were also analyzed to detect the potential use of ARs in their intentional preparation to kill predators. AR residues were detected in 38.2% of the studied animals and 34.9% may have died by AR poisoning according to the clinical information, necropsy findings, residue levels and results of other toxicological analysis. Animals considered with sublethal AR exposure had total AR residues levels (geometric mean with 95% CI) in liver of 0.005 (0.003-0.007) µg/g and animals diagnosed as dead by AR poisoning had 0.706 (0.473-1.054) µg/g. ARs were detected in 19% of baits illegally prepared to kill predators. In terms of the total incidents studied in our laboratory between 2005 and 2010 (n=1,792 animals), confirmed poisonings represented 40.9% of the cases, and 20.7% of these were due to ARs (8.5% of the total sample). Nocturnal raptors (62%) and carnivore mammals (38%) were amongst the groups with higher prevalences of secondary AR exposure, especially to second generation ARs (SGARs). On the other hand, granivorous birds showed the highest prevalences of primary AR exposure (51%), especially to chlorophacinone in a region treated against a vole population peak in 2007. The presence of haemorrhages was significantly associated with AR levels in liver, but some animals (7.2%) with elevated residue levels (>0.2 µg/g) showed no evidence of macroscopic bleeding. Previous toxicovigilance studies have found a prevalence of 26.9% of AR poisoning within confirmed cases of poisoning in non-target animals (mainly pets) in Spain. Further current monitoring studies of AR in particular species of wildlife in Spain are also discussed. The use of accumulative SGARs and the application of baits on surface (i.e. treated grain by spreader machines) should be discontinued in future EU regulations on the use of rodenticides to prevent the poisoning of non-target wildlife species.

ET09 - Global climate change: implications for environmental toxicology and chemistry

ET09-1

Global climate change and influence on chemical fate and bioavailability

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A SETAC Pellston workshop, held in July, 2011, brought together an international group of experts in environmental chemistry, toxicology, ecology, human health and risk assessment, to advance the level of scientific understanding of how global climate change may affect the environmental fate, bioavailability and toxicity of chemicals in the environment. Six workgroups addressed a range of areas that may be affected by climate change. The emphasis of workgroup 1 was aimed at assessing the influence of climate change on exposure, fate, transport, and deposition of chemicals in the environment, and how potential changes may impact environmental risk and hazard assessments. A key challenge identified relates to the complexity of interconnected environmental processes that may result in enhanced or reduced exposure of chemical contaminants. The complexity of the interactions can lead to non-intuitive results, making projections of how chemical fate and bioavailability might change in a world impacted by GCC difficult to assess. Nevertheless, efforts are made to demonstrate how models can quantify the physical-chemical properties of substances that may be more sensitive to changes in climate than others. Additionally, we attempt to demonstrate which global regions enhanced chemical exposure is more likely to occur. Utilizing an appreciation for the uncertainty and sensitivity of model parameters, as defined for both environmental fate models and climate change models, we attempt to demonstrate how model output can complement the collection of empirical data in a manner that can better inform our ability to assess potential relationships between chemical fate and bioavailability and changes in climate that can be attributed to climate change.

ET09-2

A pathway-based approach to predicting interactions between chemical and non-chemical stressors: applications to global climate change

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Incorporation of global climate change (GCC) effects into assessments of chemical risk requires an understanding of effects on mechanisms of chemical toxicity. These effects could include changes in toxicokinetics (chemical absorption, distribution, metabolism and excretion), as well as alterations in toxicodynamic interactions between chemicals and target molecules. GCC also can modify an organism's baseline physiological processes for coping with the external environment (e.g., water balance, thermoregulation, immune-endocrine-neurological systems). In organisms living in GCC modified climates, increased investment in adaptation could lead to heightened susceptibility to chemicals, disease and other stressors. Basically, GCC can cause organisms to be more sensitive to chemical stressors while, alternatively, chemical exposure can make organisms more sensitive to non-chemical (GCC) stressors. Implications of GCC interactions with chemical mechanisms of toxicity are applicable to both ecological and human health effects assessments. To better address the complexities of interactions between chemical and non-chemical stressors, we employed adverse outcome pathways (AOPs), constructs that depict linkages between molecular initiating events and subsequent responses occurring across biological levels of organization, culminating in impacts in individuals or populations that can be used for assessing risk. Through a series of examples and case studies, we demonstrate how chemical- and climate-specific interactions could lead to adverse outcomes. Scenarios are prospective, which project outcomes based on known or anticipated chemical/GCC interactions, as well as retrospective, where mechanisms are proposed for known or demonstrated chemical-climate interactions. Understanding GCC interactions along AOPs provides opportunities for extrapolation across species and biological levels of organization, and between different exposure scenarios, facilitating development of hypotheses and focal areas for further research, and improved inputs for risk assessments.

ET09-3

Combined impacts of global climate change and toxicants on populations and communities

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SETAC convened a workshop in July 2011, organized into 6 workgroups, to address a variety of issues associated with the potential impacts of global climate change (GCC) on chemical fate, exposure, toxicity and risk assessment. Climatic changes such as higher temperature and CO₂ levels have documented negative impacts on many species, e.g. amphibians and coral reefs. GCC-related stress and toxicant exposure can therefore be regarded as multiple stressors, where the combined effects can result in synergistic or antagonistic interactions. Moreover, GCC can also directly and indirectly impact on population and community processes, e.g. disrupt the timing of predator-prey interactions. A great challenge for ecological risk assessment is to predict the how toxicants effects at the individual level (e.g. reduced survival) will be transferred to the population level (e.g. population growth rate) or community level (e.g. species richness). Given the large complexity of direct and indirect GCC impacts on the environment, it is not possible to give general predictions for combined climatic and toxicant impacts will propagate from the individual to higher ecological levels. Our approach in this paper, therefore, is to describe relevant ecological mechanisms that will influence the responses of ecosystems to toxicant stress under climate change. In particular we will focus on these topics: (1) Resistance, resilience and recovery from disturbances; (2) Acquired tolerance to stressors and associated costs; (3) Species traits and vulnerability in a landscape context. Within this framework, we use case studies from various aquatic ecosystems to illustrate the complexity of joint effects of GCC and toxicants on populations and communities.

ET09-4

How will global climate change affect human health risk assessment?

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Global climate change is predicted to alter long-term weather characteristics in different regions. These changes, including increased temperature, greater precipitation extremes, and loss of glacial and polar ice, have implications for human exposure to chemical contaminants. Climate change may also directly and indirectly affect the vulnerability of humans to chemical exposures. Changes in human exposure may arise from altered use, inputs, fate and transport of chemicals due to climatic and other drivers. Human vulnerability may be affected directly by heat and other weather-related stressors, or indirectly through altered co-exposures or disease patterns. To further explore the implications of climate change for the assessment and management of chemical risks, the authors examine four specific risk contexts: natural toxins, pesticides, air pollutants, and legacy chemicals (e.g., mercury, POPs). For the specific types of decisions to be made in each of these contexts, we examine how assessments and management decisions may be affected by climate change, and how significant the impacts of climate change may be.

Climate change is likely to both increase and decrease human exposures, depending on the specific contaminant and specific region or other exposure context. There is limited evidence that climate change will increase the sensitivity of humans to chemical exposures. But small changes in exposure variability or human vulnerability can translate into significant changes in population risk profiles.

To assess and manage chemical risks effectively, exposure data sources will need to be regularly updated and defaults and assumptions used in exposure assessment evaluated in a context of changing climate. Monitoring and sampling should be done with frequency sufficient to capture variability, which is likely to increase in many places. There are many research gaps in interactions between climate and weather parameters and human responses to chemical exposures. These factors will all exacerbate gaps in chemical protection between developed and developing countries.

ET09-5

Ecological risk assessment in the context of global climate change

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This paper originates from the SETAC Pellston Workshop entitled "The Influence of Global Climate Change (GCC) on the Scientific Foundations and Applications of Environmental Toxicology and Chemistry" and specifically, from the workgroup charged with determining how ecological risk assessment (ERA) will encompass the

challenges presented by GCC. Although the basic ERA structure remains useful, the process needs to broaden beyond contaminant issues per se to include interactions with changing habitats that alter biologic communities and recognize that landscape ecology needs to be embraced to optimize effective environmental management decisions in the context of GCC. We propose seven principles for conducting ERAs under GCC: 1) evaluate a priori whether climate-related factors are likely to impact to a given ERA process and management decisions; 2) develop and express assessment endpoints as ecosystem services; 3) recognize that responses, (changes in ecosystem services), can be positive or negative; 4) recognize that the risk assessment process requires a multiple stressor approach with non-linear interactions; 5) develop conceptual cause-effect diagrams that consider context-dependent management decisions, at the appropriate spatial and temporal scales while ranking direct and indirect effects; 6) determine the major drivers of uncertainty by estimating and bounding stochastic uncertainty spatially, and across time; and, 7) plan for adaptive management to account for changing environmental conditions and consequent changes to ecosystem services. Given the complexities and uncertainties associated with GCC, good communication is essential for making risk-related information understandable and useful for managers and stakeholders.

ET09-6

Environmental contaminants and global climate change: implications for environmental damage assessment and restoration/rehabilitation

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A SETAC international workshop, held in July, 2011, brought together an international group of experts in environmental chemistry, toxicology, ecology, human health and risk assessment and natural resource damage assessment, to advance the level of scientific understanding of how global climate change (GCC) may affect the environmental fate, bioavailability and toxicity of chemicals in the environment. Six workgroups addressed a range of areas that may be affected by GCC. The emphasis of the restoration workgroup was environmental damage assessment and restoration/rehabilitation in relation to GCC, specifically addressing: (i) legislation relating to contaminant management, damage assessment and restoration/rehabilitation requirements; (ii) experience with damage assessment and restoration implementation; and (iii) potential direct and indirect influences of GCC on damage assessment and restoration/rehabilitation processes. An example is provided in the U.S., where legislated requirements for damage assessment and restoration incorporate chemical exposure and ecosystem effects from site contaminants to assess the injury to resources and estimate damages paid for restoration of those resources. GCC will impact the processes of assessing injury and rehabilitating/restoring and conserving resources by affecting the magnitude of impact of contaminants on natural resources and altering potential restoration/rehabilitation efforts. Baseline/reference conditions for estimating resource injury and restoration/rehabilitation also may shift significantly and exhibit greater variability due to GCC, representing a significant challenge to practitioners. This presentation will discuss how such future environmental damage assessments and restorations might be influenced by GCC and provide recommendations of research needs in the field.

ET10 - Long-term ecotoxicological impact: trans-generational effects and evolutionary responses to pollutants

ET10A-1

How evolutionary concepts may enhance ecotoxicology: tracing the genetic background of differential cadmium sensitivities in invertebrates

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Species sensitivity towards pollutants and risk assessment parameters are often regarded as random variables in a statistical approach. Beyond statistics, however, toxicant sensitivity of every species assumes a biological significance, especially if we consider that sensitivity traits have developed in lineages of species with common evolutionary roots. The significance of such a perception will be stressed by reviewing and reporting examples of work from our own lab.

In the first case we explored the possibility that populations of *Tubifex tubifex* from different European rivers (Danube, Oder and Dniepr) may exhibit differential sensitivities towards Cd. This hypothesis was based on the fact that the populations had developed in river systems that are geographically separated and may have experienced, in addition, different contamination histories during the past decades. Populations were screened for Cd toxicity and a mitochondrial gene marker (16S rDNA). All natural populations contained, to different degrees, distinct genetic lineages or even cryptic species of *Tubifex* differing significantly with respect to their Cd sensitivity. This suggests to consider, whenever possible, potential genetic differentiation in populations or species when performing ecotoxicity experiments, or when evaluating ecotoxicity data for purposes of risk assessment. We are currently planning to investigate the physiological basis behind these intra-specific sensitivity traits in tubificid worms.

The second example is focused on the evolution of Cd-specific MT isoforms in terrestrial snails. In this case, evolution has led to functional diversification of MT isoforms through shaping of their metal-specific features in a large monophyletic group (the pulmonate snails). As a result of this evolutionary process, terrestrial gastropods possess a Cd-specific MT isoform that must be regarded as a major trait conferring enhanced Cd tolerance to these animals. Interestingly, however, emerging differences evolved in both, regulatory and coding regions of pulmonate CdMT genes give rise to differential transcriptional regulation and expression of deviating gene products in a population- and species-specific manner. This may have implications on Cd tolerance of the affected individuals.

It is concluded that both, differences and similarities in sensitivity traits of related invertebrate species can only be understood if we also consider the underlying evolutionary processes and mechanisms.

ET10A-2

The potential for adaptation in a natural *Daphnia magna* population: narrow-sense heritability of net reproductive rate under Cd stress at two temperatures

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The existence of genetic variability is a key element of the adaptive potential of a natural population to stress. In this study we estimated the additive and non-additive components of the genetic variability of net reproductive rate (R0) in a natural *Daphnia magna* population exposed to Cd stress at two different temperatures. To this end, life-table experiments were conducted with 20 parental and 39 offspring clonal lineages following a 2[GREEKX]2 design with Cd concentration (control vs. 3.7 µg Cd/L) and temperature (20°C vs. 24°C) as the factors. Offspring lineages were obtained through inter-clonal crossing of the different parental lineages. The population mean, additive and non-additive genetic components of variation in each treatment were estimated by fitting an Animal Model to the observed R0 values using restricted maximum likelihood estimation (REML). From those estimates narrow-sense heritabilities (h2) of R0 were calculated. Significant values of h2 (=0.23) were only found in the 24°C + 3.7 µg Cd/L treatment, suggesting that the ability to produce more offspring under this stressful condition may be inherited across sexual generations. In the three other treatments these values were all low (h2 range: 0.04-0.06) and not significantly higher than 0. Collectively our data indicate that both the asexual and sexual reproduction phases in cyclic parthenogenetic *D. magna* populations may play a role in the long-term adaptive potential of *Daphnia* populations to chemical stress (with Cd as the current example) and that environmental variables other than the chemical itself may influence the adaptive potential to that chemical (with temperature as the current example).

ET10A-3

Do pesticides influence evolutionary processes in natural populations of non-target species? A study in the freshwater snail *Lymnaea stagnalis*

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Pollutants present in the environment may affect the evolution of natural populations in several ways. Fitness reduction can result from direct effects on the germ-line (mutagenic compounds), or it may be the consequence of a negative impact on genetic diversity, through directional selection (when the population becomes locally adapted) or through amplification of random genetic drift (when local demography and dispersal patterns are impaired). Pollutants may thus affect the evolution of genes under selection as well as neutral regions of the genome. The issue of evolutionary impact is therefore conceptually important for ecotoxicologists and also for ecological risk assessment and management.

With respect to human induced pollutions, some conditions are expected to increase the risk of genetic change in natural populations. Among such conditions, freshwater lentic habitats located within agricultural landscapes are likely to be exposed to recurrent contamination by pesticides, through various modes of transfer from the treated parcels. Non-target species occupying these habitats are thus exposed to a high risk of evolutionary impact, especially when they have low dispersal ability (e.g., when the whole life cycle is aquatic) or opportunity (weak connectivity among occupied sites, e.g., marshes or ditches).

The present study investigated the effect of historical exposure to pesticides on population genetics patterns, using the freshwater gastropod *Lymnaea stagnalis* as model species. A set of 14 populations was sampled from two contrasted types of landscape, expected to differ in terms of pesticide pressure (sites within zones of intensive agricultural activity vs sites distant from such zones). Patterns of population genetic differentiation were compared between neutral genetic markers (microsatellites) and quantitative traits related to fitness (life history traits) for the two types of populations. Additionally, the project includes a quantitative analysis of population divergence for gene expression at the transcriptome level (RNAseq, qPCR). The aim of the study was to test the hypothesis that chronic exposure to pesticide cocktails had a selective effect on the studied populations. The applicability of the presented approach to ecological risk assessment will be discussed.

ET10A-4

Rapid evolution in a *Caenorhabditis elegans* (nematodes) population: evaluation of resistance costs

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Anthropogenic disturbances affect more and more the evolution of contemporary populations. Pollution, one of the anthropogenic stress, amplifies the intensity of selection pressures which occur in natural populations. Consequently, micro-evolutionary changes may occur very quickly in response to these new forces. Environment can usually undergo simultaneous changes in various conditions, and this will likely increase with the increase in human activities. These changes may accentuate stress, affecting the life, the growth and the reproduction of individuals, with strong demographic and longer-term phenotypic and genetic consequences.

We started knowing the evolutionary response of a population to changes in one environmental parameter. However, much less is known on the evolutionary reactions of a population to more realistic, multi-variate changes in environmental conditions. We therefore investigated firstly the evolutionary responses of *Caenorhabditis elegans* population exposed to uranium (U) or salt (NaCl) stress for 22 generations. We confirmed the adaptation to stressed population through fitness increasing for both polluted selection regime. In parallel, common garden experiments in control confirmed resistance costs associated to genetic divergence. Indeed individual fitness was lower from NaCl and U-populations. Moreover we conducted a reciprocal transplant experiment with U and NaCl from the generation 18. We found resistance costs of the primary selection regime in other stress environment. NaCl-populations reduced their brood size in U compared to control-populations, but it is not true for U-populations in NaCl. Consequently, There can be an addition of new stress that populations suffered and the action of past selection regime on the reduction of genetic diversity. However, we still need to understand the selection mechanisms.

These results confirm that a population can be affected by pollutants even after contamination will, for instance, be diluted in environment. They also highlight the importance of taking into account the adaptive effects of pollution on population in ecological risk assessment.

ET10A-5

Parental exposure to genotoxicant leads to reproduction impairment in the three-spined stickleback

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The aquatic environment is considered as the ultimate receptacle for anthropogenic compounds that frequently exhibit a genotoxic potential towards organisms. The reproductive process could influence the recruitment rate and hence the population dynamics. The link between the loss of DNA integrity in spermatozoa following paternal exposure to genotoxicant and reproduction impairment has been recently investigated in two fish species. These results have demonstrated a decrease in the progeny survival when sperm DNA was damaged and so, possible long term effects of environmental genotoxicant in aquatic systems. Such results needed to be confirmed through studies carried out in other fish species and the contribution of oocytes to further progeny defects had to be highlighted, as recently done in aquatic invertebrates. The aim of the present study was to investigate the link between DNA damage and reproductive impairment after in vivo exposure of stickleback to the genotoxic compound methyl methane sulfonate (MMS) during the breeding season, paying special attention to the contribution of genetic load brought by each sex to the observed progeny defects. Adult fish were acclimated and were then exposed or not through water to different MMS concentrations (0 / 0.05 μ M / 0.5 μ M / 5 μ M). In vitro fertilization were realized with mature fish and DNA damage in spermatozoa and erythrocytes (as a biomarker of exposure for both sex) were assessed by the comet assay. The morphological abnormalities of larvae due to parental MMS exposure were studied and the progeny survival at embryogenesis and larval development key stages was evaluated. Parental exposure of three-spined stickleback to the genotoxicant MMS induced a significant decrease in early life stage survival which was less important when only females were exposed. When males or both genders were exposed, DNA damage level measured in spermatozoa ($p < 0.01$) and exposure duration ($p < 0.01$) were shown to be significant factors associated with progeny mortality. Whatever the gender, parental exposure to MMS resulted in a 60 fold increase in progeny abnormality frequency. This study underlines that male genotoxic message transmitted to progeny could predominate on the female one. Hence, this work confirms that spermatozoa are susceptible to accumulate DNA damage under chronic and low-dose exposure to genotoxic compounds, leading to drastic offspring defects in stickleback, as recently shown in other aquatic species.

ET10A-6

Genotoxicity, fertility reduction and regeneration in a multigeneration study using zebrafish after chronic exposure to an alkylating agent

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There is still a significant lack of understanding the mechanisms linking genotoxic effects of anthropogenic pollutants to the decline of fish populations in aquatic ecosystems. Anthropogenic chemical pollution is probably a key aspect of the problem. For a better understanding, investigations into the relationship between genotoxicity and detrimental effects on reproduction in fish are required. For this end, there is a need to further focus on DNA fragmentation and micronucleus formation in somatic and - above all - reproductive tissues as well as on development and spawning capacities of exposed fish and their offspring. In the present study, zebrafish were exposed in vivo to 2 and 0.2 mg/L of the alkylating model genotoxin methyl methanesulfonate (MMS) from fertilisation to the age of one year in a continuous flow-through system. F0 fish were mated over six months to check for first reproduction, egg number and fertilisation rate. Embryonic and larval development of the F1 generation was analyzed daily. In addition, primary cells were isolated from the F0 gonads, liver and gills for analysis of DNA fragmentation in the alkaline comet assay. Micronucleus frequency was measured in histological sections of the same organs. Analogously to the F0 generation, F1 fish were mated. In the F1 and F2 generations, larval development and survival were recorded. In contrast to the F0 generation, F1 fish grew up without exposure in order to allow for regeneration. The time point of the first spawning in MMS exposed F0 fish was delayed. Over subsequent months, the egg production in exposed fish increased, but remained lower than in controls. Weight and length of MMS-exposed fish as well as larval survival in their offspring were reduced. In the comet assay, significant genotoxic effects were found in liver cells from both sexes, female gills and male gonadal cells. Exposure of zebrafish (F0) to MMS thus leads to DNA fragmentation in several organs, delay of spawning, reduction of egg production, as well as to reduced larval survival of the F1 generation. This suggests that anthropogenic genotoxic compounds may play a considerable role in the decline of field fish populations. Currently, micronucleus formation and carcinogenesis in chronically exposed fish as well as regeneration of reproduction parameters in F1 fish are under investigation.

ET10B-1

Patterns of mitochondrial and nuclear polymorphism and DNA -methylation in an arsenic tolerant earthworm population

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Evidence is emerging that earthworms can develop tolerance to trace element enriched soils, thereby allowing populations to exist at polluted sites. There have so far been few studies that have sought to establish the basis of such tolerance earthworms, and in particular whether it is adaptive or evolutionarily based. Here we report a analysis which combines mitochondrial (cytochrome oxidase II) and nuclear (amplified fragment length polymorphism) genotyping and analysis of genome methylation analysis to investigate whether previous observation of arsenic tolerance in the earthworm *L. rubellus* can be ascribe an underlying mechanism. Both mitochondrial and nuclear genotyping indicate that *L. rubellus* is a complex of two major cryptic lineages. Mitochondrial genotyping failed to indicate population selection, but AFLP analysis, especially when analysed by lineage, highlighted genome differences that were associated with site physiochemical properties (including metal/metalloid concentrations). DNA methylation was strongly influence by pollutant exposure, clearly separation mine site and reference site populations. Patterns of DNA methylation showed greatest divergence between the control and intermediately polluted sites, suggesting that epigenetic change may be a prevalent response at intermediate pollution levels. Taken all together these results suggests that earthworm adopt a multi-layer strategy to cope with adverse soil conditions, employing epigenetic mechanism to potentially change gene expression possibly as an initial adaptive mechanism and undergoing micro-evolution that can be linked to the development of tolerance traits.

ET10B-2

Genomic mechanisms of co-tolerance of cadmium-adapted *Daphnia pulex* populations to cyanobacterial stress

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Natural populations are characterized by genetic variability which may allow populations to adapt to a stressor, which is manifested by an increased tolerance to that stressor. As a consequence of this genetic adaptation, populations may become more (co-tolerant) or less tolerant (cost-of-tolerance) to other stressors. In aquatic ecosystems, stressors rarely occur isolated and the number of stressors are predicted to only increase with global climate change (e.g. cyanobacteria). This study aimed to unravel the genomic basis of the co-tolerance of Cd-adapted *Daphnia pulex* to cyanobacterial stress.

Gene-expression profiles of a Cd adapted and a non-Cd adapted clone in response to *Microcystis aeruginosa* (MC, a cyanobacterium) were characterized using a micro-array platform. Molecular pathway analysis was performed on significantly differentially expressed genes based on KEGG Orthology classification. Genes in pathways and sets of

multi-copy genes that were regulated by MC in different ways in both clones were validated through real-time qPCR in an extended set of 14 clones. Additionally, expression of genes found to underlie the differences in Cd tolerance between Cd adapted and non-Cd-adapted clones was studied. These genes may also be involved in the among-clone differences in MC tolerance due to co-tolerance with Cd.

Molecular pathways and sets of multi-copy genes that were regulated by MC in different ways in both clones were the oxidative phosphorylation and the ribosomes pathways, glutathione transferases, trypsin, neurexins and Apoptosis Inducing Factors (AIF). qPCR expression analysis resulted in three genes for which expression correlated with MC tolerance. Such correlation of a trypsin and an AIF validates the involvement of these genes or the pathways which they belong to in the tolerance to MC. Expression of Keap1, an inhibitor of oxidative stress response tended to be more downregulated in more MC sensitive (thus Cd-non adapted) clones.

This study showed possible genomic mechanisms of co-tolerance of Cd-adapted *D. pulex* populations to cyanobacterial stress. As micro-evolutionary changes in populations and their consequences (such as co-tolerance) for the toxicity of unrelated stressors gain more and more interest in risk assessment, it is beneficial to understand the mechanisms that drive these consequences. E.g., if we understand mechanisms of co-tolerance we can perhaps ultimately predict which combinations of stressors may show co-tolerance.

ET10B-3

Multi-generational exposure of *Folsomia candida* to Cd: survival, reproduction and metallothionein gene effects

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Few studies are currently available on multi-generational effects, particularly with soil invertebrates. In the present study, the effects of cadmium multigenerational exposure were studied in *Folsomia candida*. Organisms were exposed consecutively to cadmium [EC10 and EC50] along 23 generations of 28 days. Assessed endpoints were survival, reproduction and size. Additionally, the expression level of the metallothionein coding gene has been measured by quantitative real time PCR. Continuous exposure to a concentration of 32 mg Cd/kg (EC10) caused an improvement in performance until F6, after which it started to decline and failed at the 12th generation. Continuous exposure to a concentration of 60mg/kg (EC50) caused an improvement in performance (total number of juveniles) until F6, after which it started to decline but continued at lower level. Population changes could be related to the Mt induction, as continuous exposure to Cd triggered Mt induction which corresponded to reproduction in higher numbers. Interestingly, the higher tolerance observed in the population exposed to the EC50 in comparison to the one exposed to the EC10 could be related with the higher Mt induction levels which may confer larger tolerance and longevity to the particular stress. Further studies are ongoing.

ET10B-4

Global DNA methylation in *Daphnia magna* is influenced by genotype and a wide variety of environmental stressors

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Epigenetics is the study of mitotically or meiotically heritable changes in gene function that occur without a change in the DNA sequence. An important epigenetic control mechanism is DNA methylation, which was recently discovered in the water flea *Daphnia magna*, an ecotoxicological model species. It has been shown that exposure to relatively high concentrations of certain chemicals can alter the degree of global DNA methylation in *D. magna* and that this altered DNA methylation level is still found in two subsequent unexposed generations. Here we investigated the impact of a range of environmentally relevant stressors on global DNA methylation. *D. magna* of 14 days old from two different clones were exposed for 48 h to one the following stressors: chemical predation cues, strains of the cyanobacteria *Microcystis aeruginosa*, nutritional quality (essential fatty acids & sterol content), heat stress, metals (166 µg/L Pb, 6 µg/L Cd, 1000 µg/L arsenite) or salinity (5 g/L NaCl increase). All exposures were conducted in triplicate exposure aquaria, resulting in three biologically independent replicates per treatment. DNA was enzymatically digested to nucleosides (including 5-methyl-2'-deoxycytidine), which were analysed by UPLC-MS/MS. Overall, the global degree of cytosine methylation varied between 0.17 % and 2.54 %. An unexpected but interesting observation was the relatively high variation in global DNA methylation level between triplicates of some treatments, with relative standard deviations (RSDs) up to 71%. The extent of this variation between replicates differed between the various stress exposures, with fish predation cues inducing the largest RSD for both clones. Significant differences in global DNA methylation between treatments were observed. For instance we found 0.83% cytosine methylation in clone Xinb3 daphnids exposed to *Triops* medium, which is significantly higher than 0.35% and 0.44% in Xinb3 daphnids exposed to salinity or microcystin-producing *Microcystis*, respectively. Finally, differences between the two clones were observed, with global DNA methylation in the Xinb3 clone generally higher than in the linb1 clone, except for daphnids exposed to higher temperature. In conclusion, this study demonstrated that global DNA methylation in *D. magna* is dependent on genetic (clone) and environmental (exposure) factors. Further research is aimed at addressing the biological function of DNA methylation in *D. magna* and its potential long-term impact.

ET10B-5

Micropollutants' induced trait evolution in natural phytoplankton communities

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Evolution is a fundamental part of ecological theory and ecology and evolution together determine the dynamics of all living systems. Rapid and strong contemporary evolution can occur due to imposed selection, anthropogenic activity and environmental change including pollution. Trait-based approaches focus on phenotypic traits as the fundamental unit to understand adaptation in natural populations. Advances in scanning flow-cytometry afford high precision measurement of individually expressed phenotypic descriptors in phytoplankton. We report of natural phytoplankton communities exposed to micropollutants in the field. Effects were assessed as changes in mean phenotypic traits per population over a period of 7 generations post initial pulse exposure. Effects were also scaled to community level and shifts in trait-distributions compared to expected patterns from eco-evolutionary theory of community assembly. We tested the effects of triclosan, a common bactericide used in personal care products and routinely found in surface water, as a model micropollutant. We exposed natural lake phytoplankton communities to 0.1, 1 and 10 µg/L of triclosan, covering the range of environmental concentrations, in a novel type of permeable microcosms. These allow the exchange of nutrients and physical conditions with the external environment, while confining plankton and pollutants inside. At generation 7 post-exposure, both phytoplankton diversity and density responded to initial dosing of triclosan with a humped shaped "hormetic" relationship. We found that phenotypic traits associated to cell size and pigment content were significantly under selection in communities exposed to triclosan. We detected statistically significant shifts in the mean and range of size-related trait distributions, suggesting an "environmental filtering" effect. The rates of phenotypic change within phytoplankton populations showed to be significantly impacted by triclosan compared to control levels. The rate of size evolution was higher in treatments, while the rate of change in pigment content appeared to be reduced by the effect of triclosan compared to controls. We provide original findings that advocate for a more careful consideration of sub-lethal doses and environmentally relevant exposure scenarios in the risk assessment of chemicals, since evolutionary processes can occur at pulse exposure to low levels of anthropogenic chemicals in natural and complex communities.

ET11 - Marine environmental chemistry and ecotoxicology

ET11A-1

Using an integrated assessment framework for contaminants and biological effects to determine good environmental status under the Marine Strategy Framework Directive

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The use of biological effects tools offer enormous potential to meet the challenges outlined by the European Union Marine Strategy Framework Directive (MSFD) whereby Member States are required to develop a robust set of tools for defining eleven qualitative descriptors of Good Environmental Status (GES), such as demonstrating that "Concentrations of contaminants are at levels not giving rise to pollution effects" (GES Descriptor 8). Here we discuss the combined approach of monitoring chemical contaminant levels, along-side biological effect measurements relating to the effect of pollutants, for undertaking assessments of GES across European marine regions. Using data collected as part of the UK's Clean Seas Environmental Monitoring Programme (CSEMP) we outline the minimum standards that biological effects tools should meet if they are to be used for defining GES in relation to Descriptor 8. Adopting the recommendations of the ICES Study Group for the Integrated Monitoring of Contaminants and Biological Effects (SGIMC) we present a case study demonstrating how such an approach, using contaminant (e.g. metals, PAHs, PCBs) and biological effects (e.g. EROD, bile and pathology) data, in sediment, water and biota could be used to determine GES in the marine environment.

ET11A-2

PAH and biomarker measurements in fish from condition monitoring in Norwegian waters from 2005 to 2011

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Condition monitoring in fish from open seas are performed in Norway every third year. The objectives have been to investigate whether fish from Norwegian seas contain elevated levels of components that originate from discharges from offshore oil and gas production. Focus has been on the Tampen region, as this is the region with highest discharges of produced water. In 2010, 128 mill ton produced water were discharged in the Norwegian sector of the North Sea, and 59 % (76 mill ton) were discharged at the Tampen region. Condition monitoring in 2002 demonstrated changed levels of several parameters in haddock from Tampen, compared with haddock from the Egersund Bank, including 2-4 ring PAH metabolites in bile, DNA adducts in liver, and the ratio of n-3/n-6 in muscle (Balk et al., 2011).

These results were followed up in 2005 and 2008. The main focus has been the North Sea (Tampen and the Egersund bank), but samples from the Norwegian Sea and the Barents Sea (reference area) were also analysed for comparison.

NPD and PAH measured in fish muscle and liver from cod and haddock in 2005 and 2008 were found to be below LOQ for all regions. The main contributor to sum PAH metabolites in bile at Tampen and at the Egersund bank measured in 2008 was 1-hydroxy phenanthrene. Levels of alkylphenols in bile were below LOQ. Levels of Vtg in blood of male cod were generally low from all regions.

DNA adducts in haddock liver were significantly higher at Tampen compared with Egersund Bank in 2005 and 2008, but to a lesser extent (2-fold in 2005 and 2008, compared to 5-fold in 2002).

Lipid content in the liver was significantly reduced in haddock from Tampen in 2008. Fatty acid profiles showed that haddock from Tampen had relatively high levels of arachidonic acid, and the ratio between omega-3 and omega-6 (n-3)/(n-6) poly unsaturated fatty acids were significantly lower in the lipid classes in haddock from the other regions.

Cod and haddock were also collected in 2011 and analyses are under process.

Reference

Balk L et al., 2011. PLoS ONE, Volume: 6(5), article no: e19735.

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ET11A-3

Biomarkers in marine mussels (*Mytilus edulis*) exposed to environmental concentrations of oil field corrosion inhibitors

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Biomarker assays were applied to the marine mussel *Mytilus edulis* exposed for 24 hours to environmental concentrations ranged from 0.001 to 1.0 mg/l of Produced Water (PW), Quaternary Ammonium Salts (QUATs), Imidazoline (IMD) and Phosphate ester (PE), all commonly used oilfield chemicals. The Comet assay was used to determine DNA damage in individual haemocytes and gill cells; furthermore Neutral Red Retention (NRR) was also used to determine the lysosomal membrane stability (LMS), also a suite of oxidative stress endpoints, TBARS and Superoxide dismutase (SOD) activity. On the other hand, the mass spectrometry was used to assess the body burden of PW, QUATs, Imidazoline and phosphate ester. The result showed a significant increase in DNA damage in haemocytes and gill cells, as well as TBARS in gill cells at all QUATs, IMD, PE and PW concentrations. Lysosomal membrane damage was significant increase at concentration ≥ 0.5 mg/l of QUATs and ≥ 0.3 mg/l of IMD. Moreover, SOD activity was significantly increased at concentrations > 0.01 mg/l of QUATs and at concentrations ≥ 0.001 mg/l of IMD, PW and PE at concentration ≥ 0.01 mg/l. These results may have consequences for offshore disposal of produced water.

ET11A-4

Accumulation of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in deep-sea biota and sediments from a submarine canyon (NW Mediterranean)

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Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) are a family of persistent organic pollutants (POPs) consisting of 210 possible congeners. Seventeen of these congeners (2,3,7,8-substituted) have been described as the most toxic. Submarine canyons are particular environments characterized by a fast transport of sedimentary material down slope resulting in an efficient mobilization of particle-bound contaminants from surface waters to deep-sea. No data has been reported on PCDD/Fs in biotic or abiotic compartments of submarine canyons to best of our knowledge. The objectives of the present study are: (1) to provide with the first assessment of PCDD/F levels in deep-sea crustaceans and fish as well as sediments in a submarine canyon (NW Mediterranean Sea); (2) to investigate the dioxin accumulation in crustacean and fish species from different water column's zones in relation to their habits (pelagic, nekto-benthic and benthic). Higher $\Sigma 2,3,7,8$ -PCDD/Fs levels were found in general in crustaceans ranging from 220 to 795 pg g⁻¹ lipid weight (l.w.) (13 - 90 WHO98-TEQ pg g⁻¹ l.w.), whereas levels in fish varied from 110 to 300 pg g⁻¹ l.w. (22 - 33 WHO98-TEQ pg g⁻¹ l.w.). Nekto-benthic species (fish and crustaceans) exhibited the highest dioxin levels in the Blanes submarine canyon and adjacent open slope (in agreement with trophic chain studies that ranked nekto-benthic organisms at the highest trophic level). Dioxin concentration in sediment were generally in the range of those reported for surface sediments in the Mediterranean sea coastal areas and in general in low to moderate anthropogenic impacted aquatic ecosystems world-wide. Differences in congener-specific accumulation were found between the pelagic crustacean and the benthic and nekto-benthic crustaceans. Therefore, species habits seem to have an influence in the differential congener-specific accumulation of PCDD/Fs. A combination of factors such as biota habits, differential uptake of water column dioxin (dissolved and particle-bound fractions), dioxin pattern in the sediment and different metabolism capabilities and rates (CYP-mediated metabolism) can most probably explain the differences observed between crustacean species and between fish and crustaceans in the Blanes submarine canyon. However, further research is needed in order to evaluate the relative predominance of individual factors.

ET11A-5

Echinoderm early life development bioassays reveal effects of marine POP's

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Persistent organic pollutants (POPs) tend to accumulate in the food web reaching concentrations that potentially cause toxic effects. Early life stages (ELS) of vertebrates as well as invertebrates are regarded as the most sensitive to toxic effects of POPs. In addition several vertebrate species (e.g. amphibians, flatfish) comprise a thyroid hormone (TH) dependent metamorphosis which is known to be sensitive to thyroid hormone disrupting compounds (TDCs). The TH-regulated metamorphosis of echinoids (Echinodermata: Echinoidea), such as sea urchins, also is potentially susceptible to the effects of TDCs. In our study we have developed a prolonged ELS bioassay and a metamorphosis assay with the sea urchin *Psammechinus miliaris*. In addition a short term bioassay was developed to study the impact of marine POPs on the multixenobiotic resistance (MXR) mechanism. In this mechanism cellular efflux transporters form an important first line of defense against xenobiotics.

P. miliaris early life development was rather sensitive to HBCD (EC₅₀ 54 nM) with a remarkably steep dose-response curve. The most sensitive endpoint to quantify adverse effects on development was the 'penalty points' approach determined at 16 days post fertilization. This delayed development is an important sub-lethal effect that cannot be determined in common echinoid ELS tests since the observation period ends between 48 and 72h post fertilization. TCS was acutely toxic during embryo development (≥ 500 nM) and at concentrations ≥ 1000 nM no hatching occurred. In contrast to the high sensitivity of amphibians and fish, *P. miliaris* early life development was not sensitive to dioxin-like toxicity (NOEC > 0.03 nM TCDD-equivalents (TEQs)). In the newly developed metamorphosis assay thyroxine (EC₅₀ 0.09 nM) as well as an environmentally relevant PBDE mixture (EC₅₀ 219 nM) accelerated metamorphosis, while TBBPA delayed it (IC₅₀ 97 nM). Compounds tested in the cellular efflux transporter inhibition assay showed a clear dose related inhibition within 90 min. of exposure. The most potent compound was TCS (EC₅₀ 1 μ M), followed by PFOS (EC₅₀ 5 μ M) and BPA (EC₅₀ 146 μ M).

The development of echinoids as an animal model for marine ecotoxicological studies is of great ecological relevance, and in addition it may contribute to the ethically desired reduction of the number of vertebrates currently used for testing endocrine disruption and teratogenicity.

ET11A-6

Species sensitivity distributions for ocean acidification effects

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Climate change can have large impacts on marine ecosystems via ocean acidification. Already for decades it is recognized that a continuous increase in greenhouse gas emissions leads to increased uptake of carbon dioxide into the oceans and possible ocean acidification that can affect marine species. The overall impact on marine ecosystems of ocean acidification is, however, not quantified before. The goal of our work is to assess the effect of ocean acidification on marine species groups via Species Sensitivity Distributions (SSDs). SSDs are commonly used to derive the proportion of species affected at different exposure concentrations of toxic pollutants. In this case, SSDs for ocean acidification express the cumulative fraction of species affected (PAF) as a function of pH in the marine environment. The concentration chosen as critical for a specific species is the pH value at which 50% of the population is affected (defined as EC50). To derive EC50 values, data was extracted from over 100 papers, covering over 100 marine species. SSDs are established for different species groups. Depending on the species group considered the SSD largely differs. First preliminary results indicate that when considering all species, around 15% of the marine species will be affected if the current pH drops to 7.8. The results of our work can be used within risk assessment to incorporate the effects of climate change on marine species via ocean acidification, next to the effects of other pollutants that are already considered.

ET11B-1

Automatic monitoring of chemical pollution in marine water: opportunities for cost effective solutions

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The European Union has recently established the Marine Strategy Framework Directive (MSFD, 2008/56/EC). The directive foresees the introduction of Environmental Quality Standards (EQS) in territorial waters (roughly 12 nautical miles from the coast line) for a range of chemicals, with particular focus on organic contaminants. Signatory countries are thus required by law to gain adequate infrastructures and tools to perform monitoring and demonstrate fulfillment of the standards. Measurements of organic micropollutants in marine surface waters are challenging and expensive, and often require high logistic costs, for example in relationship to ship hiring and mooring in deep waters.

We present here a new device which allow fully automatic monitoring of priority chemicals using regular line ferries or other fixed stations such as, for example, off-shore platforms. The system allows spatial and temporal integration of the monitoring data, and can be controlled from any computer or mobile device in any part of the world through internet and satellite communications. In addition, the sampler provides automatic procedures for preserving the samples after the collection is completed. Sampling performance is currently under testing both on land based marine stations and on board of a ferry. We present here, in details, the features of the new device and the preliminary results from the proof of concept phase.

ET11B-2

Using ecotoxicological information in an integrative framework for the objective classification of ecological status of marine water bodies

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The European Water Framework Directive (WFD) introduced a new philosophy in environmental management and an ambitious goal: achieving a “good ecological status” of all surface water bodies for 2015. The classification of ecological status relies on setting arbitrary, type-specific reference values for a closed list of biological and chemical quality elements, among which neither biomarkers nor bioassays are considered. Due to natural variability of the WFD-proposed biological quality indicators, multiple problems have surfaced as soon as comprehensive efforts to standardise and harmonise those indicators have started, which is delaying marine water body evaluations beyond the deadlines established in the Directive. As an example, the transitional water bodies of the Minho estuary, the main estuary in the NW Iberian Peninsula, have been evaluated by neither the Spanish nor the Portuguese authorities due to the inexistence of appropriate intercalibrated indices.

An alternative methodology for the classification of ecological status using multivariate analysis, is proposed and applied to a pilot study only for the purpose of illustration of the method. The scale of the study was not designed for classification of ecological status of water bodies. This approach does not depend on the arbitrary definition of fixed reference values and ratio-to-reference boundary values, and it is based on the application of non-metric multidimensional scaling (MDS) and cluster analysis to multivariate data sets encompassing chemical, ecotoxicological (biomarkers, bioassays) and ecological (community indices) information. A multiyear data set was generated from the investigative monitoring of marine pollution in Ría de Vigo (NW Iberian Peninsula) from 2004 to 2006, conducted in collaboration with the Spanish Institute of Oceanography (IEO) and the University of the Basque Country (EHU), that included water and sediment chemistry, sediment toxicity assessed by the sea-urchin embryo test, mussel bioaccumulation, mussel physiology (SFG), and benthic macrofaunal richness and diversity. The application of multivariate analyses to a comprehensive ecotoxicological data-set allowed objective and robust classification of sampling sites into discrete categories of ecological status in a highly productive coastal ecosystem. This approach allowed integration of biomarkers and bioassays in the evaluation of coastal and transitional waterbodies according to the objectives of the European WFD.

ET11B-3

Managing environmental risk in marine coastal systems: development of an innovative expert decision support system

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Following the EU Marine Strategy Framework Directive (MSFD), member States should attain / maintain / restore a Good Environmental Status (GES) in marine areas in the next years. Risk-based frameworks are useful to objectively assess environmental quality on the basis of both chemical and biological investigations. An innovative Expert System has been developed to support decision makers in managing marine coastal areas and, in particular, sediments.

The Expert Decision Support System integrates chemical data by considering the toxic pressure due to all contaminants in sediments: concentrations are compared to TELs (threshold effect levels) and a chemical risk index (ChemRI) is calculated applying an additive model to the pollutant mixture. Ecotoxicological high-level endpoints (i.e. survival and reproduction) are used to calculate the ecotoxicological risk index (EtoxRI), while sublethal parameters (i.e. biomarkers) are integrated into the biological stress index (BSI). With the aim to optimize resources, ecotoxicological test selection should follow a 2-tiers framework: where chemical concentrations overcome PEL (probable effect level) high level endpoints (i.e. survival and reproduction) on different model organisms should be evaluated, while if contaminants are in the range between TEL and PEL, sublethal parameters should be added to the ecotoxicological battery.

Finally the Sediment Risk Index (SedRI) is calculated by combining ChemRI and EtoxRI. SedRI ranges from 0 (no risk) to 1 (strong risk): comparing SedRI with specific thresholds it is possible to rank the risk level of the sediment and consequently to manage it by planning opportune interventions (e.g. land-filling, remediation, re-use after dredging). SedRI values in the intervals 0.75-1.00 and 0.50-0.75 respectively indicate high and medium risk; SedRI values in the ranges 0.25-0.50 and 0.00-0.25 individuate mild risk and no risk.

The Expert Decision Support System has been applied to integrate data from the scientific bibliography about sediment quality in some estuarine areas of Spain. Sediments are classified in terms of both chemical contamination and ecotoxicity and recommended interventions are indicated for each site.

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ET11B-4

Preliminary evaluation of a new tool for assessment of in situ biological exposure and effects in aquatic environments

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This paper summarizes recent activities associated with the development and demonstration of an integrated exposure and effects assessment approach involving multiple, concomitantly-linked in situ measurements towards improved ecological risk assessment. When conducted properly, in situ bioassays are a means of reducing uncertainty associated with traditional laboratory-based characterization of contaminated sediments and surface waters [1-3]. In situ exposures can increase realism through reduced sample manipulation and integration of a variety of site-specific factors, which affect contaminant bioavailability and toxicity. Incorporation of passive sampling devices (PSDs) into in situ studies has recently proven valuable as an additional line of evidence towards evaluating contaminant bioavailability [4]. In some situations, in situ characterization of sites is the only relevant means of accurately assessing exposure and effects. These scenarios include assessment of (1) in situ-based sediment remedy (e.g. reactive amendment) effectiveness; and (2) time varying stressors (e.g., storm water discharges, tidally influenced groundwater seepage, underwater unexploded ordnance/ discarded military munitions, and oil spills).

The Sediment Ecotoxicity Assessment Ring (SEA Ring) is a recently developed, field deployed device capable of housing an array of in situ bioassay chambers, PSDs, and water quality sensors. Exposure chambers are oriented such that various exposure routes (e.g. surficial sediment, sediment-water interface, water column) can be investigated. A research prototype SEA Ring has been successfully deployed and recovered at marine, estuarine, and freshwater sites varying in water depth and hydrology. These studies have largely incorporated minor modifications to standard laboratory methods using common test species [5,6].

The prototype SEA Ring is currently undergoing a series of refinements to standardize/control in situ exposure conditions, increase user-friendliness, and reduce dependence on divers. An overview of recent use of the SEA Ring, status of refinements to the device/approach, initial validation of new prototype performance, and discussion of efforts towards regulatory acceptance of the approach will be presented.

ET11B-5

DGT-copper flux measurements predict bioaccumulation and toxicity to bivalves in sediments with varying properties

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Many regulatory frameworks for sediment quality assessment include consideration of contaminant bioavailability. Analyses of pore waters or acid-volatile sulfide (AVS)-

simultaneously extractable metal (SEM) relationships frequently provide this information, but have significant limitations. Porewaters measurements are non time-integrated 'snap-shots'; acid-volatile sulfide (AVS) over-emphasises the importance of sulfidic sediments; and both these methods inadequately assess metal bioavailability for benthic organisms living in the surface layer (i.e. majority of organisms). The use of inappropriate or inadequate information for assessing metal bioavailability in sediments may result in incorrect assessment decisions. The technique of diffusive gradients in thin films (DGT) enables the in situ measurement of metal concentrations in waters and fluxes from sediment pore waters. Field deployments of this simple device can provide time-averaged information on site-specific in-situ metal fluxes from sediments to porewaters under the natural conditions. We believe these fluxes may provide the best measure of the 'release potential' of metals from sediments and thereby provide excellent information on metal bioavailability. We used the DGT technique to interpret the bioavailability of copper to the benthic bivalve *Tellina deltoidealis* in sediments of varying properties contaminated with copper-based antifouling paint particles. For a concentration series of copper-paint-contaminated sediment types (sandy, silty-sand and silty), DGT-probes were used to measure copper fluxes to the overlying water, at the sediment-water interface and in deeper sediments. The DGT-Cu fluxes were shown to provide excellent relationships with both the copper concentrations and properties of the sediments which influenced the bioavailability. Strong dose-response relationships were observed when DGT-copper flux was used to predict the bioaccumulation and lethal effects occurring to the copper-sensitive benthic bivalve, *T. deltoidealis*. The study demonstrates the strength of the DGT technique, which we expect will become frequently used for assessing metal bioavailability in sediments.

ET11B-6

Imposex in the dogwhelk (*Nucella lapillus*): monitoring around England and Wales since 1992

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Surveys of imposex in the dogwhelk (*Nucella lapillus*) have been conducted UK-wide on four occasions over the past eighteen years (1992, 1997/1998, 2004 and 2007) to assess the effectiveness of legislation controlling the use of TBT on yachts and ships in the marine environment. As a continuation of this assessment, a further survey was conducted between October 2010 and January 2011. Dogwhelks were collected from 76 sites around England and Wales and analysed for imposex (VDSI and RPSI) and data were assessed according to the criteria developed by OSPAR (Oslo Paris Commission). The results show a significant decline in the level of imposex. In 1992, 100% of sites showed VDSI values above 2, (OSPAR assessment class C) while in 2010/2011, only 10% of the sites showed VDSI values above 2 and 42% were below 0.3 (OSPAR assessment class A). The data confirm that the legislation brought in by the International Maritime Organisation for large ships, and implemented during 2003-2008, has been very effective in reducing the impact of TBT in the marine environment.

ET11C-1

Biomarker responses in the laboratory are confirmed in large scale field study of ten estuaries

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The use of biomarkers as a measure of ecological response to contamination is increasing in environmental monitoring programmes. Biomarker responses are complex and may be species-specific, therefore they require evaluation in species that are native to the bioregion being monitored before they can be applied by monitoring agencies. Using both laboratory experiments and a large scale field study, we validate the use of the Sydney rock oyster (*Saccostrea glomerata*) as a biomonitoring species, and assess the potential use of two cellular biomarkers; lipid peroxidation and lysosomal membrane stability. In the laboratory, oysters were exposed to various concentrations of Cu and Cd via either their food or surrounding environment (water) for 96-h. In the field, oysters were deployed for 3 months at 4-7 sites within ten estuaries in NSW with various degrees of human modification. Biomarker responses were measured in the digestive glands of oysters. In both laboratory and field experiments lysosomal destabilisation rates were significantly higher in systems with higher contaminant concentrations when compared with controls or cleaner reference sites. In the field study, lysosomal membrane stability was significantly related to individual metal (Cu, Pb and Zn) contaminant concentrations in suspended sediments. Lipid peroxidation concentrations were generally unaffected by contaminant exposures, with the exception of a low dose food-borne Cu (10 µg Cu L⁻¹) exposure where lipid peroxidation concentrations were significantly increased. Lysosomal membrane stability in the Sydney rock oyster proved to be an efficient indicator of an organism under anthropogenic stress and these laboratory and field results further validate its use for studying biological effects of environmental pollutants in estuarine systems.

ET11C-2

Embryotoxic and genotoxic effects of pesticides and heavy metals on embryos of Pacific oyster (*Crassostrea gigas*)

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Although oyster embryos are used for decades to assess marine water quality, less is known about environmental pollutant genotoxicity and the link between genotoxicity and embryotoxicity in this sentinel species. The aim of this study is to assess embryotoxicity and genotoxicity of two dissolved metals copper sulfate and cadmium chloride (Cu, Cd) and two pesticides (metolachlor, irgarol) in Pacific oyster (*Crassostrea gigas*) larvae and to investigate the relationship between those two endpoints. Embryotoxicity was measured by calculating the percentage of abnormal D-shaped larvae and genotoxicity was evaluated with DNA strand breaks using the comet assay. After 24h-exposure, the percentage of abnormal D-larvae showed a significant increase from 0.1 µg L⁻¹ for Cu ($p < 0.05$), 10 µg L⁻¹ for Cd ($p < 0.05$) and 0.01 µg L⁻¹ for both metolachlor and irgarol ($p < 0.001$) in comparison with the seawater control. Following 16h exposure, significant increases of DNA strand breaks were observed for both metals (from 0.1 µg L⁻¹ and 10 µg L⁻¹ for Cu and Cd respectively) and pesticides (from 0.01 µg L⁻¹ for both metolachlor and irgarol) ($p < 0.05$). A strong positive relationship between embryotoxicity and genotoxicity was recorded for each tested toxicant ($p < 0.001$, $R^2 = 0.71-0.88$), except for irgarol showing moderated correlation ($p = 0.0019$, $R^2 = 0.57$). The current study suggests that some of these pollutants (irgarol, metolachlor and copper) can induce larval abnormalities and DNA damage in exposed oysters at environmentally relevant concentrations.

ET11C-3

First development and validation of genotoxicity biomarkers in frozen total blood of *Xiphias gladius* and *Caretta caretta*: a novel approach to evaluate genotoxic effects in wildlife

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The aim of this study was to develop and validate genotoxicity biomarkers (comet and diffusion assays) on frozen total blood in order to assess the DNA integrity in swordfish (*Xiphias gladius*) and the loggerhead turtle (*Caretta caretta*). Only a limited number of studies have been published that use these assays with these or other pelagic marine species. This might be due to the logistical problems associated with performing the techniques in open sea or in particular conditions. Moreover no authors have solved the fresh blood conservation problems over a long period of survey and none have assessed the biomarkers of genotoxicity on the cryopreserved blood of marine and freshwater fish species and reptiles. In the first phase of this work, a methodology using frozen conservation was developed in parallel with the comet assay on the fresh and cryopreserved blood of *Dicentrarchus labrax* and loggerhead turtles. In the second phase the techniques were validated on the blood of the loggerhead turtle and swordfish. No statistical differences were observed in the DNA fragmentation and apoptotic cells between the fresh and frozen blood of the *Dicentrarchus labrax* and loggerhead turtle. Regarding the comet assay, a positive correlation was found between the fresh and frozen blood of the loggerhead turtle. An age-related decrease in DNA fragmentation and an age-related decrease in apoptosis in the loggerhead turtle was observed. The female swordfish showed higher values of DNA damage ($p < 0.05$) and lower values of apoptotic cells than males. This study enabled a whole blood cryoconservation protocol applied for the first time in an ecotoxicological investigation. This technique led to the evaluation of possible DNA integrity in two species never studied before in this field, such as the loggerhead turtle and swordfish. The findings strongly suggest that comet and diffusion assays in frozen blood can be used to assess genotoxic damage in a very high variety of species, from threatened species to species used for the environmental monitoring of remote areas. In addition, this innovative methodology could be carried out in all field sampling conditions and not only during laboratory experiments as has been the case until now.

ET11C-4

Temporal trend of organochlorine contamination in stranded and free-ranging striped dolphin (*Stenella coeruleoalba*) specimens in the Mediterranean Sea

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Mediterranean cetaceans are known to accumulate very high levels of xenobiotic contaminants, such as organochlorines (OCs). In addition they are susceptible to effects of these anthropogenic contaminants such as endocrine-disrupting effects, including consequences on reproduction and immunity. In 1990-1992, a severe Morbillivirus infection affected striped dolphins (*Stenella coeruleoalba*) along the Mediterranean coasts, starting from Spain and progressively reaching France, Italy, Greece and Turkey. In this period, the organochlorine levels in the blubber of stranded striped dolphin specimens resulted statistically higher than the levels of all other periods and the PCB concentration exceeded those estimated to be a threshold level in aquatic mammals for observed effects on reproduction and immunity. However, the precise roles of these xenobiotic contaminants in the epizootic are unknown. Several hypothesis were formulated in order to explore the potential link between high OC levels and Morbillivirus infection. The aim of this work is to investigate the temporal trend of the levels of certain organochlorine xenobiotics: dichlorodiphenyltrichloroethane (DDT) and its metabolites and polychlorinated biphenyls (PCBs) in subcutaneous blubber of free-ranging and stranded striped dolphin specimens sampled in the Mediterranean area from

1988 to 2011, especially considering that a new *Morbillivirus* infection is present in the Mediterranean Sea. In fact, it has been isolated a new virus strain closely related to the dolphin *Morbillivirus* that was isolated during the previous epizootic in 1990. The levels of organochlorine contaminants result always statistically higher in stranded specimens than in free-ranging specimens. Temporal trends of DDTs and PCBs of both stranded and free-ranging striped dolphins highlight that the alleged decline of these xenobiotic compounds, expected after the ban of DDT in the late '70s and from the PCBs regulation, is not detectable by our results. In fact, the decrease between the years 1996-2003 is followed by an increase of these xenobiotics with accumulation peaks in the specimens sampled in 2005. Hence in the future, along with studies that should be conducted in these marine mammals to assess the presence and toxicity of the new generation contaminants, such as flame retardants, should not be left out of investigation these 'dinosaurs' of the environmental contamination of xenobiotic origin.

ET11C-5

Organochlorine and polybrominated diphenylether compounds in the northern Baltic Sea food web

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This study explored PCDD/Fs, PCBs; non-dioxin-like PCBs and dioxin-like-PCBs (DL-PCBs), and PBDEs in the Baltic Sea fish; Baltic herring (*Clupea harengus*), salmon (*Salmo salar*), sprat (*Sprattus sprattus*), whitefish (*Coregonus lavaretus*), and vendace (*C. albula*), and their predator Baltic grey seal (*Halichoerus grypus*). Contaminant profiles demonstrate possible connections in the food web expressing intense predator-prey relationship. The sampling area covered the Gulf of Bothnia in the time period 2002-2007. PCDD/F sums and WHO-PCDD/F-TEQs were highest in salmon and herring. PCDD/Fs in grey seal existed general in low level. PCDD/F concentration in herring clearly exceeded the PCDD/Fs in grey seal although herring is the most important prey species of grey seal. The most abundant PCDD/F congeners in fish were 23478-PeCDF and 2378-TCDF. PCDD/F profile in grey seal was different expressing high contribution of 123678-HxCDD and OCDD. The highest PCB sum was detected in grey seal, followed by salmon and herring. A same trend was seen when considering a sum of 6 indicator-PCB congeners (PCB-28/31, -52, -101, -138, -153 and -180) of which -138, -153 and -180 were the most dominating ones in grey seal. WHO-PCB-TEQ was in the highest level in salmon, which indicates effective accumulation of toxic PCB congeners in salmon: The sum of the most dioxin-like non-ortho PCBs was overwhelmingly highest in salmon that affects straight to the WHO-TEQ level seen. Also mono-ortho-PCB sum was highest in salmon. PCB (non-dioxin-like PCB and mono-ortho-PCB) profile was very similar between the fish species and also between fish and grey seal. The profiles of 6 indicator-PCBs were similar in fish species, deviating slightly from grey seal. Non-ortho-PCB profiles were similar between the fish species, when grey seal had clearly more PCB-169 and less PCB-77 than fish. Salmon had the highest PBDE level, although grey seal was almost had equal PBDE concentration. The others stayed quite far below of these two species. BDE-47 was the most dominating PBDE congener in all studied species: quantitatively its amounts were biggest in salmon (3.8 pg/g ww) and grey seal; especially grey seal from the Bothnian Bay (9.2 pg/g ww). BDE-209, analyzed only from grey seal, did not contribute significantly to the overall PBDE cluster. Toxic load (WHO-TEQ) of grey seal probably greatly results from preferring herring and perhaps salmon in the diet, especially in the Bothnian Bay.

ET11C-6

PCDD/Fs and DL-PCB levels in Seagull eggs from natural and National Parks of Spain

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Polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans were studied in Yellow-legged gull (*Larus michahellis*) fresh eggs from several Spanish Natural or National Parks. These areas represent the most important gull colonies of the Iberian Peninsula and all of them are declared as Special Protection Area for birds (SPAs). Concentration levels for PCDDs and PCDFs ranged between 3-10 pg WHO-TEQ/g-lw. For DL-PCBs, high levels were observed with values ranging from 66 to 400 pg WHO-TEQ/g-lw. No significant differences were observed between the concentrations found in the samples collected during the two year sampling campaigns (2010-2011). In addition, Audouin gull eggs (*Larus audouinii*) collected from the Ebro Delta Natural Park were analysed and the results were compared with those obtained in the Yellow-legged gulls (*Larus michahellis*) eggs.. The high PCDD/F and DL-PCB levels found in the Audouin gull eggs were attributed to different feeding habits among species and it confirms that the gull diet has an important role in the accumulation of dioxins and related compounds.

ET12 - Soil ecotoxicology

ET12A-1

Will the ecosystem services concept help to tackle multiple threats to soil?

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Dutch soil policy are investigating how to facilitate optimal use of ecosystem services by land users and local authorities, in order to achieve more sustainable land use. The Dutch Soil Protection Committee (TCB) is preparing a scientific opinion to support this investigation.

Until now, soil protection was based on regulations for a number of management practices. The question is whether the ecosystem services concept will be able to provide better protection of soils than regulation and if it will allow to reduce the number of regulations, which is a political wish.

Using the ecosystem services concept to achieve more sustainable land use changes the approaches followed so far drastically. Ecosystems and the services provided by these are placed central in the concept instead of an environmental compartment (soil) or a threat (contamination). Therefore the use of the ecosystem services concept in environmental policies may in theory lead to integration of separate policies, over environmental compartments and over multiple threats. Another important aspect of the concept is that it focuses on the benefit people obtain from ecosystems. It is expected that the use of ecosystem services-language will motivate stakeholders better to use soil in a sustainable way, because it is for their own benefit.

Optimization of particular ecosystem services is considered to be more sustainable when the trade off against other services is limited as much as possible. Guidelines for the sustainable use of ecosystem services and for balancing benefits of optimising one service against the effects this may have on other services, have been described.

The ecosystem services provided by soils are mainly supporting services that contribute to the provision of final ecosystem services of which mankind directly benefits.

The maintenance of intermediate services may be seen as sustainable land use and is of crucial importance for the provision of final services. It seems plausible that this relationship works also the other way: overexploiting final services may lead to lesser quality of intermediate services. It is concluded that optimal use of ecosystem services may lead to sustainable land use, provided that the ultimate goal for 'optimal use of ecosystem services' is clear and guidelines are respected. This approach has the potential to address multiple threats, as mentioned in the European Soil Strategy.

ET12A-2

Sustainable risk-based land management on polluted sites

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There is an increasing request for biomass for biofuel production. However, the use of land to produce such biomass has raised a range of concerns about the sacrifice of food growing land, food security, food poverty and habitat conservation issues. In parallel brownfields exist for which there is no economic urge for restoration to conventional functional re-use and/or no realistic prospect for 'hard' re-use. The combination of biomass cultivation and soil rehabilitation could provide leverage to bring such degraded land back into use. Benefits from this kind of land use might include providing: a self-funding land management regime, economic activity to deprived areas, a long term improvement in land values and environmental benefits.

The project Rejuvenate started as a literature and interview study regarding advantages and disadvantages of non food biomass crop on marginal, especially contaminated, land and a decision support tool (DST) for biomass production on marginal contaminated land was developed. In the second phase case studies are performed which focuses on ecological risk assessments, environmental impact assessments and assessments of economical and social aspects for the demonstration sites in Sweden and Romania. Test work has begun at one of the sites, where a site specific ecological risk assessment (Triad) was performed prior to cultivation and the addition of soil amendments and will be executed again during the project in order to gain insight in the effect of soil tillage and the addition of soil amendments. At all other sites chemical analyses of soil (including available fractions of pollutants) were performed, prior to cultivation and the addition of soil amendments and during the project. In addition chemical analyses are performed on different plants that are cultivated at the sites. The availability and uptake of pollutants in plants at treated and non treated soil plots will be compared in order to assess the effects (both positive and negative) of soil amendments.

This summer the analyses after one year of crop growth have been performed. The results will be presented at the meeting. At one site in Sweden a sewage sludge treated plot was compared with a non treated plot. At the sewage sludge treated plots the uptake of cadmium by plants (*Salix*) is decreased. The uptake of all other metals is in the same

order of magnitude in both test plots. The results of the other sites will be interpreted during the months to come.

ET12A-3

From single-species laboratory toxicity tests to assessing effects on soil biodiversity: how far can we jump?

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This contribution will review developments in soil ecotoxicology, emphasizing currently available tools and their potential to predict effects on soil biodiversity and soil ecosystem functioning.

In 1984 the first short-term toxicity tests with earthworms and plants were standardized, while tests were already available for determining effects on microbial processes.

By early 2000 toxicity tests using sublethal endpoints were standardized for terrestrial plants, enchytraeids, earthworms, Collembola and predatory mites, and new tests developed for soil microorganisms. For earthworms and enchytraeids, avoidance behaviour tests and a bioaccumulation test have been described.

Most standard toxicity tests use single species, except for the ones on soil microorganisms. To assess toxic effects in more realistic settings, micro-ecosystems were developed to allow quantifying effects at the community level, taking into account species interactions. A field test is available for assessing pesticide effects on earthworms that can be combined with a litter bag test.

For reasons of standardization, most toxicity tests with soil invertebrates use artificial soil, or LUFA 2.2 soil as a suitable and natural alternative.

The quest for "putting more eco into ecotoxicology" triggered the focus on more ecologically relevant test designs, integrated approaches with responses at different levels of biological organization, taking into account the normal operating range of ecosystems. More complex issues receive attention, including ecological vulnerability, trait-based analysis and effects on functional endpoints (ecosystem services).

Many of the tests developed for assessing the toxicity of single chemicals are also used for assessing contaminated land, together with chemical analysis and field observations (TRIAD approach). To support field observations and monitoring, sampling methods for soil invertebrates have been standardized. Interpreting results of field sampling is hampered by difficulties to find suitable references. Sampling efforts like the Dutch Biological Soil Quality network may provide useful reference data.

Properly predicting effects on ecosystem structure and functioning in field soils will require moving to more complex test systems and further development of trait-based approaches and the ecosystem vulnerability concept. It will also ask attention for long-term effects of low exposures to persistent pollutants, possibly in combination with other stress factors.

ET12A-4

The use of a small-scale terrestrial ecosystem to evaluate pesticide mixtures

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Most studies in soil ecotoxicology evaluate the effects of one chemical in one test species using a reference soil, thus here is a need for a more comprehensive, holistic, hierarchical and integrated approach to assess the impacts of chemical pollutants on soil ecosystems. An attempt to accomplish higher "realism" in ecotoxicity evaluation was achieved through a higher tier experiment, using a small-scale terrestrial ecosystem ("STEM") containing soil from an agricultural field. Effects of the herbicide glyphosate, the insecticide dimethoate and the acaricide spirodiclofen to soil non-target organisms were evaluated using three concentrations: the field dose, i.e. recommended application dose, 5 and 10 times the field dose. The concentrations were chosen based on the predicted environmental concentration (PEC) according to the FOCUS model for each pesticide. Pesticides were applied at the soil surface, and effects were observed after a 28 days of exposure. Earthworms (*Eisenia andrei*), isopods (*Porcellionides pruinosus*), turnip seeds (*Brassica rapa*) and bait-lamina strips were used to survey the effects in single and binary combination exposures of the three pesticides. Results of the individual and binary exposures are discussed taking in account pesticide dissipation along exposure period and effects mainly driven by pesticide or due to interactions occurring in the soil system. In conclusion, the work made with the STEM provided a good insight about the effects of pesticide mixtures to non-target soil organisms, both in functional and structural endpoints, and permitted an evaluation of the interactions between organisms at different trophic levels after exposure to pesticides.

ET12A-5

Assessment of the interaction between Cu and temperature changes - a multispecies approach

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Temperature is an important aspect in the study of effects of climate change. Temperature may e.g. affect the reproduction, growth or survival of species. If combined with pollution, temperature effects may be even more severe to ecosystems. In order to assess the effects of the temperature alone and in combination with copper pollution in soil population and its processes, a two factorial soil multi-species experiment was performed. Six species representatives of different functional groups were tested over three exposure durations. Feeding activity and OM breakdown were also assessed. A range of temperatures resembling the annual variation for Denmark was used. Results showed that the food-web was significantly affected by Cu pollution and temperature increase. Different species reacted very differently to the distinct treatments applied but the major changes in the abundance of species were in general, attributed to the first 28 days of exposure. Litterbags and bait laminas showed Cu and temperature effects on the OM breakdown.

ET12A-6

Evaluation of the risk for soil organisms under real conditions

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It has been questioned in the last years if the risk assessment of Plant Protection Products (PPP) in soil addresses properly the relationship between pesticide exposure and effects on soil organisms. Thus, it is necessary to develop a new approach especially regarding the estimation of environmental concentrations within the soil profile. A study was conducted in Terrestrial Model Ecosystems (TME) analysing on the one hand the effects of two model pesticides with different physico-chemical properties (Lindane, Imidacloprid) on soil organisms (collembolans, oribatid mites, enchytraeids, earthworms). On the other hand, the exposure of soil organisms, i.e., the pesticide movement was monitored in the soil profile over time. The pesticides were applied on 68 TMEs (467 mm, height 400 mm), in two concentrations each; untreated TMEs served as controls. Additional TMEs for chemical analyses, for soil moisture/temperature monitoring, and for quantification of percolation water were installed. Different soil layers (0-2,5 cm, 2,5-5 cm, 5-10 cm, 10-20 cm), were separately analysed and results are shown for soil arthropods (collembolans, oribatid mites) colonization and pesticide contents. The results of the present study enlighten the spatial and temporal pathway of pesticides along with the effects on soil organisms in different soil layers. This project will provide an insight into relevant issues for the risk assessment of PPP based on results under realistic environmental conditions.

ET12B-1

Monitoring microbial diversity in European soils: ongoing projects and challenges

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According to the Convention of Biological Diversity (Rio de Janeiro, 1992), biodiversity is the variation in life from genes to species, communities, ecosystems, and landscapes. Soils represent a huge reservoir of biodiversity which varies in terms of taxonomic richness, relative abundance and distribution according to soil types, climatic conditions, vegetation and land uses. The key functions supporting ecosystem services as identified in the Millennium Ecosystem Assessment (MEA, 2005) largely depend upon organisms that inhabit the soil. Thus, the design and implementation of a sustainable soil management strategy requires a better knowledge of soil biodiversity. Against this background,

soil biodiversity is subject to various threats. The most serious are caused by anthropogenic activities which can impair soil biodiversity and functioning with negative consequences on ecosystem service delivery, with consequent effects on primary production and soil sustainability. Determining the range of biodiversity and its impact on soil functioning and ecosystem services is therefore a critical challenge which needs to be addressed.

This talk will give a short overview, with a specific focus on microbial biodiversity, on national (France, The Netherlands, United Kingdom) and European (EcoFINDERS) initiatives which have been undertaken to assess variations of biodiversity at large spatial scales according to soil and climate types and land uses. The EcoFINDERS (Ecological Function and Biodiversity Indicators in European Soils) project supported by the European Commission was launched in order to gain information on soil biodiversity (including both microorganisms and fauna). This project will result (i) at the scientific level in increasing our knowledge of soil biodiversity and its role in ecosystem services across different soils, climate types and land uses, (ii) at the technological level in the standardization of methods and operating procedures for characterizing soil biodiversity and functioning, and the development of bioindicators, (iii) at the economic level in the assessment of the added value brought by cost-effective bioindicators, and of cost effectiveness of alternative ecosystem service maintenance policies. The management of such large sampling schemes requires the development of platforms allowing the extraction, storage and analysis of the DNA, such as the GenoSol platform (www2.dijon.inra.fr/plateforme_genosol/).

ET12B-2

Evaluation of soil biodiversity in Germany - Compilation and analysis of soil status with regard to the implementation and advancement of the national strategy on biological diversity

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According to the German Law on Soil Protection (1998), one of the natural functions of the soil is its ability to act as a habitat for organisms (mainly invertebrates and microbes). So far, this function is not considered when assessing the quality of soils, e.g. in the context of landscape planning. Therefore, in a project supported by the German Federal Environmental Agency (UBA), the following issues were addressed:

- Critical compilation of existing concepts and approaches in the area of biological soil classification and assessment;
- Assembly of a data-base on soil biodiversity in Germany, in particular collection of data from German Permanent Soil Monitoring Sites (BDF) and from literature (Collembola, Oribatida, Lumbricidae, Enchytraeidae and microbes);
- Preparation of recommendations for the implementation of soil biological monitoring concepts and methods in Germany.

In this contribution, an overview on the results is given. After a short description of the data base (structure, content), the distribution of species in correlation to site conditions is presented. In addition, ecological profiles of relevant species from different groups have been prepared. Most importantly, the composition of communities (i.e. species assemblages) for different biotope types is shown. These are the first steps to develop reference values (= normal operating range of species and communities). Finally, recommendations concerning suitable organism groups and standard methods (including - mainly microbial - genetical indicators) for a nation-wide soil biodiversity monitoring program are given.

ET12B-3

Bacterial community composition and Cu tolerance across a soil pollution gradient: exploring the bacterial domain for Cu-responding taxa by pyrosequencing

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Toxic metals affect the composition and metal tolerance of microbial communities, but very little is known about specific bacterial taxa responding to Cu toxicity in soil. We used bar-coded pyrosequencing of the 16S rRNA gene to determine the long-term impact of copper (Cu) on bacterial community composition within a soil contamination gradient (20 - 3537 µg Cu g⁻¹) established by CuSO₄ pollution more than 85 years ago. In parallel, we monitored microdiversity of culturable *Pseudomonas* spp. by universally primed PCR (UP-PCR) genotyping. Community composition were linked to pollution-induced community tolerance (PICT) data generated by the [³H]leucine incorporation method and to detailed physicochemical characterization of the soil Cu gradient soils including analyses for bioavailable Cu ([Cu]_{bio}) by a whole-cell bacterial biosensor.

Relative abundances of the most abundant phyla (Actinobacteria, Proteobacteria and Acidobacteria) were not correlated with [Cu]_{bio}, but abundances of specific classes belonging to these phyla frequently correlated to [Cu]_{bio}. Relative abundances of several less abundant phyla (Bacteroidetes, Verrucomicrobia, Chloroflexi, WS3, and Planctomycetes) were all negatively correlated with [Cu]_{bio}. The genus *Ilumatobacter* was abundant in low-Cu soils (2-3 % of all sequences), but its abundance was strongly decimated in the most contaminated soils. Remarkably, the number of unique operational taxonomic units (OTUs; 97 % similarity level) was not correlated to [Cu]_{bio}. Likewise, rarefaction analysis and calculated diversity indices did not indicate any trend between [Cu]_{bio} and diversity. By contrast the microdiversity of culturable *Pseudomonas* spp. was negatively affected by Cu as witnessed from a reduced number of unique UP-PCR genotypes in High-Cu soil. Bacterial community tolerance to Cu was strongly correlated to all measured Cu exposure descriptors indicating that the community was indeed strongly selected by Cu. Principal component analysis performed at the taxonomic class level reinforced the role of Cu as selective agent as PC1 was strongly correlated (P < 0.001) to [Cu]_{bio}.

Our data demonstrates that Cu exposure selects for Cu tolerant bacterial communities with changed community composition, but unaltered "species" richness at the taxonomic resolution obtainable using 16S rRNA gene sequencing. In addition, we propose that the unexplored genus *Ilumatobacter* may represent a sensitive indicator group for soil Cu toxicity.

ET12B-4

Soil microbial biodiversity indicating adverse effects of pharmaceutical antibiotics

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Agricultural soils receive pharmaceutical antibiotics with contaminated manure used for fertilization. Many antibiotics, being bioactive agents, tend to persist in soil for month. Hence, it is expected that antibiotics adversely affect the functional and structural diversity of soil microbial communities. Vice versa, endpoints addressing biodiversity might be suitable for the ecotoxicological testing of antibiotics in soil. Results are presented on the effects of pharmaceutical antibiotics and especially sulfonamides on soil microorganisms, tested in microcosm, mesocosm and field experiments. Contaminated manure was used for soil spiking. Phospholipid fatty acids (PLFA) and 16S rRNA genes were analyzed as markers of the soil community structure and functional enzymatic activities were determined. Manure had a strong influence on functional and structural parameters, increasing the activity of selected enzymes, basal respiration, microbial biomass and PLFA parameters. Following introduction with manure, the extractable concentrations of antibiotics and especially the CaCl₂-extractable antibiotic fraction (defined as bioavailable) quickly dissipated in soil, while effects on microorganisms tended to increase on a mid-term. This led to an apparent concentration independence of antibiotic effects. Some of the effects of the sulfonamide SDZ on soil microorganisms were significant for several months and observed for up to 120 d. Effects of SDZ on single functional parameters were often small and insignificant. However, combining test results to functional patterns more clearly revealed effects and it became evident that key-functions, e.g. of the N-cycle, were adversely affected. Distinct effects of SDZ on microbial biomass and structural diversity were determined from PLFA and 16S rRNA gene analysis, which effects interacted with the manure application rate. Both manure and SDZ induced shifts in the community structure, making communities of different soils and soil compartments more similar. Derived ratios of bacteria-to-fungi and gram⁺-to-gram⁻ bacteria indicated changes in the structural diversity induced by SDZ. Furthermore, manure-borne bacteria, designated as antibiotic-resistant, survived several weeks in soil treated with SDZ contaminated manure as was shown by sequencing of selected bands.

ET12B-5

Using soil microarthropod community testing to increase ecological relevance of effect data in pesticide risk assessment

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In the present study, a new complementary approach combining the use of the natural soil microarthropod community and conventional test methods was used. The effects of soil contamination with the insecticide carbofuran on two geographically microarthropod communities (Warm Temperate and Tropical) were evaluated under in their soils of origin/controlled laboratory conditions.

After contamination of two agricultural soils from Portugal and Brazil, a gradient of concentrations was prepared. Soil cores were taken from the respective uncontaminated surrounding areas and the mesofauna of three cores was extracted directly to the test soil. After extracting the microarthropod communities to the test soil, these were incubated under laboratory conditions for 4 weeks, after which the mesofauna was extracted again. The organisms were assorted into higher taxonomic groups and Acari and

Collembola were respectively assorted into order/sub-order/cohort and family. Collembolans were still classified according to morphological traits and used as a case-study of trait-based risk assessment (TERA, Baird et al, 2008) of pesticides.

The exposure to insecticide contamination caused the impoverishment of the taxonomic diversity in both communities. Significant shifts in the microarthropod community structure in the different carbofuran treatments were found for the both soils, although effects were more pronounced in the assay performed with the soil from Brazil. Collembolans were the most affected group with a strong decline in their abundance. A dose response relationship was observed, showing a consistent decline on the relative abundance of Isotomidae, closely followed by an increase of Entomobryidae. Contrastingly, Acari (especially Oribatida) tended to increase their numbers with higher concentrations.

Trait-based analysis of Collembola data suggested that a shift in the functional composition of the communities occurred due to carbofuran soil contamination and that species adapted to deeper soil layers were more vulnerable to insecticide toxicity.

ET12B-6

Effects of insecticidal Cry proteins on nematodes - implications for the ecological risk assessment of a stacked Bt-maize variety (MON89034xMON88017)

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ET12C-1

Assessment of contaminated soil in the Canadian boreal forest using standardized toxicity tests

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There is an increasing need in Canada for soil toxicity test methods applicable to the boreal forest and northern regions to serve the needs of both industry and government for assessment and site remediation of thousands of industrial sites in these habitats. To meet this need, Environment Canada and the Saskatchewan Research Council are developing a suite of single-species terrestrial toxicity test methods. Ecologically-relevant boreal plant and soil invertebrate species were selected and brought into culture in the laboratory. Seeds were obtained from Canadian collections or suppliers or were field-collected while candidate invertebrate species were collected through heat-extraction of various boreal reference soils from across Canada. Growth of plants and survival and reproduction of invertebrates was measured in clean reference soils to establish performance measures. Test method development has focused on seven plant species, an oribatid mite species, two collembolan species and two earthworm species. A case study of toxicity testing of a soil impacted by weathered hydrocarbons will be presented and test results indicate that the use of a test battery of both plants and invertebrates was important for a complete assessment of eco-risk at the site

ET12C-2

Development of a microbial test method suite for assessment of contaminated soil toxicity to indigenous boreal forest microbial communities

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Environment Canada (EC) has developed test methods to evaluate soil contaminant toxicity to invertebrate and plant species in agronomic soils. As soil microorganisms play a significant role in nutrient cycling and organic matter decomposition, the need to develop a test method to assess the impact of soil contaminants on indigenous soil microbial community health is apparent. The boreal forest region of Canada accounts for over 50% of Canada's land mass. As such EC is currently working to develop suitable test methods, including soil microbial community health, to assess contaminated soils in this region. The proposed test method for the evaluation of soil contaminant toxicity to indigenous soil microbial communities in boreal forest regions incorporates a suite of tests, each with different endpoints, to reflect the reality that endemic soil microbial communities vary from site to site. The test suite evaluates soil microbial biomass, activity, and community structure using the following tests: fumigation-extraction, nitrification, ammonification, organic matter decomposition, substrate-induced respiration, basal respiration, bait lamina, enzyme assays, community level physiological profiling, and denaturing gradient gel electrophoresis. Results of freshly-collected reference and petroleum hydrocarbon contaminated boreal forest soil from Alberta, Canada will be presented.

ET12C-3

Ecotoxicological assessment of soils contaminated primarily with petroleum hydrocarbons (PHCs) and/or metals and salt

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In Canada, soils contaminated with petroleum hydrocarbons are managed on the basis of four hydrocarbon fractions. There are Tier 1 Canada-wide soil standards for each fraction designed to protect ecological receptors exposed via the direct contact exposure pathway. If these standards are exceeded by fraction-specific concentrations in the soil, then the proponent has the option to conduct a Tier 2 ecotoxicological assessment to demonstrate that 1) the exposure pathway can be excluded; 2) PHC residuals are stable and represent minimal risk to soil organisms; or 3) data generated can be used to derive site-specific remedial objective (SSROs). In practice, Tier 2 ecotoxicological assessments are conducted primarily to demonstrate that PHC residuals are stable and represent minimal risk to soil organisms. The data from the toxicity assessment must satisfy criteria established for different land uses classes. This is called the Tier 2 Pass/Fail approach. If the site passes, then no further remediation or action is required and site closure can be obtained when other lines of evidence corroborate minimal risk. However, if the site soils fail to satisfy the criteria, the proponent must select management alternatives to mitigate risk. Alternatively, the data generated from the Tier 2 ecotoxicity assessment can be used to derive site-specific remedial objectives to guide future remediation. The current challenge facing regulators, assessors, and managers alike is the lack of a framework or process for the derivation of these Tier 2 SSROs. A case study demonstrating the successful application of the Tier 2 Pass/Fail Approach will be presented. A case study where the approach failed will also be presented. A process that is scientifically defensible will be presented to demonstrate how to derive SSROs where the data signal to noise ratio is small.

ET12C-4

What does ecotoxicity testing tell us about dredged sediments?

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Research is focused on the actual environmental issue - a dredged sediment application on soils and preceding evaluation of their ecotoxicity to prevent possible ecological risks. High volumes of sediments are removed from rivers and ponds routinely. Although they may have a lot of positive properties (e.g. high nutrient levels) there are reasonable suspicions about contaminants present accumulated sediments. Therefore, an assessment scheme is necessary which helps to decide, if the land application will be allowed for every dredged sediment. This assessment cannot be based only on chemical analyses and should be accompanied by ecotoxicological testing. In the Czech Republic, novel directive 257/2009 Coll. for the dredged sediments application on agricultural land was ratified to determine the conditions when it is allowed to use sediment on agricultural soils. Innovation of this directive is the inclusion of four contact bioassays into the assessment. The most important question is, which bioassays should be included in the test batteries for the complex material ecotoxicity testing. The no-less important question is if the ecotoxicity bioassays reflect the contamination of the samples or their physico-chemical properties of other factors. The aim of this study was to answer the question about the appropriate battery of ecotoxicity bioassays for the dredged sediments testing and to investigate the driving factors affecting the results of the bioassays. Extended battery of traditional and new soil bioassays as well as tests of eluates was used for testing thirty-six different sediment samples.

ET12C-5

Eco-restoration of large-scale anthropogenic disturbances in the Boreal Shield: effects of moisture content on zinc availability and toxicity to the mite *Oppia nitens* and collembolan *Folsomia candida*

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Contamination of soil is often the most common form of anthropogenic stress in ecosystems and can pose threats to human health and the environment. Metal toxicity in soil ecosystems affects microbial, fungal and invertebrate populations. These effects are often compounded by other physical stressors like acidification and moisture stresses. The Flin Flon MB, Canada is a prime example of a boreal area experiencing forest dieback and a loss of plant biodiversity associated with mining and smelting activities for metal ores especially copper and zinc but also cadmium and lead. For many years some effort (mainly liming) has yielded moderate output on restoration in some areas, while other areas are recalcitrant. As part of the effort geared towards understanding the factors that might modulate metal toxicity and/or recovery in the area, we assessed the effect of moisture on Zn toxicity to two invertebrates with contrasting uptake routes but occupying similar trophic levels; the oribatid mite *Oppia nitens* and the collembolan

Folsomia candida. We singled out Zn after a relative to Cd toxicity model (TCD) proposed by Hopkin and Spurgeon (2001) identified Zn as the metal of most concern in the Flin Flon area. Initial trials to assess the effects of moisture on Zn toxicity using contaminated field soils (with or without organic matter adjustments) were not successful due to complicating effects of soil properties and low reproduction/survival of the invertebrates. We therefore used an artificial soil system and adjusted the moisture contents to 30, 45, 60 and 75% of the maximum water holding capacity of the soil. After four weeks, we assessed availability of the metals using an ion exchange resin membrane fractionation procedure and compared survival and reproduction as well as metal accumulation in the organisms. The results showed that with increased moisture level, there was increased availability of Zn in the substrates and increased accumulation in the mites. This was further confirmed by the result of life-cycle effect for both invertebrate species which showed increased Zn toxicity as moisture level increased. The results indicate that irrespective of invertebrate species involved, increased moisture had a surging effect on Zn toxicity, and therefore, methods aimed at keeping soil moisture low in remediated areas would be of importance in eco-restoration of contaminated sites. The implications in risk assessment of Zn in contaminated sites are discussed.

ET12C-6

Applicability of the *Caenorhabditis elegans* survival, growth and reproduction test to assess the effects of biosolids used in agriculture

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Modern high productivity agriculture has led to an impoverishment of nutrients and organic carbon in field soils, and as a consequence plants nutrition complements like fertilisers or wastes have to be used to keep high yield. These complex materials can also be a source of toxicants, depending on their origin, so there is a necessity to assess their environmental effects before field use. In this study, biosolids used in agriculture were tested as a mix with standard soil at different field dose through ecotoxicity test on the nematode *Caenorhabditis elegans*, endpoints for this organism being survival, growth and reproduction. In order to run these tests we first followed the standardized protocol for sediment and soil testing with *C. elegans* (ISO 10872, 2010). These results showed that the mixtures could be significantly different according to the type of soil (e.g. two phases system with overlaying water). Indeed, soil moistening in the standardized protocol is based on soil dry weight which means that the same amount of water is added for every soil, regardless of the water holding capacity (WHC) of the samples. Moreover, some biosolids can have a high WHC what increase humidity's differences among soil and soil mixed with biosolids when moistened as recommended by this protocol. To prevent this, improvements of the standardized protocol were developed, in which water addition is based on soil WHC instead of soil dry weight. Moreover, food volume added to the samples was lowered in order to be able to include it in the volume for soil moistening. In this context and in order to validate this new method, comparisons between standardized and optimized protocols were carried out for endpoints responses in five soils with different textures. This optimized protocol was used to assess the effect of different biosolids (limed sludge, manure ...) mixed with standard soil at different field rates and an example for a limed sludge is given. Regarding these results, improvements of the standardized protocol are adapted to assess the effects of these materials on *C. elegans*.

ET13 - Natural toxins and bioactive compounds

ET13A-1

Hydroxylated / Methoxylated analogs of Polybrominated Diphenyl ethers: biotransformation products of PBDEs or natural products

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Brominated compounds are ubiquitous in the aquatic environment. The polybrominated diphenyl ether (PBDE) flame retardants are anthropogenic compounds of concern. Results of some studies have suggested that PBDEs can be biotransformed to hydroxylated brominated diphenyl ethers (OH-BDE) and subsequently to methoxy BDE (MeO-BDE). However, the efficiency of transformation of PBDE to OH-BDE formation observed has been extremely small and could be accounted for by trace contaminants in the experimental materials. OH-BDEs have also been identified as natural compounds produced by some marine invertebrates. Another class of compounds, the methoxylated BDEs (MeO-BDEs), has also been identified as natural compounds in the marine environment. Both the OH-BDEs and MeO-BDEs bioaccumulate in higher marine organisms. Recent studies have demonstrated that MeO-BDEs can be biotransformed to OH-BDEs and this generates greater amounts of OH-BDEs than could be generated from PBDEs. Consequently, MeO-BDEs likely represent the primary source of metabolically derived OH-BDEs. Given that for some endpoints OH-BDEs often exhibit greater toxicity compared to PBDEs, it is prudent to consider OH-BDEs as chemicals of concern, despite their seemingly "natural" origins.

ET13A-2

Release and distribution of the bioactive compound artemisinin in the soil in and near *Artemisia annua* plantations

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Growing of biomedicine crops, i.e. bioactive compounds produced in plants and extracted for pharmaceutical products, require monocultures of plants with high contents of the desired bio-active molecules. Artemisinin is produced by *Artemisia annua* L. and used as active ingredient in anti-malaria drugs. Cultivation of *A. annua* in field scale implies high amounts of artemisinin produced and potential high losses to soil and leaching to the aquatic environment, where artemisinin could impact vulnerable organisms. Knowledge of the release routes of active compound from plant to soil, soil concentrations as well as within and around plantations is pivotal to risk assessments of biomedicine plantations.

To evaluate the release routes *A. annua* was grown in the green house, where leaf content, total soil content as well as contributions to total soil content from debris of dead leaves, rain off and root exudation of artemisinin were measured every second week over a full growing season. The dynamics of artemisinin in a field soil was investigated under Danish conditions measuring artemisinin in depth integrated samples (0-100 cm) and along a transect (0-20 m from the plantation). Artemisinin was measurable up to 15 ms away from the *A. Annua* plantation in the direction of the dominating wind. Large amounts of precipitation during the experiment facilitated transport of artemisinin to lower soil layers at the level of the drainage pipes. Hence, leaching of artemisinin to streams and lakes is a considerable risk.

Rain off contributed to the release of artemisinin to the soil environment, but the biggest contributor was dead leaves. Root exudation was a minor contributor. The amount of artemisinin in rain off followed the leaf concentration, whereas the total concentration in soil followed the amount released from dead leaves. The artemisinin content in soil reached steady state around week 21, suggesting a degradation rate in the similar magnitude as the input rate.

A model of inputs and outputs of artemisinin to the soil environment will be provided at the symposium, as a tool for risk assessment and risk managing when cultivating plants producing a bioactive compound.

ET13A-3

Production and emission of mycotoxins from a *Fusarium* infected winter wheat test field

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Mycotoxins are naturally occurring secondary metabolites of fungi colonizing a variety of cereals, fruits, vegetables and organic material in the soil, but can also arise due to moist conditions during storage. Mycotoxins have been studied intensively for decades due to their occurrence in food and feed and, hence, their potential threat to human and animal health.

Recently published data on two prominent mycotoxins, i.e., deoxynivalenol (DON) and zearalenone (ZON), indicates that the aquatic environment can also be exposed to mycotoxins. The identified main input sources of these mycotoxins into the aquatic environment included 1) run-off and drainage water from fields cultivated with cereals, like wheat or corn, 2) manure application and excretion from grazing livestock and 3) human excretion via sewer systems. This raises the question about the emission of other similar compounds from agricultural areas cropped with cereals. Therefore, the production of mycotoxins on a *Fusarium* infected winter wheat test field and their emission via drainage water was studied. Data from this campaign will be presented here.

ET13A-4

Risk assessment of *Bacillus thuringiensis* var. *israelensis* and spinosad on *Polypedilum nubilifer* and *Tanytarsus curticornis* (Diptera: Chironomidae) in coastal wetlands

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Larvicides used for mosquito control are introduced into aquatic ecosystems where mosquito larvae develop (marshes, ponds, sanitation devices). A number of natural products have been proposed as 'environment-friendly' insecticides and some of them exhibit selectivity towards certain insect taxa which promotes their use for mosquito control. This is the case for the bacterial larvicide *Bacillus thuringiensis* var. *israelensis* (Bti), widely used for mosquito control all over the world, and for Spinosad, a mixture of spinosyns A and D known as fermentation products of a soil bacterium. Therefore, the present study was undertaken to assess the impact of Bti and spinosad on Mediterranean coastal wetland populations of *Polypedilum nubilifer* (Skuse) and *Tanytarsus curticornis* Kieffer (Diptera: Chironomidae). Unlike Bti, spinosad had a strong lethal effect on *P. nubilifer* population and seems to affect *T. curticornis* at presumed recommended rates for field application. But differences observed in term of sensibility

between this two chironomid populations confirm that the assemblage of the arthropod community as well as the population dynamic need to be known for the evaluation of risk of pesticides at the population level.

ET13A-5

New vectors of PSP, spirolides and okadaic acid in the North Atlantic: implications and impacts in marine ecosystems

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This work was done under the project ATLANTOX, aiming the discovery of emerging marine toxins in the Atlantic ocean, increased by the impact of anthropogenic inputs and climate change. For that purpose the Portuguese coast was surveyed between 2009 and 2010 for three distinct biotoxins (Saxitoxin group (PSP), Spirolides (SPX) and Okadaic Acid (OA)) in 15 benthic species of molluscs and echinoderms. These species were selected by their importance in the food-chain and to search for the presence of new vectors. The invertebrates were collected along the Portuguese coast since the summer of 2009 till the end of 2010.

For PSP's samples were analysed by the Lawrence method, for SPX LC-MS/MS technique was performed, and UPLC-MS/MS for OA analyses.

Results show that we are in presence of 16 first reports of these toxins in these endemic species: PSP's in *G.umbilicalis*, *N.lapillus*, *Monodonta* sp., *P.lividus*, *M.glacialis* and *A.depilans*; SPX in *G.umbilicalis*, *N.lapillus*, *Monodonta* sp., *M.glacialis* and *P.intermedia*; OA in *G.umbilicalis*, *N.lapillus*, *Monodonta* sp., *P.lividus* and *M.glacialis*. These results show that in the north Atlantic area new vectors of PSP, SPX and OA were found. The values obtained for some species such as the sea stars and the gastropod *N.lapillus* show that toxin transfer along food chain occur via mussels. New toxin routes especially including edible ones provide evidences that monitoring of marine toxins should be extended to other species than bivalves in order to prevent human health risks.

ET13A-6

Variations in the microcystin congener composition of lakes

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Cyanobacteria can produce a wide range of compounds which are toxic to other organisms including humans. The microcystins appear to be the most widely encountered cyanotoxins in freshwater and over 70 different types of congeners have been described. The drinking water standards are based on microcystin-LR and less attention has been given to other congeners. However, other microcystins and the actual composition observed in lakes should also be of interest since some microcystins are much more toxic than others. Unfortunately only about a dozen microcystins are commercially available and can thus be routinely quantified. At present, the factors that regulate the composition of microcystins in the environment are largely unknown. Culture studies and empirical observations of European lakes has suggested that light and nitrogen availability may influence the observed microcystin congener composition of lakes. Using ongoing water intake and lake monitoring data as well as literature data we examined the effect of lake trophic state on the relative abundance of microcystin congeners. Observations from lakes in central Canada (Ontario and Quebec) suggest that microcystin-LA, which has a similar mammalian toxicity as -LR, may be more common than in more nutrient enriched European lakes. Microcystin-LA concentrations appeared negatively correlated with microcystin-LR concentrations and the proportion of microcystins as microcystin-LA declined with increasing total microcystins.

ET13B-1

Trade-offs in herbicide-stressed *Microcystis aeruginosa*: growth vs. toxin production

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Organisms allocate assimilated resources into competing metabolic demands, such as maintenance, growth and defense. This pattern of allocation may change in response to stress and result in an increment of resources allocated to one trait and a decrement to another trait. The aim of the study was to test for the existence and magnitude of a potential trade-off between growth and toxin production in *Microcystis aeruginosa* exposed to the herbicide glyphosate at different light regimes. The ecological costs of microcystin production were estimated from differences between growth rates in a wild type strain of *M. aeruginosa* (PCC 7806) and its mutant unable to produce microcystins (PCC 7806 ΔmcyB). The trade-off (negative functional interaction between traits) triggered by glyphosate was only detected when cultures were incubated under photoperiods longer than 8 h of light (12:12 and 16:8), with different patterns for the microcystin analogues, time of incubation, and exposure concentration. Growth was the prioritized trait (ratio > 1), especially under the shortest (8 h) and longest photoperiods (16 h) during the first week of exposure. The magnitude of the trade-off ranged from 3-5[GREEKX] for the most toxic microcystin analogue MC-LR and and 2.5-12[GREEKX] for its demethylated variant [D-Asp3]MC-LR. The ecological costs involved in microcystin production were higher during the first week of incubation compared with the second. The mutant unable to produce microcystins had generally a higher growth rate than the wild type, regardless of presence or absence of glyphosate and especially during the first week of incubation. Glyphosate conferred additional ecological advantages to the mutants expressed in terms of tolerance and benefits to the wild type cultures during late exponential growth phase by either increasing toxin production or inhibiting the extracellular releases to the medium. The negative production rates calculated for [D-Asp3]MC-LR, during the first week of incubation might imply that the [D-Asp3]MC-LR pool is not stable and that the compound is synthesized, used and replenished constantly under the given culturing conditions. Growth, seems to be a conservative trait in *M. aeruginosa* because is preserved over toxin production which limits its application as ecotoxicological endpoint in risk assessment of pesticides. Microcystin production seems more responsive (sensitive) to glyphosate in time and at lower concentrations than growth.

ET13B-2

Physiological costs for biotransformation of cyanobacterial toxins in *Dreissena polymorpha* and *Unio tumidus*

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Freshwater mussels such as *D. polymorpha* and *U. tumidus* with high filtration may accumulate cyanobacteria and their toxins during cyanobacterial blooms. The population of Unionidae is endangered due to shoreline construction and water pollution as well as by competition for food with *D. polymorpha*. The two species are not competing for the same habitat, as the Unionidae submerges in soft sediment, whereas *D. polymorpha* attaches to hard substrat. In contrast to the native *U. tumidus*, the invader *D. polymorpha* establishes sustainable populations in urban watercourses. *D. polymorpha* seems to be a moderate sensitive species.

This study compares the freshwater mussels *D. polymorpha* and *U. tumidus* with regard to their physiological costs for the biotransformation of microcystin. For this, the energy reservoirs (glycogen- and lipid content) and the glutathione pool (total GSH, reduced GSH and GSSG) were compared in the invasive *D. polymorpha* and in the native *U. tumidus* in response to cyanotoxin exposure (10 and 50 µg-L⁻¹) for 24 h and 7 d. A further aim was to compare the effect of cyanotoxin exposure to fatty acid patterns in the two species.

Energy reserves and GSH pool of *D. polymorpha* were measured in whole mussel tissue and of *U. tumidus* in digestive gland, remaining tissue, gills, mantle and foot. Fatty acid composition were analysed in whole mussel tissue of both species. The glycogen content decreased after 24 h in both species indicating the energy requirements due to the stress caused by the MC-LR exposure. The lipid content was merely not affected in *D. polymorpha*, whereas the lipid content in remaining tissue, digestive gland and foot of *U. tumidus* significantly decreased. The decrease in the lipid content in *U. tumidus* indicates the additional consumption of the long-term energy reserves to deal with microcystin. The GSH pool was merely not affected by microcystin exposure in both species.

Exposure to MC-LR or cyanobacterial crude extract enhanced the requirement for energy, as indicated by reduced glycogen content in both mussel species and reduced lipid content in *U. tumidus*. Together with results from a further study on capacities of biotransformation of microcystin and the ability to cope with oxidative stress in *D. polymorpha* and *U. tumidus*, it seems that *D. polymorpha* is better adapted to cyanobacteria contaminated water courses in comparison to *U. tumidus*.

ET13B-3

Do cyanobacteria produce estrogenic compounds?

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Number of studies document hazardous potential of cyanobacterial metabolites. Despite the observed effects on reproduction related parameters, growth and development, there is little information on endocrine disruptive potential of compounds from cyanobacteria. Our research aimed to determine if complex water bloom of cyanobacteria collected from the environment contain compounds that would interact with the signaling pathways of arylhydrocarbon receptor (AhR), androgenic receptor (AR), estrogenic receptor (ER), glucocorticoid receptor (GR) or retinoid acid receptor (RAR). In vitro reporter gene trans-activation assays were used to determine the potency of samples to elicit receptor-mediated responses. Estrogenicity was detected for most environmental samples, their estimated estrogenic equivalents ranged from 19 to 2200 ng 17β-estradiol /g dw. Some of them also interfered with the signalling of androgen receptor. The follow-up study investigated in greater detail estrogenic potency of both inner content and exudates of individual cyanobacteria and algae cultured in laboratory. The results document estrogenic potential of compounds produced by cyanobacteria

and algae, generally more for the compounds excreted to their surroundings. Exudates from two algal species and from most tested pure strains of cyanobacteria elicited estrogenic activity with concentrations of estrogenic equivalents in the range of ng/L, which could be considered relatively large since equivalent potencies of estrogens have been shown to cause reproductive toxicity to aquatic animals. Aqueous extracts caused significant estrogenicity only in case of both algal species and one cyanobacteria (*Aphanizomenon gracile*) with estrogenic equivalents ranging from 15 to 280 ng 17 β -estradiol/g DW. There does not seem to be any clear relation between the estrogenic potency and concentration of known cyanotoxins. The research was supported by the Czech Science Foundation grant No. P503/12/0553 and by the project CETOCOEN (CZ.1.05/2.1.00/01.0001) from the European Regional Development Fund.

ET13B-4

Seasonal variation of immune parameters in mussels (*Elliptio complanata*) exposed to natural bloom of cyanobacteria

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Toxic cyanobacteria can have harmful impacts on aquatic organisms. In Lakes located in the Eastern-township (Québec, Canada), *Microcystis aeruginosa* blooms drift into littoral areas. We studied the effects of *M. aeruginosa* on the immune system of a common mussel (*Elliptio complanata*) during an algal bloom. In order to verify the toxic potential of these algae, we immersed caged mussels during 4 weeks for three consecutive months, from August to October, in four different sites. Among these sites, three of them belong to the Yamaska Basin, which is contaminated by anthropogenic pollution (agricultural and industrial). Two of the sites (Boivin, Choiniere) are prone to cyanobacterial blooms during summer, a third site represented the Yamaska River outlet (Yamaska) into the St-Lawrence River and the fourth site was located in the Lac St-Pierre and is considered the cleanest site (LSP). *M. aeruginosa* bloom occurred in Choiniere in September. The immune parameters consisted in haemocyte integrity and phagocytosis were analyzed each month by flow cytometry. Two-way ANOVA (interaction term) indicated that immune parameters exhibited marked temporal variation in different sites. Haemocyte viability remained constant throughout the experiment in Boivin and Choiniere but a significant decrease was measured in September and in October in both Yamaska and LSP sites. At Choiniere, phagocytosis activity remained relatively constant after each monthly exposure even though a significant decrease was observed in both LSP and Yamaska in September. Mortality of haemocytes could be a sign of cytotoxicity and mechanical or chemical disturbance, which cyanobacteria bloom does not seem to be. Yamaska outlet incorporates all sources of pollution both agricultural and industrial but LSP results are quite surprising as it represents the cleanest site. A decrease of the phagocytic index is usually observed response to in vivo or in vitro exposures to toxic concentration of xenobiotics or a hormesis phenomenon. Consequently, ingestion of algal during a bloom by mussels does not provide evidence of a depressed immune status during *M. aeruginosa* bloom. Furthermore, the present study will investigate other effects of these toxins on the intracellular thiols production, reactive oxygen species (ROS) production, cyclooxygenase activity (COX) and nitrate production in mussel haemolymph.

ET13B-5

Impact of natural cyanobacterial biomass containing microcystin-LR on larval stages of the amphibian *Xenopus laevis*

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Cyanobacteria are known to produce several compounds and especially their secondary metabolites, so-called cyanotoxins, might have severe effects on vertebrates according to their chemical structures. The main occurring cyanotoxins, microcystins (MCs), are considered as being potent hepatotoxins causing a threat for health of vertebrates including humans. Despite aquatic vertebrates are primarily exposed to cyanobacteria and especially to cyanotoxins, in their natural habitat, most studies with MCs were performed using non-aquatic vertebrates e.g. mammals. In aquatic vertebrates most experiments have been carried out in fish by unnatural exposures via injections of pure cyanotoxins. In amphibians, only a few data are available to assess MCs impacts during embryonic development. However, only postembryonic larval stages are characterized as the most sensitive period concerning development and sexual differentiation. In the present study, tadpoles of the South African clawed frog *Xenopus laevis* were exposed for 21 days to MC-LR provided with diet as a natural exposure route. Effects of diets containing natural cyanobacterial biomass resulting in considerable amounts of 42.8 and 187.0 μ g MC-LR/g diet, respectively, were investigated concerning bioaccumulation, development, stress and detoxification in order to determine for the first time potential physiological impacts of cyanobacteria on postembryonic stages of amphibians. The fate of MC-LR at concentrations of taken up via ingestion was determined in whole body using liquid chromatography-with tandem mass spectrometry detection. Beside mortality and weight effects on metamorphosis were assessed by recording developmental stages. In parallel, corticosteroid levels were determined as stress biomarker. In addition, gene expression of endocrine parameters associated with metamorphosis and sexual differentiation as well as of detoxification enzymes of all three biotransformation phases was investigated. Surprisingly, exposure to MC-LR containing cyanobacterial biomass applied via natural exposure neither resulted in bioaccumulation of MC-LR nor affected survival and development, only slight impacts on weight and corticosteroid levels were found. It seems likely that *X. laevis* underwent evolutionary adaptations to cope physiologically with toxic cyanobacteria occurring in the same habitat, enabling survival even at MC-LR concentrations being rather toxic for mammals.

ET14 - Bringing ecological processes into ecotoxicological risk assessment

ET14-1

Fungicidal effects on a decomposer-detritivore-system

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In heterotrophic streams, leaf litter breakdown is a fundamental process that is primarily realized by decomposing microorganisms, especially fungi, and detritivorous, leaf-shredding macroinvertebrates. Although fungicides, due to their unspecific modes of action, may affect both groups of organisms, up to date no test design exists to assess combined effects (i.e. direct toxicity and food quality related, indirect effects) on shredders. Therefore, the present study evaluated this scenario using a five-week semi-static test design with *Gammarus fossarum* as test organism and tebuconazole (65 μ g/L) as model fungicide. Hence, gammarids directly exposed to the fungicide additionally received leaves conditioned (i.e. colonized and altered by microorganisms) in the presence of tebuconazole. Fungicide exposed gammarids produced significantly less faeces (~20%) resulting in a significantly increased assimilation (~30%). This increase probably compensated for direct, toxic as well as indirect (i.e. lower quality of food) effects. The latter were indicated by a significantly reduced fungal biomass (~40%) and a significantly reduced sporulation (~30%) associated with tebuconazole exposed leaves. However, the significantly reduced lipid content (~20%) of gammarids exposed to the fungicide implies that combined effects of tebuconazole were not fully compensated by the increase in assimilation. The present study, thus, indicates that fungicides may affect energy processing in gammarids, a key species in leaf litter breakdown. This may eventually translate into alterations in energy transfer in heterotrophic streams. Consequently, test designs similar to the one used during the present study - assessing combined effects - should be used in the future to validate the protectiveness of environmental risk assessment for decomposer-detritivore-systems and consequently leaf litter breakdown.

ET14-2

Trophic niche metrics based on stable isotopes (d13C and d15N) for the assessment of the functional effects of toxic substances on aquatic food webs

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Toxic substances may have negative impacts leading to cascading effects on food webs through e.g., changes in the nature and abundance of available food resources or inter-species relationships. This may lead to effects on various functional processes that ultimately translate into changes in ecosystem properties. The identification of parameters that may provide information on the effects on ecosystem functioning therefore constitute a challenge for the future of ecological risk assessment. Measurements of the natural abundances of stable isotopes of various elements in environmental samples are widely used in ecological studies to characterize the trophic position of species and to identify the sources and pathways of matter across food webs. Various quantitative metrics of trophic niche based on such analysis have recently been proposed, that proved to be efficient in identifying the effects of environmental degradation on aquatic food webs.

In this study the characteristics of the trophic niche of a freshwater snail species, *Radix peregra*, were characterized in outdoor control and fungicide-exposed artificial streams. Streams (length: 40 m, depth: 50 cm) were exposed to a dithiocarbamate fungicide, thiram. Two concentrations (nominal values: 35 and 170 μ g L⁻¹), were used, with two replicates per concentration. Four untreated cosms were used as controls. Streams were continuously exposed for three weeks, followed by a two month-long recovery period. Samples of snails and their potential food sources (filamentous algae, biofilm, litter) were collected in the streams just before the beginning and immediately after the end of the exposure period, and at the end of recovery period. Stable carbon (C13C) and nitrogen (C15N) isotope signatures of samples were measured using mass spectrometry. The results were used to compute the values of various trophic niche metrics that were compared between control and contaminated systems. The results clearly show a significant effect of thiram on the characteristics of the trophic niche of *R. peregra* and on their dynamics. Changes in the structure of benthic food web in the streams were associated with changes in the nature and abundance of food sources used by *R. peregra*. Stable isotope signature of snail tissues mirrored these changes therefore suggesting that this approach may prove to be useful to assess some of the functional consequences of toxic substances on aquatic food webs.

ET14-3

The do's and don'ts of putting eco into ecotoxicology

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In 1988 John Cairns Jr. published a paper in Regulatory Toxicology and Pharmacology entitled 'Putting the eco into ecotoxicology'. In this paper he made a visionary plea for assessing effects of chemicals using mesocosms and to include this information in the ecological risk assessment process. The hope was to bridge the gap between the data gathered for the risk assessment (standard laboratory tests) and the protection goal (ecosystems). In the field of (chemical) stress ecology, ecological tools are used and adapted for inclusion in risk assessment. In this paper I will review the studies that use species traits and the processes between species traits and the determinants of ecosystem response to chemical stress. The do's and don'ts will be identified and discussed as well as a way forward. Van den Brink (2008) defines vulnerability as a combination of intrinsic sensitivity, recovery potential and ecosystem interactions. Although sensitivity is not an ecological trait its inclusion in the ecological risk assessment of chemicals is pivotal. For the establishment of the relationships between traits and sensitivity or when sensitivity is used in retrospective risk assessment, mode of action specific approaches need to be developed. For a successful use of indices of chemical stress, their specificity should also be proven by assessing their correlation with other types of stress, like sedimentation, nutrient and habitat destruction. Recovery is determined by the sensitivity and life history characteristics, like dispersal ability and reproduction, of the species and the structure of the landscape under consideration. Therefore, population models are ideal tools to estimate of recovery for a particular species in a defined landscape. In order to improve the ecological foundation of risk assessments, there is a great need for modelling and experimental studies that addresses the importance of habitat quality, multiple stress and both intra- and interspecific interactions for the recovery of affected populations. Micro- or mesocosm experiments are considered a realistic and useful approach to directly assess chemical effects on ecosystem structure. Measurements of ecosystem functioning, however, are much more scarce. Food web models can either be used to assess the effects on ecosystem function from an existing mesocosm data set or can be used for the assessment of chemical risk for a certain ecosystem.

ET14-4

Predicting community effects of toxicants considering interaction with stressors

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Environmental risk assessment aims at predicting effects of PPP's on non-target communities so that unacceptable effects are avoided. To do so, a tiered approach is used that ranges from lower tier to higher tier testing, characterised by an increasing realism. Relevant processes to be considered in this context are interactions of toxicants with biotic and abiotic stressors. Such interactions can act in two directions: they may either reduce or enhance effects of toxicants. The identification of these effects is highly relevant to further improve risk assessment of toxicants. The aim of this contribution is to identify the possibilities and limitations of a systematic prediction of magnitude and direction of toxicant effects when interacting with biotic and abiotic stressors in aquatic ecosystems. We show that interactions of toxicants with biotic and abiotic stressors alter the sensitivity of populations. They can vary up to two orders of magnitude. Ecological traits that are relevant to predict outcomes of interactions were identified. Finally the results were related to the trait based indicator system SPEAR to validate predictions of toxicant interactions with stressors. We conclude that considering interactions with biotic and abiotic stressors greatly enhances the quality of the effect assessment of toxicants

ET14-5

Competition matters: modelling long-term population level effects of contamination

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Prospective risk assessment of pesticides aims at insuring that there are no adverse effects of the substances on the environment. Recently, experimental studies revealed the high relevance of species competition for toxicant effects on the population and community level. Hence, for risk assessment to be realistic and to include all relevant ecological processes, competition should be taken into account.

Here, we use a generic individual-based model (IBM) of two competing species to investigate how acute and chronic toxicant effects on individuals translate to population level effects. In addition, we investigate the role of life cycle traits on population recovery. Furthermore, we compare our modelling results with data of population dynamics observed in a semi-field study.

We found that competition substantially prolongs population recovery after contamination: Without interspecific competition the population of the sensitive species recovered within 9 time steps after a contamination event that killed 50% of the individuals. In contrast to that, recovery time increased to up to 34 time steps in the presence of an insensitive competitor. Furthermore, chronic toxic effects on the individuals that decreased reproduction capacity or survival probability increased the adverse effects on the population level. In particular, species with lower reproduction capacity were strongly affected in the presence of competitors and additional chronic effects. In extreme cases populations of such species eventually went extinct even with low acute and chronic effects of the toxicant on the individuals. We showed the structural realism of the model as it produced comparable pattern of the relationship between the two competing species abundances as observed in the semi-field study.

Our findings imply that both experimental and modelling studies that do not consider the interaction between interspecific competition and toxicant effects will largely overestimate the speed of recovery processes. The inclusion of species interaction in ecotoxicological models aids a more realistic prediction of long-term effects on the population level from effects on the individual level.

ET14-6

Using a modelling approach to compare sensitivities to Triphenyltin at the individual and population levels for three planktonic organisms

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Extrapolation of toxic effects from the individual to population level requires the consideration of detailed underlying processes. Species differ in life-history strategies and consequently in their responses to toxic stress, population dynamics and time to recovery.

In this study an individual-based population modelling (IBM) approach for the freshwater cyclopoid copepod *Mesocyclops leuckarti*, the multivoltine aquatic phantom midge *Chaoborus crystallinus* and the cladoceran *Daphnia magna*, was used to simulate the responses of populations to Triphenyltin (TPT). Acute toxicity data for *M. leuckarti* with TPT (LC₅₀ between 50 and 60 µg/l for copepodites and adults), *C. crystallinus* (LC₅₀ 19 µg/l) and *D. magna* (LC₅₀ 30 µg/l for neonates, 50 µg/l for adults) for constant exposure to TPT came from laboratory experiments. We used a newly developed IBM for *M. leuckarti* and existing IBMs for *C. crystallinus* and *D. magna*. The IBMs for each of these species are based on their respective physiological parameters and life-history. Each IBM is distinct in terms of the life cycle of the species it describes. The General Unified Threshold model of Survival (GUTS) was employed to simulate the toxicokinetics (TK) and toxicodynamics (TD) of TPT. The endpoints evaluated were extinction probability for constant exposure and time to recovery for a single peak application.

For similar acute sensitivities in laboratory experiments, which is also what we observed, microcosm experiments by Roessink (2008) showed different responses at the population level e.g. NOEC values for chaoborids, cladocerans and copepods in clean sediments were 10 µg/l (week 8 to 42), 30 µg/l (week 0.4 to 12) and 1 µg/l (week 2 to 8) respectively. For a single application of TPT, cladocerans and copepods recovered in 4 weeks and 12 weeks post application at 30 µg/l whereas chaoborids did not recover at all. We used our IBMs to simulate these data sets and to try to predict the observed varied sensitivities and recovery times.

In conclusion, for species with similar individual level responses to toxicants, population level responses may be vastly different due the influence of different life-history strategies. It is possible to simulate these differences using the power of population modelling and make educated predictions for better risk assessment.

ET15 - Pesticide fate and ecotoxicology

ET15A-1

Implications of the inclusion of acute tests with *Americamysis bahia* and/or *Chironomus riparius* as new data requirements for the aquatic effect assessment of insecticide

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Under the new PPP regulation the acute toxicity to aquatic invertebrates will, in first instance, be assessed on basis of (1) the acute lab 48-h EC50 for *Daphnia* and (2) the acute 48-h EC50 for *Americamysis bahia* and/or *Chironomus riparius*. In the first-tier effect assessment an Assessment Factor (AF) of 100 will be applied to the lowest 48-h EC50 value. The tier-1 Regulatory Acceptable Concentration (tier-1 RAC) thus obtained should not exceed the highest predicted peak concentration (PECmax) in edge-of-field surface water. We intend to evaluate the implication of the new aquatic data requirements (inclusion of acute tests with *A. bahia* and/or *Chironomus* spp.) for the tier-1 acute effect assessment for insecticides by using threshold concentrations for treatment-related effects as observed in micro/mesocosm studies treated with insecticides.

Our evaluation demonstrates that for insecticides with very specific modes-of-action, such as neonicotinoids and insect growth regulators (IGRs), the concentration on basis of the EC50 of *Daphnia* and the application of an AF of 100 may not always protect sensitive arthropods in micro/mesocosm tests. The tier-1 effect assessment procedure on basis of the new data requirements (acute EC50 values for *Daphnia magna* and *Americamysis bahia* and/or *Chironomus*) appears to be protective for the vast majority of insecticides evaluated in micro/mesocosms. For the combination *D. magna* and *A. bahia* only in 2 out of 21 cases the tier-1 RAC was not protective, while that was 1 out of 25 cases for the combination *D. magna* and *Chironomus* and 1 out of 29 cases for the combination *D. magna* and either *A. bahia* or *Chironomus*. Overall, the current evaluation

by comparison of RACs obtained from the new first-tier data requirements to ecological safe threshold concentrations for arthropods communities as derived from semi-field tests implies that the new data requirements in case of insecticides and using acute toxicity data from *D. magna* and *A. bahia* or an OECD-chironomid seems to be protective in most cases and is an improvement compared to the old data requirements.

ET15A-2

A focal fish species for pesticide risk assessment in the EU

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Extrapolation is needed to link standard toxicity endpoints to protection goals. Ecological modelling can serve as an extrapolation tool from the individual to the population level and from test to exposed species. However, it is crucial to decide on a suitable species to be modelled. For this purpose, we established a procedure to identify a focal species, a realistic-worst case which is thus representative of the life histories that might occur in the field, on conservative basis. We adopted the vulnerability principle which consists of (a) intrinsic sensitivity, (b) external exposure and (c) ability to sustain a population. The procedure allows the definition of a focal species assuming that all fish can be intrinsically sensitive to certain levels of plant protection products (PPPs).

Out of 579 fish species living in European freshwaters, 27 were found to be native to Europe, widespread in at least one of the EU regulatory zones of authorization of PPPs, and inhabiting streams, ditches or ponds. Based on the reviewed studies on species assemblages, the presence of these species in agricultural settings was verified, and hence their potential external exposure to PPPs.

Deterministic age-based matrix models (time step 1 year) are being parameterised from published field demographic studies. The procedure was exemplified so far for four species whereby proportional effects of alterations in juvenile survival, adult survival or fertility on population multiplication rates (λ) were compared. Since fish are protected on the individual level against visible mortality, the most important factor in our analysis is the reduction in fertility and its effects on the population multiplication rate of a species.

The focal species will be identified and presented; a fish species that is native to Europe, widespread in the EU, lives in edge-of-field water bodies and has the lowest ability to sustain its population (highest proportional effect or elasticity of λ) as compared with the other species in case a PPP reduces its fertility and thus compromises this ability.

ET15A-3

In situ GamTox for assessment of acute and chronic pollution in small streams

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ET15A-4

Sub-lethal effects of imidacloprid pulses to aquatic invertebrates

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Observing behavioural changes of organisms evoked by relatively short and low concentrated stress in ex-situ studies is a useful tool to address xenobiotic impacts in a more environmentally-relevant manner. Ecosystems are usually exposed to low concentrations of toxicants and environmentally-relevant concentrations rarely cause mortality nor are continuously present.

We aimed to contribute to the understanding of the time course of sub-lethal effects of short-term exposure with xenobiotics to non-target species by undertaking ex situ studies with a recovery phase. The feeding rate of the shredder *Gammarus pulex* and a range of sub-lethal endpoints for the crustacean *Daphnia magna* were observed. We measured the influence of the neonicotinoid insecticide imidacloprid to the feeding rate of *G. pulex* within a feeding activity assay consisting of a four day exposure phase extended with a five day recovery phase. The impact of a one-week exposure of new born *D. magna* to imidacloprid was observed measuring growth and reproduction subsequent to exposure frequently over four weeks.

We found that concentrations within the range of measured and estimated environmental concentrations caused significant decreases in the feeding activity of *G. pulex* during exposure, and that a strong rebound in feeding activity occurred after exposure. Effects of imidacloprid to the growth and reproduction of *D. magna* were observed within and after exposure. Both endpoints were still concentration dependent and significantly affected four weeks after exposure. Inhibition in growth was observed at the lowest concentration tested (0.15 mg/L) whereas inhibition in reproduction was observed at concentrations of at least one order of magnitude higher.

Lethal and sub-lethal endpoints can be still valid beyond the presence of the toxicant. Observing the endpoint of interest above the direct impact can lead to a better understanding of the effect. Whether or not sub-lethal effects of toxicants at an individual level are ecologically relevant needs investigation. Ecotoxicological modelling, in particular individual-based population modelling, is a rising research area trying to overcome the mismatch between laboratory testing on individuals and the aim of environmental risk assessment to protect populations. Data as shown here not only contribute to the understanding of effects, but they can also be used for development and evaluation of models.

ET15A-5

New methods for assessing the effects of insecticides on insect larvae and adult emergence in freshwater outdoor microcosms

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In the classic test designs that follow current recommendations for freshwater microcosm studies, (HARAP, CLASSIC, and OECD), the effects of insecticides on the emergence of insects from aquatic systems are generally based on counts of their numbers in emergence traps that occupy a relatively small proportion of the water surface. In order to allow a more critical examination of insecticide effects, in this presentation we describe the application of completely new methods for assessing effects on larvae and on adult emergence, as well as effects on the reproduction of some species. Field microcosms were covered by enclosures to form a walk-in aerial microcosm (24 m³) which retained emerging insects for collection and also provided the opportunity for insects in the enclosure to reproduce and re-introduce larvae back into the aquatic microcosm.

The results of two studies on insecticides with differing physicochemical properties are described. In the first, over 155,000 insects from 13 families were collected. Emergence times for some species e.g. Damselflies (Coenagrionidae) and Mayflies (Baetidae) were clearly defined whereas others e.g. Caddis (Lymnephilidae) and the Phantom midges (Chaoboridae) were protracted and spanned almost the entire experimental period. Insect numbers were sufficient for multivariate and univariate analysis but differences in emergence periods highlighted the need for a knowledge of life history to allow correct data interpretation. In the second study, where emerged insects were allowed to survive within the enclosures, larval counts in invertebrate colonisers placed into microcosms showed that at least three insect taxa i.e. Lymnephilidae (Caddis), Coenagrionidae (Damselfly) and Lestidae (Damselfly), reproduced and deposited eggs back into the microcosms from which they themselves emerged.

The methods described here can be used to assess the effects of plant protection products on insect emergence and reproduction under realistic semi-field conditions which satisfies a key requirement for the determination of the environmental safety of insecticides. The insects identified in these studies that were capable of emerging and reproducing in the aerial microcosms formed by the field enclosures, provide a valuable resource for extending field methods in ecotoxicology.

ET15A-6

Low concentrations of imidacloprid cause mortality of *Gammarus pulex* by interfering with feeding behavior

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Neonicotinoids are insecticides which interfere with invertebrate behavior, e.g. feeding, by mimicking neurotransmitter acetylcholine and stimulating the nervous system. If feeding behavior is impaired, neonicotinoids might cause mortality even in concentrations far below acute toxicity levels. We studied effects of the neonicotinoid imidacloprid on the aquatic invertebrate *Gammarus pulex*. *G. pulex* is an European amphipod decomposing organic material in streams. It is highly affected by imidacloprid: acute toxicity to *G. pulex* (LC50 = 270 µg/L) is almost 800 times below the concentrations for fish.

We carried out two experiments (14d and 21d long), where we measured survival, feeding rate and lipid content. Both included two treatments (A, B) with two 1-d exposure pulses and one treatment with constant exposure to the corresponding time-weighted average concentration (C: 15 µg/L). Treatments A and B differed in their times between the chemical pulses (A: short, B: long). In the 1st experiment, we observed only survival while during the 2nd experiment, we also observed feeding rate by weighing the food before providing it to *G. pulex* and after removal from experimental beakers. Lipid content was measured from organisms sampled at the end of the 2nd experiment, by the gravimetric method. For food we provided horse chest-nut leaves, inoculated with the fungi *Cladosporium herbarum*.

At the end of the 1st experiment, survival in treatment A was 70%, in treatment B 63%, in treatment C (TWA) 46% and in controls 82%. The difference in survival curves between treatment C and controls was highly significant (one-way ANOVA: $p = 0.0001$) and also the pulse treatments differed from controls (A: $p = 0.091$, B: $p = 0.014$). Feeding rate measured during the 2nd experiment did not differ significantly between controls and pulsed treatments (Repeated Measures ANOVA: $p > 0.05$) while between

treatment C (TWA) and controls, the difference was highly significant ($p < 0.001$, C: 1.94 ± 0.85 mg/individual/day, controls: 4.42 ± 1.82 mg/individual/day). In addition, lipid content at the end of the 2nd experiment differed between treatment C and controls (t-test: $p = 0.027$, C: $1.1 \pm 0.7\%$, controls: $1.5 \pm 0.6\%$). The difference between survival curves of treatment C and controls was not significant ($p = 0.076$).

Altogether, the results indicate that low concentrations of imidacloprid inhibit feeding of *G.pulex*, which may lead to mortality due to combined effect of the compound and starvation.

ET15B-1

Using 'species sensitivity distribution' to assess herbicides toxicity on benthic diatoms assemblages

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Herbicides in the littoral zone of lakes are often present in higher concentrations with greater molecule diversity than in pelagic zone. Benthic diatoms comprise a significant portion of the fixed biomass in this zone, but the herbicides tolerance threshold of these non targeted organisms is not well known. With the view of assessing the ecological risk of herbicides for aquatic microorganisms, some models, such as Species Sensitivity Distribution (SSD), have been developed on phytoplanktonic species of the pelagic zone. Often, benthic communities are not considered in lake from an ecotoxicological point of view. However, these zones could be exposed to specific contamination and studying the sensitivity of benthic organisms could provide a new approach for ecotoxicity assessment in lacustrine environments. Our study aims to determine if SSD models are adapted to describe the sensitivity of benthic diatoms to herbicides. To achieve this goal, we assessed the sensitivity of 11 benthic diatoms species to 5 herbicides (diuron, isoproturon, terbutryn, atrazin and metolachlor).

First, we constructed a database of toxicity thresholds for each herbicide-species combination using 96 hours monospecific bioassays and an evaluation of growth inhibition. From each dose-response curve, an effective concentration that inhibits 50% of growth (EC50) was extrapolated. EC50 values showed a great variability of sensitivity among species for a same herbicide and among herbicides for the same species. A SSD curve (SSD-EC50) was built for each herbicide, based on EC50 values of each species exposed to that herbicide. According to Hazardous Concentrations (HC) obtained from SSD-EC50 curves, the increasing order of herbicide toxicity was metolachlor, atrazin, isoproturon, terbutryn and diuron. Different pools of diatoms were defined according to their tolerance level. The most obvious pools distinction was associated with photosystem II (PSII) inhibitors. Four strains (*Nitzschia palea*, *Craticula accommoda*, *Gomphonema parvulum*, *Eolimna minima*) of the 11 tested species were always the more resistant. This tolerance could be explained by the trophic mode and the motile guild. Indeed, N-heterotroph and motile guild species seemed to be more resistant to PSII inhibitors than N-autotroph and other profile guild species.

These initial results are encouraging and support the use of SSD models for more complex investigations based on benthic diatoms data.

ET15B-2

Ecotoxicity testing of pesticides at the soil surface using a non-target terrestrial gastropod

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Fate and effects of pesticides in agrosystem were studied using a non target species living at the interface soil-plant-air, the landsnail *Helix aspersa*. Fate in and effects on snails of 8 commercial herbicide and fungicide formulations (Roundup® and Bypass® = glyphosate; Basta® = glufosinate; Valiant® Flash = folpet + cymoxanil + foseetyl-Al; Coral® = tebuconazole; Cabrio® Top = pyraclostrobin + metiram-Zn; Thiovit® = sulphur; Bordeaux mixture = copper salts) commonly employed in vineyards were studied in the field and for some of them, in laboratory experiments. Here we present a synthesis of a four-years program that aims to develop various biological methods for in situ biomonitoring and life cycle exposure under controlled conditions.

A field study in real conditions of pesticide application revealed few effects on the survival and growth of juvenile snails exposed in microcosms to each different treatment.

Glyphosate (4 mg kg⁻¹) and AMPA (8 mg kg⁻¹) were detected in snail tissues. The transfer of pyraclostrobin and tebuconazole was also demonstrated. Internal concentrations were low but however higher than LMR drawn for animal products.

The effects of two herbicides, Bypass® and Basta® were studied on the whole life cycle of snails in laboratory conditions. No toxicity was found on survival and growth although an accumulation of glyphosate was revealed in snails (6 mg kg⁻¹) exposed continuously to contaminated food (30 mg kg⁻¹).

Bioassays for embryotoxicity testing of chemicals in solution or in the soil showed that all pesticides inhibited egg hatching success at lower concentrations than those applied in the field and the influence of soil characteristics on the toxicity of the pesticides was demonstrated. As bioassays using eggs are considered as in vitro methods, efforts should be given to improve and promote the use of such method.

This work led to the proposal of complementary tools available for pesticide risk assessment in terrestrial environment. Analytical difficulties were encountered and it was not possible to finalize the analytical methods for all substances studied. Thus, the determination of pesticide residues in animal tissues still represents an essential challenge for current environmental concerns.

ET15B-3

Dust drift during sowing into adjacent areas - potential emission during sowing of seeds treated with pesticides

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Seeds of many crops are pesticide treated to protect young seedlings against pests. In 2008 bee poisonings of about 12000 hives was observed in Germany after sowing of maize. Relevant amounts of insecticides drifted into adjacent flowering crops in the form of contaminated dust. Analysis of dust abrasion from different seed batches resulted in up to several g a.s. / ha in the form of loose dust.

In field experiments using seed batches with known content of loose dust and a.s. in dust different passive samplers (petridishes, adjacent crops, gauze nets) were used to check for potential exposure in the neighbourhood of sown fields. Data of field experiments carried out between 2008 and 2011 will be presented which show potential exposure due to sowing of different crops and seed qualities. Differences in spray and dust drift measurement will be presented.

Residue analysis in adjacent crops compared to values in petri dishes on uncovered soil at the same distance of sowing machinery indicate between about 1 - 5 times higher amounts of residues in crops calculated per m² than in petri dishes on bare soil. Adjacent crops seem to filter dust, so higher rates per m² were detected near to the sowing area in plants compared to petri dishes. Residues in vertical gauze nets were about 3 - 10 times higher compared to petridishes in the same distance of sowing.

Therefore drift residue data of petridishes should only be used carefully to predict exposure for non target organisms such as honey bees in adjacent 3D structures such as plants.

ET15B-4

How important are pesticides for the ecological integrity of large rivers and what are their effect thresholds in freshwater ecosystems?

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Several field studies in small agricultural streams ranging from a local to regional scale have demonstrated that pesticides may affect freshwater ecosystems. However, it is unclear whether effects are limited to small streams in agricultural areas, whether pesticides are more important than other chemicals and what are the effects thresholds in the field. In order to answer these questions we compiled two data sets. First, the detection frequencies and concentrations for 331 organic compounds measured in the four largest rivers of North Germany over a period of ten years were analysed including an assessment of the ecotoxicological risk to standard test organisms. Second, we compiled data from nine regional field studies from Europe, Siberia and Australia in order to derive thresholds for the effects of pesticides on macroinvertebrate communities and the ecosystem function leaf breakdown. For the rivers in the first data set, the detection frequency for most organic compounds decreased significantly over the ten year study period and polycyclic aromatic hydrocarbons (PAHs) were most frequently detected. Nevertheless, pesticides were the most important chemical group concerning toxicity for the standard test organisms and occurred in concentrations envisaging acute toxic effects. Regarding effect thresholds, dose-response models for the relationship of pesticide toxicity with the abundance of sensitive macroinvertebrate taxa showed significant differences to reference sites at levels of 1/1,000 to 1/10,000 of the median acute effect concentration (EC50) for *Daphnia magna*. Hence, the analysis revealed effects well below the threshold of 1/100 of the EC50 for *D. magna* derived from mesocosm studies that corresponds to the threshold incorporated in the European Union Uniform Principles for pesticide registration. The invertebrate leaf breakdown rate was linearly related to the abundance of sensitive species in the communities and decreased in concert with a decrease in the sensitive macroinvertebrates, though only for two of the three countries for which data was available. Our results suggest that pesticides should be taken into account as a relevant stressor in river basin management and that the current thresholds used in the risk assessment framework are not protective for field communities.

ET15B-5

Pesticides effects on soil and adjacent water systems under Mediterranean crop-based exposure scenarios using a semi-field methodology

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Exposure and effects of pesticides on target and non-target organism may differ according to the natural variability of the ecosystem due to differences in climate and soil characteristics among others. Therefore, the need to study pesticide effects under Mediterranean conditions is of critical importance due to the lack of information on pesticide behavior and its effects on terrestrial biota for this region. In order to advance the knowledge on pesticide behaviour in soils and its routes of entry into adjacent aquatic systems as well as to study their lethal and sublethal effects on biota a semi-field experiment (soil-water simulator) was undertaken. The experimental design was based on simulations of realistic “worst case scenario” applications of recommended doses of three pesticides (azoxystrobin, chlorothalonil, ethoprophos) on highly irrigated crops (onion, potato, maize) using natural soil under Mediterranean climate (temperature and rain-event). Soil and water samples were analyzed for pesticide residues and tested for acute and chronic toxicity with *Folsomia candida*, *Eisenia andrei* and *Daphnia magna*. Azoxystrobin moved to the water compartment, mainly through leaching most likely due to the high irrigation. As a result, toxic sublethal effects due to leachate and lethal effects due to runoff samples were observed towards *D. magna*, at the applied dosages. Runoff waters from rain events 10 days after chlorothalonil application can cause effects on the aquatic microcrustacean. Both scenarios do not threaten non-target invertebrate soil biota under the applied dosages. Ethoprophos stayed practically immobile in the soil nevertheless pesticide residues detected on leachates and runoff samples were sufficient to cause lethal effects on the cladoceran. A high impact on both terrestrial species survival and reproduction was observed. The studies proved that water compartment close to agricultural fields and terrestrial communities are affected when highly irrigation is present and natural soil characteristics influence pesticides properties.

ET15B-6

Consequences of the Three Gorges Dam in China - Conceptual approaches to study the fate, bioaccumulation and effects of organic micropollutants in aquatic food webs and sediments of the Yangtze

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The construction of the Three Gorges Reservoir caused the flooding of former urban, industrial and agriculturally used areas. Consequently, substantial amounts of organic and inorganic pollutants are continuously released into the reservoir. Beyond further contaminants and nutrients (e.g. nitrate, phosphate) enter the reservoir by runoff from adjacent agricultural areas as well as from sewage of industry, aquacultures and households. Periodical changes in water level cause flooding events and thereby a relocation of contaminated water, particulate matter and sediment onto agriculturally used areas along the reservoirs shore. The main aim of the presented project is to identify major processes that determine the fate and degradation of organic contaminants, their potential bioaccumulation and the ecotoxicological effects of polluted sediments on aquatic organisms within the TGR. We focus on the specific alterations of the behavior and the effects of organic pollutants that are caused by the unique conditions within the new reservoir body [1]. We present our conceptual approach including the definition of contamination scenarios. First fate studies of 14-C radiolabelled model pesticides in water-sediment systems point to potential bioaccumulative metabolites. The simulation model AQUATOX was modified and used for preliminary modeling experiments which revealed the particular importance of the nutrient regime on the composition of the aquatic food webs and its implications for the bioaccumulation. Sediment samples of the Kaixian region have been tested in the Fish Embryo Toxicity Test and the Sediment contact Assay. These findings already serve as a starting point for a subsequent project on management strategies to reduce the pollution in Yangtze flooding areas.

Reference

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ET16 - Quantitative Structure Activity Relationship (QSARs) and similar models for predicting the toxicity of chemicals, mixtures and combined stress

ET16-1

VEGA, a new platform combining QSAR and read across for the prediction of chemical properties

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Under European regulations, including REACH, QSAR and read across are identified as acceptable methods for obtaining valid estimations of the properties of chemicals.

The VEGA platform (*Virtual models for Evaluating the properties of chemicals within a Global Architecture*) offers free access to specialist software designed for QSAR predictions and read-across. VEGA is freely available online: <http://www.vega-qsar.eu>.

There are several QSAR models within VEGA, taken from CAESAR and T.E.S.T. software. VEGA can be used to predict BCF, fish and daphnia toxicity, logP, mutagenicity, carcinogenicity, developmental toxicity and skin sensitization. The models can be used on line, or you can download them.

VEGA introduced an applicability domain index (ADI) to evaluate if the result of the QSAR models is reliable. The ADI includes an assessment of the experimental values of the most similar compounds, and of the predictions of these compounds. Thus, the ADI goes beyond the typical approaches, which are based on chemometric measurements. However, even if the evaluation of the QSAR model through the ADI may identify critical issues, the user should evaluate the results obtained for the similar compounds, and then decide if the prediction is reliable. We verified that the predictions for the chemicals with higher ADI values have on average lower prediction errors. A stricter ADI reduces the prediction coverage, of course.

VEGA implemented a series of tools showing the role of relevant descriptors, or the occurrence of important fragments. For each model you can see the role of descriptors and fragments for the chemical of interest and the similar compounds.

VEGA shows the uncertainty associated with the prediction, depending on the different ADI. On the basis of these errors, in order to provide a conservative classification, we added a safety value to the predicted one. Thus, the prediction uncertainty is used to set the confidence interval for the prediction.

VEGA has been checked for robustness by testing it on 4 million unique chemical structures.

Acknowledgement

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ET16-2

Intracellular localization of chemicals

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Intracellular localization can to a large part be predicted from common physico-chemical properties, such as log K_{ow}, pK_a, valency and molar mass. The selective distribution of molecules in intracellular compartments such as membranes, mitochondria and lysosomes will also lead to specific diseases. Consequently, intracellular distribution may be a determinant of specific toxic effects of molecules. The cell model was used to predict the chemical space of compounds that localize in cellular subcompartments, such as cytosol, acidic vesicles (vacuoles or lysosomes), nucleus, mitochondria and oleosomes. Accordingly, weak acids localize in cytosol and stronger in mitochondria. Weak mono- and bivalent bases accumulate in acidic vesicles such as lysosomes and vacuoles. Strong bases (i.e., cations) and weak bases that form cations with delocalized charge are attracted by the electrical potential of mitochondria. Lipophilic molecules (log K_{ow} > 5) partition of course primarily into membranes and lipids. Similar predictions are obtained using the Horobin QSAR model. A validation study for mitochondriotropics gave about 2/3rd agreement. Disagreement could be explained in most cases. The intracellular localization is not always selectively, making literature references sometimes ambiguous. A recent experimental study gave good agreement of cell model predictions with the experimentally determined dynamics of uptake. In conclusion, the prediction of intracellular localization from molecule properties by QSAR and pharmacodynamic models is feasible and eventually may be exploited to identify new, specific modes of action.

Horobin RW, Trapp S, Weissig V. 2007. Mitochondriotropics: A review of their mode of action, and their applications for drug and DNA delivery to mammalian mitochondria. J Contr Release 121:125-136

ET16-3



Developing read-across justifications in REACH

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Under REACH, 'alternative methods' such as Read-Across and Grouping/Category are key tools for preventing unnecessary animal testing and reducing costs for dossier preparation. However, relatively little guidance on the identification of valid analogues and categories and whether categories will support only 'interpolation' or also 'extrapolation' is available. In this poster, we will present and develop practical examples of what we think are valid argumentation for these approaches. Read-across Read-across implies that measured data for one substance are used as surrogate data for another closely related substance, under the assumption that the chemical similarity of the two substances will result in biological similarity. Read-across can range in scope from a single end point to an entire dataset, and may involve using available source end point data to substitute for unavailable target data or to argue that tests need only be done for the designated source. A testing proposal submitted by a client for 2 closely related alkylphenols argued that the test was only needed for one substance, with subsequent read-across to the other; prompted by a rejection from ECHA, we developed more thorough argumentations for read-across based on available toxicological data and an in-depth description of the chemistry of both substances. Category approach implies that classes of closely related chemicals (usually homologous series) exist for which it is possible to qualitatively interpolate properties for 'missing' substances (analogously to the quantitative argument for classical QSARs). For a long chain alcohol, we prepared an argumentation that category approaches may also be used for near-end extrapolation and category extension. 'Mixtures' Data availability is often a problem for UVCB substances; moreover, their variable nature presents significant challenges for testing and interpretation of results. Treating complex substances as mixtures allows the use of available data for individual constituents, if these are known, in an exercise akin to read-across, but prescribed methods often lead to over-prediction of hazard and classification. ENVIRON used a mixture approach to develop an integrated data set for a well-defined reaction product. By careful evaluation of the data set, supported by limited testing and refining evidence for degradation and bioconcentration, we were able to justify more reasonable PNECs and avoid classification of the substance.

ET16-4

Immobilised artificial membrane (IAM) chromatography : an investigation of the effect of pH on ionisable species

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The octanol/water partition coefficient (log P) is used in many areas of ecotoxicology and modelling as a measure of hydrophobicity or lipophilicity. However it is well known that for ionisable chemicals or surfactants the measurement of log P is difficult due to the tendency of these types of materials to sit on the octanol/water barrier. Similarly, the accuracy of the prediction of log P for these types of materials is variable depending on the model used to calculate it. However, even accurate determination of log P does not always lead to accurate in silico correlation with toxicological endpoints. In part this is due to differences between octanol/water partitioning and partitioning across biological membranes. Phosphatidylcholine (PC) is a ubiquitous phospholipid which is a major component of biological membranes. Immobilised Artificial Membrane (IAM) Chromatography utilises a phospholipid membrane made of PC. Using PC as a stationary phase and aqueous or organically modified buffers as a mobile phase, a partition coefficient (k-IAM) for chemicals can be determined as a measure of the ability of a chemical to partition into the liposome layer (phospholipophilicity). This technique has been shown to have good correlation with ecotoxicological endpoints for neutral compounds where log k-IAM is measured. As in biological systems and in buffers, ionisable compounds exist in different ionic states which means that they interact to a different extent with biological membranes. The methods used to determine log P in these systems do not sufficiently allow for this which is why in part that correlation of ionisable chemicals with ecotoxicological endpoints becomes reduced. This study was designed to determine the effect of the pH of the system on the retention of the chemicals in their ionic and neutral forms using IAM chromatography. As expected, the data indicated that the retention of ionisable chemicals is affected by the pH. Chemicals had a value for the neutral form (log kIAM) and the ionised form, (log kIAM (ion)) under relevant extreme pH conditions. However, at pH between these extremes, retention of the chemicals were based on an aggregate of both neutral and ionised species. It was also recorded that this aggregate retention indicated that the extent of ionisation of the system was not directly linked to the pKa of the compound under aqueous conditions but was shifted positively along the pH scale for acids and negatively for bases.

ET16-5

Simulation of metabolism and modelling environmental fate and (eco)toxicity of chemicals

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Information regarding the metabolism of xenobiotic chemicals plays a central role in regulatory risk assessments. Because so many new chemicals are being produced than can be assessed for potential hazards, setting assessment priorities among the thousands of untested chemicals requires methods for predictive hazard identification. Metabolites and breakdown products may occur in every environmental compartment, in animal and human food causing unexpected changes of evolutionary established biological communities or chronic adverse effects. For this reason data on the extent of formation of metabolites is required by regulatory authorities as specified in technical guidance documents. The aim of this presentation is to share our experience in the computerized management of metabolic data and development of simulators of metabolism for predicting environmental fate and (eco)toxicity of chemicals. Mathematical formalism and its adaptation for modeling environmental fate (abiotic and microbial degradation, bioaccumulation) and (eco)toxicity of chemicals (acute aquatic toxicity, skin sensitization, genetic toxicity) will be presented. Model performance, applicability domain and interpretation of model predictions and supplementary information will be also discussed.

ET16-6

Integrated screening classifier for substance-specific waiving of biodegradation assays in PBT assessments

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The implementation of the REACH regulatory framework requires the assessment of persistent, bioaccumulative and toxic (PBT) potential for substances that are produced or imported in amounts of 10 or more tonnes per year. The PBT assessment entails detailed substance-specific testing, which involves substantial use of animals and significant economic costs. In this context, the use of intelligent testing strategies (ITS) emerges as a cost-effective assessment scheme. A key element for the success of the ITS is the use of non-testing methods as complementary sources of evidence to waive specific bioassays. Therefore, the use of ITS to optimize the costs of regulatory risk assessment requires the development of highly reliable non-testing methods suitable for test waiving.

Persistence is a fundamental property that strongly determines the potential environmental impact of a chemical. Consequently, the objective of the current work was to develop and validate an integrated screening classifier specifically designed to identify non-ready biodegradable chemicals with high reliability (i.e. low risk of false negatives). Results obtained demonstrated that the use of ensembles of weak classifiers, tuned to distinguish between ready and non-ready biodegradable chemicals, constitute an appropriate approach to develop screening models suitable to identify non-ready biodegradable chemicals with high accuracy (99.7% and 100% respectively). The low false negative rate of the above ensemble classifiers allows its integration within the decision-making process of an ITS to waive biodegradation testing for certain chemicals. The bioassay waiving that may result from the use of this approach will impact PBT assessments in terms animal use and economic resources needed, thus providing a cost-effective implementation of the REACH regulations.

ET18 - Tropical ecotoxicology

ET18-1

Assessing the toxicity of herbicides mixtures used in sugarcane culture in Brazil using zebrafish

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Brazil is a pioneer in large-scale use of ethanol, a renewable fuel that contributes to the reduction of greenhouse gas emissions by combustion of fossil fuels. The high productivity of sugarcane crops demands the intensive use of equipment and inputs that can contaminate water bodies adjacent. Herbicides are the most common pesticide used in this crop, but the impact of this practice on non-target organisms is not well known. Moreover, possible interactions between different herbicides used simultaneously are not understood. Most studies into herbicides ecotoxicology are related to temperate ecosystems thus, there are still many gaps about its effects in a tropical climate. The aim of this work was to evaluate the effects of the mixture of ametryn (AMT) and diuron (DIU), herbicides widely used in sugarcane crop, to the tropical zebrafish (*Danio rerio*). Binary combinations of DIU and AMT were tested as follows: 0, 4.8, 6.8, 9.6, 13.6, 19.2 mg L⁻¹ of DIU and 0+ (solvent control); and 0, 0+, 9.7, 13.7, 19.4, 27.4 and 38.8 mg L⁻¹ of AMT. The trials were based on the OECD guideline on Fish Embryo Toxicity Test. Several sub-lethal and lethal endpoints were evaluated. The activity of the biomarkers CHE, LDH and GST was determined after 96 h of embryos exposure to the binary mixture: 0, 0+, 2.5, 3.5, 5.0, 7.1 and 10.01 mg L⁻¹ DIU; and 0, 0+, 5.1, 7.2, 10.1, 14.2 and 20.0 mg L⁻¹ of AMT. Sigma Plot 10.0 package was used for statistical analyses and ToxCalc spreadsheet were used to calculate LC50-96h and determine the model that best describes the mixture. LC50-96h values for zebrafish exposed to AMT and DIU were, respectively, 48.46 (2.2) mg L⁻¹ and 23.93 (1.24) mg L⁻¹. The model which best describes the mixture was the independent action, with toxicity dose level-dependent: synergism in low and antagonism in high dose. GST activity was induced by AMT. However, when the mixture had higher concentrations of DIU we observed a reduction in activity. We observed an inhibition of CHE in higher concentrations of the two components of the mixture and no influence on LDH activity. We determined that the model which best describes biomarkers activity for these enzymes are concentration

addition. Our results show that there is a synergism between ametryn and diuron, which enhances its effects on *Danio rerio*. Risk assessment which uses data from the single substances may underestimate the real risk of these substances, as it is almost impossible to find them isolated in nature.

ET18-2

Baseline levels and trophic transfer of persistent organic pollutants in sediments and biota from the Congo River Basin (Democratic Republic of Congo)

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The present study aims to evaluate the occurrence of persistent organic pollutants (POPs) in the Congo River Basin (CRB) and to investigate the trophic transfer of POPs through a tropical freshwater aquatic food web using stable isotopes. To our knowledge, no data on levels of POPs in sediment and biota from the CRB are present in the literature. In addition, studies on biomagnification profiles of POPs using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ are scarce in tropic regions.

Samples of sediment and biota (i.e. invertebrate and fish species) were collected from three tributaries (Lomami, Aruwimi and Itimbiri) and the Congo River itself to determine the degree of contamination by POPs (PCBs, PBDEs, DDTs, HCHs, CHLs and HCB) with a GC-MS. Stable isotope ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) measurements in plant, invertebrate and fish species were performed using an EA-IRMS.

POP levels in the sediment and biota are low compared to results of other studies from temperate and tropical regions, with exception of total PCB levels found in fish from the Itimbiri River (average $\Sigma\text{PCB} = 1465 \pm 920$ ng/g lipid weight lw). The PCB levels are of the same magnitude as found in industrialised basins. This might be related to the presence of industrial and agricultural (palm oil) activity in the Itimbiri River Basin. Of the POPs analysed, total PCBs were the predominant contaminants with concentrations ranging from 21 to 3664 ng/g lw, followed by DDT and its metabolites at 4 to 504 ng/g lw, PBDEs at 2 to 188 ng/g lw and HCHs at 2 to 66 ng/g lw.

The values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in biota ranged from -40.5‰ to -22.6‰ and from -2.3‰ to 16.5‰, respectively. On average, $\delta^{15}\text{N}$ increased from primary producers to invertebrates to fish. The understanding of the trophic transfer of environmental contaminants in biota of the CRB is critical to evaluate the influence of these contaminants on ecosystems and human life. The observed increase in $\delta^{15}\text{N}$ with increasing PCB 153 concentrations indicates that trophic levels play an important role in the movement of contaminants through the Itimbiri food web ($R^2 = 0.66$, $p = 0.0012$). The slope of the regression equation (0.35) is an index for biomagnification. These correlations are currently determined for all individual contaminants. Slopes will be compared with results from other studies around the world and will be used for risk assessment.

ET18-3

Avian risk assessment for the Neotropics: methods to find appropriate species

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In the context of pesticide registration a lot of emphasis has been given to European farmland birds during the last decade. In Europe the concept of focal species has been implemented by the EU authorities and a wealth of data has been gathered. We conducted several field studies in the neotropics to evaluate the usefulness of different survey methods given the local conditions of high species diversity, different behavioural traits and different taxonomic affiliations of the birds and to gain some preliminary insight into bird communities that are found on neotropical agricultural land.

ET18-4

First evidence of linked physiological and demographic effects of chlordecone in the freshwater shrimp *Macrobrachium faustinum* (Decapoda, Palaemonidae) in the Pérou River in Guadeloupe (FWI)

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From 1972 to 1993, the organochlorine insecticide chlordecone was intensively used as Kepone and Curlone against the banana root borer in the French West Indies. Chlordecone is resistant to degradation in the environment, and it is adsorbed for hundreds of years in soils of Caribbean islands where it was used. Driven by water cycle, it steadily gets transferred to aquatic ecosystems. Chlordecone has a high potential for bioaccumulation in freshwater fish and shrimps. From 2009 to 2010, a field study was undertaken to characterize the physiological and demographic effect induced by the bioaccumulation of chlordecone in the freshwater shrimp *Macrobrachium faustinum*. Individuals were sampled at 7 dates upstream and downstream of the Pérou River located southeast of the Basse-Terre island of Guadeloupe (FWI). Tissue chlordecone concentration and biochemical parameters specifically related to the mode of action of the molecule (Glutathione S-transferase and Na⁺/K⁺-ATPase activities; 20-hydroxyecdysone and vitellogenin levels) were simultaneously measured in the organisms, so that a direct relation between bioaccumulation and physiological alterations can be established. In parallel, body length of the individuals was used to follow size cohorts and to study the dynamics of the population. The results show a correlation between water concentration in chlordecone and the amount of compound accumulated in the tissue of *M. faustinum*. Water and abdomen tissue concentrations were higher downstream, as compared to upstream, with concentration factors by *M. faustinum* of 2600 and 1000, respectively. Glutathione S-transferase activity was inhibited in all the individuals whereas no change was observed for Na⁺/K⁺-ATPase. The level of 20-hydroxyecdysone was higher in the females sampled downstream, and vitellogenin was detected in all the males found in the samples. Population dynamics analysis showed that chlordecone did not increase mortality but it affected individual growth. Moreover, population modelling showed that whatever the season, the maximal mean individual size (L) was lower downstream as compared to upstream. Slower growth of the individuals sampled downstream may result from high 20-hydroxyecdysone levels measured in those individuals. For the first time, it has been indicated that bioaccumulation of chlordecone by a freshwater shrimp resulted in physiological alterations that might affect population dynamics.

ET18-5

Environmental risk assessment of pesticides used in sugarcane plantations in the Central Amazon - Brazil

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The sugarcane production has registered a significant increase in Brazil during the last years mainly due to the increasing demand of biofuels. The Amazon region has the highest indices of sugarcane production expansion in the country. Sugarcane is the fourth crop in consumption of pesticides in Brazil, relying on a wide variety of herbicides and insecticides. Pesticides used in sugarcane plantations of the Amazon are likely to reach down-stream aquatic ecosystems that are considered to be hot-spots of species diversity. In this study, pesticide risks for freshwater ecosystems surrounding sugarcane plantations were assessed considering pesticide spray-drift and runoff as the main source of pesticide entry into aquatic ecosystems. The PRIMET model was used to calculate Predicted Environmental Concentrations (PECs) of pesticides produced by spray-drift deposition. Pesticide runoff inputs were calculated using the Reus runoff formula. Risks were calculated using Toxicity Exposure Ratios (TERs), calculated by dividing PECs by Predicted No Effect Concentrations (PNECs). PNECs were derived using toxicity data for standard aquatic test organisms. For the pesticides that showed risk, a refined risk assessment was performed using semi-field toxicity data with the PERPEST model. A clear risk was estimated for 3 pesticides, 2,4-D amine, diuron and metribuzin, out of the list of the 14 pesticides evaluated. Probabilities to find clear effects higher than 25% were estimated for fish and tadpoles, macrocrustaceans and macrophytes for 2,4-D amine. Molluscs were the only taxonomic group that presented no risks for diuron, and all the studied endpoints showed probabilities to find clear effects higher than 25% for metribuzin. The results of this study suggest that pesticides used in sugarcane plantation in the Amazon are posing risks for aquatic biodiversity in surrounding ecosystems, showing that the model of biofuels production that is currently established in the Amazonian region can be environmentally unsustainable due to the intense pesticide applications.

ET18-6

Is the risk of pesticides in tropical countries different from that in other parts of the world?

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Literature surveys show that pesticides tend to dissipate faster in tropical countries than in temperate regions. This fact has led to the assumption that ecological effects and environmental risks of pesticides in tropical countries are reduced, since exposure times in such countries are considerably shorter than in temperate regions of the world. Given that the toxicity of most organic compounds to tropical species is practically no different from their toxicity to non-tropical species, the question remains whether the actual risk of pesticides is less under tropical conditions. To elucidate this query, a comparison of environmental risks of pesticides between tropical and non-tropical regions was carried out, using experimental data from the literature and fugacity modelling. Tropical field data included dissipation and sorption characteristics of 73 pesticides, which were compared to non-tropical data for the same compounds obtained from databases. Exposure modelling was based on the physico-chemical properties of the pesticides, and outputs were obtained for the two scenarios to be compared, i.e. tropical and non-tropical conditions. Results from modelling show that except for a few cases, the level of risk of exposure for most pesticides in tropical agriculture is similar to that in other climatic regions of the world. Generally, dissipation of pesticides increases under the warm and wet conditions of the tropics, with most of the dissipation occurring through hydrolysis in water and biological degradation in water and soil.

High temperatures in the tropics also foster volatilisation rates, while high precipitation and poor soils tend to increase losses into runoff and, for certain chemicals, their leaching behaviour. The environmental risk appears to be determined by a balance between soil types, soil organic carbon, pH and the rates of degradation in the various environmental compartments. Therefore, the actual risk of pesticides depends more on the specific soil types and water properties of the areas concerned than on climatic features of the tropical regions.

ET19A - Veterinary medicines in the environment: basic research for risk analysis

ET19A-1

Dissipation and sequestration of veterinary antibiotics in soil under variable environmental conditions

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Knowing the impact of environmental conditions on the fate of veterinary medicines in soil is crucial for a thorough understanding and modelling of their environmental fate, especially when transferring laboratory results to the field situation. Particularly soil temperature and moisture are known to affect the dissipation and sequestration of organic contaminants in soil. To study the impact of these parameters on the dissipation and sequestration of veterinary antibiotics, we conducted laboratory experiments with varying soil temperature and moisture. Furthermore, we conducted a field experiment to validate our results under realistic conditions. All experiments were performed with two compounds of contrasting sorption behavior, the weakly sorbing sulfonamide antibiotic sulfadiazine (SDZ) and the strongly sorbing fluoroquinolone antibiotic difloxacin (DIF). Bioaccessibility and sequestration of the compounds in soil were characterised by sequential extractions of increasing harshness.

In laboratory experiments, the dissipation of SDZ proved to be accelerated by a factor of two to three with a temperature increase of 11°C. We successfully used the temperature-dependence of dissipation rate constants that was derived from these laboratory experiments to predict temporal changes of SDZ concentrations in the field experiment. When the soil moisture was either very high or low, however, systematic deviations between measured and predicted concentrations were observed, particularly for the sequestered antibiotic fraction. We conclude that temperature is the main factor controlling the dissipation rates of SDZ. Sequestration is additionally affected by soil moisture, presumably due to conformational changes of soil organic matter with wetting and drying of the soil. Experiments with drying-wetting cycles in climate chambers are currently conducted to further clarify the effects of soil moisture on the dissipation and sequestration of SDZ. The results of these experiments will be presented. Soil temperature and moisture surprisingly had no impact on the dissipation of DIF. The residual concentrations of this compound remained on the same constant level across all experiments. In contrast to the weakly sorbing SDZ, the strong sorption of DIF presumably limits the impact of environmental conditions on its fate. We conclude that the fate of strongly sorbing pharmaceuticals is less dependent on environmental conditions than the fate of weakly sorbing compounds.

ET19A-2

Fate, metabolism and uptake plant uptake of selected veterinary drugs in the rhizosphere of maize

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Root-soil interactions in the rhizosphere include various processes influencing the soil solution chemistry. A rhizosphere box system is well suited to demonstrate the influence of the root on the fate and metabolism of antibiotics. By the help of this micro ecosystem it is possible to measure the metabolite patterns in the phase and in rhizosphere pore water without further sample clean up and at high spatial and temporal resolution. In addition the usage of 14C-labelled compounds will reveal the mass balance of the applied drug including formation of NER (non-extractable residues) and mineralization.

The influence of the maize rhizosphere on the fate and behaviour of two veterinary antibiotics sulfadiazine (SDZ) and difloxacin (DIF) and their metabolites in soil was investigated in suitable microcosms. Radioactivity was traced and LC-MS/MS analyses of pore water and soil was performed. The pore water analysis of soil at various distances from the rhizosphere of maize plants revealed no influence on the fate of the applied compounds. After incubation for 55 days with SDZ the soil analysis at various distances from the roots of the plant revealed the considerable impact of this biological hotspot. The mineralization rate near the roots was 10 times higher than for bulk soil. Furthermore the results revealed that up to 70% of the applied compounds in the root area were degraded to unknown metabolites. In order to correlate chemical and biological data, results on the abundance and transferability of bacterial antibiotic resistance genes were analyzed in parallel and confirmed that the effects were most pronounced under conditions of maximum SDZ transformation. Development of a new analytical method to detect plant uptake of the applied drugs yielded results revealing only trace amounts (maximum of 1% of the applied compounds) mainly in the roots (99%) of the maize plants. The experimental setup using the fluoroquinolone DIF revealed only a minor impact of the rhizosphere on the fate of the drug applied. The mineralization rate (<0.5%) and the plant uptake (< 0.5%) were negligible over the whole experimental period. The radioactive balance revealed that 99% of the applied radioactivity was still detectable in soil.

ET19A-3

Fate of sulfadiazine and its effects on abundance and transferability of antibiotic resistance in rhizosphere and bulk soil

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Veterinary antibiotics reaching agricultural lands with manure pose the risk of spreading of antibiotic resistance. The rhizosphere is a hot spot for horizontal gene transfer but might also facilitate the degradation of antibiotics. On the one hand, it was shown that the addition of artificial root exudates increased the bacterial community tolerance towards the antibiotic compound sulfadiazine (SDZ), indicating that the rhizosphere might be a hotspot of resistant bacteria. On the other hand, the exposure of bacteria to SDZ is presumably reduced in the rhizosphere since the dissipation of bioaccessible SDZ-concentrations was recently shown to be accelerated in rhizosphere soil. However, little is known about the abundance and dynamics of sulfonamide resistance genes in the rhizosphere. We therefore compared the fate and effect of SDZ in bulk- and rhizosphere soil in mesocosms planted with maize and in field plots planted with maize or grass. Both were treated with manure from pigs which either received SDZ or not. A sequential SDZ-extraction protocol for soil yielded antibiotic fractions of increasing binding strength serving as a proxy for the bioaccessible concentration. Following the application of manure, easily extractable concentrations of SDZ and its metabolites decreased rapidly in bulk soil whereas residual concentrations decreased much slower. In rhizosphere soil, easily extractable and residual concentrations of SDZ were mostly lower than in bulk soil indicating an accelerated degradation. Quantitative real-time PCR detection in total community DNA showed that the application of manure containing SDZ increased the relative abundance of the SDZ resistance genes *sdz1* and *sdz2* in bulk- and rhizosphere soil of maize, which may be linked to the increased abundance of LowGC-type plasmids. In the field experiment, this increase was prolonged only in the rhizosphere of grass and maize even at bioaccessible SDZ-concentrations below previously reported effective doses. Likely, the continuous selection of resistant bacteria in the rhizosphere was fostered by a higher microbial activity together with a constant but low exposure to SDZ.

ET19A-4

Degradation of sulfadiazine in lysimeters, soil microcosms, and pure cultures

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Among sulphonamides, sulfadiazine (SDZ) is one of the most widely applied veterinary antibiotics. Long-term lysimeter studies with manure from pigs treated with 14C-labelled sulfadiazine (SDZ) gave the first hint that considerable amounts of the radiolabelled SDZ applied to the soil could have been mineralized within 3 years, although, up to now, mineralization of SDZ in soils was supposed to play only a minor role. Three years after the application of the 14C-manure, 50% of the applied radioactivity was found in the non-extractable fraction (NER) of the lysimeters and the readily extractable fraction (RES) was about 10%. Small amounts of the radioactivity were recovered in 30 to 100 cm depth and 0.5% in the percolate. Overall 65% of the applied radioactivity was recovered, meaning that 35% failed in the balance. Microcosm experiments showed a mineralisation of up to 10% of the added SDZ in three months, when incubated at 45% of the WHCmax and up to 50-60%, when incubated as slurry (one part soil, 4 parts water). Thus we found strong evidence, that mineralisation of SDZ could have been the reason for the loss of radioactivity in the lysimeter experiment and that water content plays a crucial role. Furthermore, we enriched mixed and isolated pure bacterial cultures which were capable of SDZ degradation in absence of soil matrix. Extensive analysis by radio-HPLC, LC-MS/MS and FT-ICR-MS/MS revealed more details about the fate of SDZ in our cultures. Disappearance of SDZ always went along with appearance of a principal metabolite identified as 2-amino-pyrimidine. 2-amino-pyrimidine was already quoted in the literature as a photolysis product. In our experiments, 2-amino-pyrimidine originated definitely from microbial activities.

ET19A-5

Impacts of a decade of annual tylosin, chlortetracycline and sulfamethazine application on drug persistence, bacterial phylogenetic diversity and functional diversity in an agricultural soil

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Use of antibiotics as growth promoting agents in livestock production contributes to the increasingly worrisome development of antibiotic resistance. In order to evaluate the long term impacts of antibiotic exposure on soil microbial populations, a series of field plots were established in London, Ontario, Canada in 1999 that have since received annual applications of a mixture of sulfamethazine, tylosin and chlortetracycline at concentrations (0, 0.1, 1.0 and 10 mg/kg soil) bracketing that which would result from an annual application of manure from medicated swine. Following ten annual applications, biodegradation potential and persistence of these drugs was evaluated. Residues of sulfamethazine and tylosin, but not chlortetracycline, were removed much more rapidly in soil with a history of exposure to the drugs than in untreated control soil. Residues of ¹⁴C-sulfamethazine were rapidly and thoroughly mineralized to ¹⁴CO₂ in the historically treated soils, but not at all in the untreated soil. Enrichment cultures of bacteria able to degrade sulfamethazine were obtained from historically treated soils, but not from untreated soil. The abundance of viable bacteria and their relative distribution in major bacterial phylogenetic groups of bacteria was evaluated microscopically. Using DAPI and the Molecular Probes Live/Dead stain, there was no treatment effect on the abundance of viable bacteria. There were no differences between treatments with respect to the relative abundance of Alpha-Proteobacteria, Beta-Proteobacteria, Gamma-Proteobacteria, low-GC and high-GC Gram positive bacteria. There were differences in the abundance of Planctomycetes and the *Cytophaga-Flavobacterium* cluster with treatment, but these did not indicate a coherent dose-response. Long-term treatment altered functional microbial populations as detected using the pollution induced community tolerance (PICT) test evaluated with either each of the antibiotics present individually, or as a mixture. Overall, these results indicate that soil bacteria adapt functionally to long-term exposure to some veterinary antibiotics, notably resulting in sharply reduced persistence of the drugs. Accelerated biodegradation of antibiotics in matrices exposed to agricultural, wastewater, or pharmaceutical manufacturing effluents would attenuate environmental exposure to antibiotics, and merits investigation in the context of assessing potential risks of antibiotic resistance development in the environment.

ET19A-6

Effects of pig slurry co-applied sulfadiazine (SDZ) on the microbial diversity in soil microcompartments such as earthworm channels and rhizosphere soil

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Effects of veterinary antibiotics such as sulfadiazine (SDZ) on soil microbial diversity were previously reported to depend on the presence of easily available nutrient sources (e.g. pig manure) and the spatial proximity of agents and microorganisms. Antibiotic effects in rhizosphere soil and earthworm channels, however, are not well investigated. Hence, we determined antibiotic effects in two independent experiments using agricultural topsoil (Luvisol) mixed with SDZ spiked pig manure. Rhizosphere soils of maize plants were studied using the chloroform fumigation extraction (CFE) and phospholipid fatty acid (PLFA) analysis. In contrast to un-rooted bulk soil, microbial biomass and PLFA profiles substantially changed after applying more than 1ppm SDZ. Sections of *Lumbricus terrestris* L. channels were sampled after top application of SDZ spiked slurry and investigated using 16S rRNA gene denaturing gradient gel electrophoresis (DGGE) and enzyme activity measurements. The organic matter composition and wettability of earthworm channels were mapped by 2D-DRIFT-FTIR along transects. The experiment confirmed evident pseudomonas and β -proteobacteria community shifts as well as a decreased C/N ratio at the inner surface of earthworm channels. The DRIFT mapping confirmed the larger hydrophobicity of inner earthworm channel surfaces, whereas the boundary layer to the bulk soil was more hydrophilic. These results show that the fate and effect of veterinary medicine has to consider the heterogeneity of natural soil environments.

ET19B-1

Effect of repeated application of sulfadiazine-contaminated pig manure on abundance, Diversity and activity of microbes involved in nitrogen transformation in different soil compartments

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Several studies from our group have indicated pronounced effects of single application of sulfonamide (SDZ) contaminated manure to soil. The aim of this study was to investigate the effects of the antibiotic sulfadiazine in combination with pig manure on the abundance, diversity and activity of functional microbial communities involved in nitrogen (N) transformation in different soil compartments after repeated application of the antibiotic. The repeated applications of SDZ-contaminated manure to bulk soil entailed different response patterns of functional genes involved in N cycling after the first, the second and the third amendment which might indicate an adaptation of the microbial communities to the antibiotic substance.

ET19B-2

The effect of sulfadiazine on bacterial dynamics in the field

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A kinetic model that couples environmental fate processes of antibiotics with an effect model of bacteria inhibition was studied. The model uses the antibiotics concentration in soil as link. It requires a number of input parameters which are more or less uncertain. A probabilistic Monte-Carlo type methodology was used to estimate the uncertainty of selected output variables depending on uncertainty estimates of input parameters. Additionally, the contribution of single parameters to the simulated variability of the output is analyzed and expressed by the contribution to variance. The uncertainty of the output variables is characterized by the 10th and 90th percentile of the frequency distributions. As an example, sulfadiazine (SDZ) effect on the density of ammonium-oxidizing bacteria (AOB) in soil was investigated. After manure application to soil, simulated SDZ concentration decreases monotonically with moderate uncertainty in the first 25 days. The time point at which the available SDZ concentration falls below the inhibition constant of AOB (0.025 mg/kg) is predicted to be most likely between 22 days (10th percentile) and 33 days (90th percentile). Increasing AOB densities over the first 15 to 50 days are predicted due to enhanced growth after amendment of ammonium containing manure. Once all ammonium has been consumed, AOB density decreases again. Uncertainty of the input parameters manifests itself in a difference of approximately factor 1.6 between the 10th and 90th percentile of AOB densities. A delaying effect of SDZ on AOB growth is predicted by the coupled model. Since in the first 22 - 33 days available SDZ is above the inhibition constant, growth of AOB is suppressed, but starts after SDZ has dissipated. The uncertainty is larger in the coupled model because it additionally carries the uncertainty of the chemical fate parameters. The contribution of the chemical fate parameters on AOB uncertainty is small compared to the parameters of the biological model. Only the hydrolyzation rate constant contributes to more than 20%. The analysis shows that the combined parameter uncertainty does not compromise the conclusion of the delaying effect of SDZ on AOB growth after manure amendment. The probabilistic simulations show that the chemical fate model, the AOB growth model and the coupled model deliver interpretable predictions of the dynamics of the important output variables SDZ and AOB, even with the uncertainty of the input parameters.

ET19B-3

Modelling environmental risks of veterinary medicines used in Asian pond aquaculture

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A wide array of Veterinary Medicinal Products (VMPs) have been introduced during the last decades in Asian aquaculture production for treating and preventing bacterial diseases and parasitic infestations in the cultured species. In the present study, a decision support system (ERA-AQUA) was developed to assess risks of VMPs applied in Asian pond aquaculture for the targeted produce, for human health and trade, and for surrounding aquatic ecosystems. VMP concentration dynamics are modelled in four different compartments (i.e., pond water, pond sediment, cultured species, and the watercourse receiving effluent discharges) based on scenario and physico-chemical input parameters, and mass balance equations. Predicted no effect concentrations (PNECs) for the targeted produce and acute and chronic PNECs for aquatic organisms (i.e., algae, invertebrates and fish) are calculated from toxicity data and assessment factors according to international risk assessment guidelines for VMPs. Acceptable daily intakes and maximum residue levels are used to assess risks for consumers and trade, respectively. Finally, risk quotients are calculated for each of the included endpoints and the potential exceedance of the predicted exposure concentrations over the calculated "safe" concentration is provided by the model. In this presentation, the model will be presented and its potential applications will be demonstrated through a case study in which risks will be assessed for the application of oxytetracycline (antibiotic) and benzalkonium chloride (disinfectant) in an intensive striped catfish (*Pangasianodon hypophthalmus*) scenario for the Mekong Delta (Vietnam). The ERA-AQUA decision support system can be considered as a useful tool i) for performing preliminary risk assessments of VMPs applied in Asian pond aquaculture, ii) for assessing the influence of different application schemes and aquaculture practices on the environmental impacts of aquaculture production, and iii) for designing chemical and biological monitoring and other higher-tier risk assessment studies.

ET19B-4

Effects of ivermectin application to cattle on dung fauna and dung degradation: an international comparison of field studies

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It has been well documented that the application of toxic anthelmintics such as ivermectin to domestic animals can affect populations of dung-dwelling organisms and in some cases retard dung degradation. During the registration process, parasiticides such as anthelmintics need to be tested at higher tier levels (i.e., multi-species) when adverse effects on dung organisms are observed in single species toxicity tests. An outline for conducting such field tests was recently published. Following publication, an international project was set up in order (1) to assess the robustness of these field tests when conducted by different research groups at different geographic sites, varying in dung and soil faunas, and in environmental conditions, and (2) to investigate the effects of these variable conditions on the interpretation of test results. The experiments were conducted in Lethbridge, Alberta, Canada, near Montpellier, France, near Zurich, Switzerland, and in Wageningen, The Netherlands. Ivermectin was used as the test compound. The study demonstrated that there are considerable differences in the composition of the principal groups of dung insect fauna (family level) between different experimental sites in the study, as could be expected according to biogeography. The results further indicate that ivermectin does negatively affect various groups of dung flies and also dung beetles at the different study sites. However, ivermectin treatments do not seem to have an effect on the degradation rate of cattle dung in temperate climate regions. The study design is suitable to evaluate the effects of parasiticides on dung insects under field conditions such as required in higher-tier testing for risk assessment. Extreme weather conditions during the course of the experiments, however, may interfere with the abundance of certain important groups of dung insects.

ET19B-5

In vitro effects of 17 β trenbolone on the mRNA levels of steroid hormone receptors, Growth Hormone and gonadotropins in pituitary glands from rainbow trout (*Oncorhynchus mykiss*)

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Based on the high biological activity of trenbolone in fish and on the doses used by body builders, the compound might potentially pose a risk to the aquatic environment also in EU although not officially present. Previous studies in our laboratories have shown that trenbolone is capable of irreversibly changing sex ratios to 100% males in zebrafish at water concentrations in the low ng/L level. The present study was undertaken to investigate effects of trenbolone on mRNA levels of steroid hormone receptors, GH, LH and FSH in pituitary glands from rainbow trout after in vitro incubations of the excised tissues with the synthetic androgen.

IGF-1 and AR mRNA levels in liver slices incubated with trenbolone at concentrations from 5-100 ng/ml were unchanged

Pituitary glands were incubated with trenbolone from 1.35-1355 pg/ml:

- No significant changes were found in GH mRNA levels
- AR mRNA was significantly reduced at all concentrations
- FSH mRNA was significantly increased at the highest concentration
- LH mRNA was massively and inversely reduced with incubation concentrations

LC01 - Development in life cycle inventory analysis and modelling

LC01A-1

Market-based allocation of recycling benefits

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Market-based allocation of recycling benefits has already been described since the years 2000. However, this approach tends not to be largely adopted in international standards.

In practice, there are mainly three types of benefit allocation with system expansion modeling: (i) allocation of benefits to the producer bringing material on the market (that can become secondary material at end-of-life); (ii) allocation to the product incorporating secondary material and (iii) 50/50 allocation (half of the benefits of recycling at end-of-life and half of the benefits of incorporating secondary material are accounted for).

Market-based allocation is dictated by the answers to the following questions: "Which additional amount of secondary material will be exchanged on the market if supply increases thanks to the apparition of a new source of secondary material?" or "Which additional amount of secondary material will be exchanged on the market if demand increases thanks to the apparition of a new producer of a good based on the secondary material?" Analysis of price elasticity of demand and supply provides answers.

This paper aims at depicting several typical supply-demand curves and at associating these to actual market situations (for example, what if there is a back obligation or subsidized waste collection, etc.). The appropriate market-based allocation can hence be dictated in each case.

Namely, allocation to the supplier (i) is to be promoted in cases where the supply is fully inelastic and where the demand is fully elastic. Examples of such markets are, among others, PET and packaging glass.

In markets where answers to supply and demand variations are more equilibrated, a 50/50 allocation should be used, as it is the case for certain types of paper and boards.

Market situations for main materials are analyzed so as to provide sound justification for standard developments.

LC01A-2

Using a long-term energy model for the consequential and prospective life cycle assessment of the use of biomass based synthetic diesel (BTL) in France

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Consequential Life Cycle Assessment (C-LCA) has been developed recently as a modeling approach that captures environmental impacts of a product beyond direct physical relationships accounted for in Attributional LCA. This approach seems interesting for the evaluation of biofuels since their indirect impacts on other sectors (agriculture, forestry, electricity production) may be important. In this study, we use a prospective optimization model representing the French energy production and transportation sectors (agriculture is partially included due to the presence of biofuels) to evaluate the impacts of the production of BTL in France in the time horizon 2007-2030. The model was developed with the economic model generator TIMES.

Part of the work consisted in adapting the model to perform C-LCA integrating energy consumptions and emissions factors to the technologies described in the model.

Scenarios were built in order to expose the C-LCA methodological issues we aim to discuss. We observed how the model behaves when applying variations to: the technology used for BTL production, the levels of development of BTL in France and the energy policy. Global Warming Potential (GWP) was calculated for each scenario using time-dependent characterization factors for greenhouse gases.

Preliminary results show that the long-term energy model can be applied for measuring the indirect effects of the development of BTL in France. For example, it was possible to identify the source of the electricity that is going to compensate the supplementary demand for electricity due to the production of BTL using an allothermic process. This type of model presents some advantages in relation to other models previously used in C-LCA:

- It allows a fine description of transformation steps of primary resources in energy carriers.
- It allows the observation of marginal and non-marginal perturbations on the energy sector.
- Emissions are endogenous to the model. Most of the other C-LCA studies use economic equilibrium models to quantify the consumption of certain products and the emissions are integrated manually afterwards.

Nevertheless, in this version of the model, the agricultural sector is only described partially and land use changes (direct and indirect) can't be evaluated. A whole world description of the agriculture and forestry sector would be necessary for this type of analysis. One way of improving this C-LCA would be to integrate our model with a general equilibrium model.

LC01A-3

Modelling land use changes in consequential LCI: limitations of equilibrium models

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This presentation aims at discussing the use and limitations of economic equilibrium models for the development of consequential LCI, with a specific focus on the case of biomethane production from maize in Luxembourg. The core research objective is the development and application of a Partial Equilibrium (PE) model representing the market constraints and reaction to the demanded production of maize and providing: 1) the change of crop production patterns, i.e. the primary consequences on Luxembourg's agriculture system. Forage crops are included, making the link to the consequences on meat and milk production. 2) The changes of land use type and related primary consequences in terms of modified pollutant emissions and land transformation impacts. The modelled changes are then propagated inside the national economy, using a computable general equilibrium model (Luxgem), and outside the national boundaries to account for additional environmental impacts.

So far the results show no need for intensification of the existing and new areas to meet demand for maize. To study the impacts of increased agriculture prices, we increased the import prices of the "agriculture" commodity by 10% in Luxgem calibrated to the base year 2005. We find that the share of expenditure by households on agriculture products fell from 2.5% to 2%. Agriculture has a very small share in the value added in the economy and increased demand for maize which may displace existing crops will not have serious economic impacts. These findings were corroborated using the global model GTAP, which was used to evaluate the increased demand for displaced agriculture crops on account of additional production of maize. However, since the PE model is based on revenue maximization, it is difficult to properly consider non-economic constraints such as behaviours related to habits, cultural heritages or additional regulatory constraints. Also the modelling of the influences of crop production patterns on the food sectors is not trivial because of the difficulty of assigning a clear and robust aggregated market relation between forage crops and meat and milk.

Equilibrium models fall short in including non technical and non economical constraints. The complementary use of agent-based modelling could be an alternative approach for proper consequential LCI.

LC01A-4

Integrating accident-related methods and impacts into the life cycle toolbox

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Life Cycle Assessment is more and more understood to be 'only' one component of a wider environmental and even sustainability assessment toolbox. Enhancing classical Life Cycle Inventory (LCI) modelling by integrating accidents in production, use and end-of-life of products is one important step towards more complete evaluation of product systems' life cycles. LCA according to ISO 14040ff deals implicitly with non-accidental ('regular') releases only, while some accidental releases may be included due to how LCI data is collected (e.g. site's yearly average, including leakages etc.). There are however important organisational and decision-making related reasons for managing these two sets of inventories separately. Accident prevention in industry is done separately from dealing with normal releases to the environment. A separate inventory of accident related releases and casualties (Life Cycle Accident Inventory, LCAI) is hence required for effective decision support. Several studies present case-specific solutions for this issue and some general method concepts have been presented. A systematic and consistent methodological solution for full implementation is still lacking. Joining LCI methodology and tools from Risk Assessment (Event Tree Analysis and Fault Tree Analysis) yields a powerful approach for integrated analysis of the environmental and health effects from accidents and under regular operation. Moreover the methodological question comes up on how to reflect attributional and consequential modelling principles when modelling accident inventories for joint analysis with LCI data. The presentation will systematically address the question of how best to place the accident-related impacts within the life cycle toolbox and which methodological implications this has. Data sources and data management are further issues to discuss to ensure a proper approach in support of life cycle based decision making in industry.

LC01A-5

Better characterising the environmental performance of intermittent power generation with help of LCA: integrating wind power into the German electricity grid in 2006

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Intermittent power generation and in particular wind power is continuously gaining shares in the power plant parks of many countries. Given its intermittent nature, however, the true environmental impacts increased wind power integration remain an open question. This is because the seemingly equivalent functionality expressed in a kWh of electricity produced is in fact not directly comparable to conventional power generation techniques. For that reason, a more systemic approach is advocated. Relying on energy systems analysis results, the purpose of the study is to analyse to what extent considering substitutional effects and back-up can help better characterise power generation from intermittent resources. The study starts with describing the German electricity grid in 2006 with and without wind power generation. By using the results from an agent-based energy system model, the issues of intermittency and the backup needed for wind power were addressed. Through substitution of fossil fired capacities, the LCA considering these consequences led to an even better environmental performance of wind power in all of the considered impact categories (including global warming) compared to the LCA not considering these consequences, except for mineral resource depletion and natural land transformation. As this study could not fully resolve the issue of finding a functional unit that is more appropriate than the kWh for comparing different power generation techniques, other approaches are also discussed.

LC01B-1

Book, trade and claim systems in LCA: how to model certificates delinked from physical flows

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In May 2011, the association of issuing bodies AIB announced that 1 billion EECS certificates (which equals 1 billion MWh of electricity) have been issued in Europe since its start nine years ago. There is a large demand in renewable energy certificates, which helps electric utilities and companies from the manufacturing as well as service sector to reduce the environmental impacts of the electricity they purchase.

The international standards on life cycle assessment (ISO 14040, ISO 14044) do not specify how certificates should be taken into account when performing a product or company life cycle assessment (LCA) study. The recently completed carbon footprint standard are clear with regard to carbon offsetting measures (emission certificates): these are considered as an improvement measure and shall not be included in the product LCA but kept separate.

Up to now, the role of renewable energy certificates has not been discussed widely. This presentation shows the mechanism and volume of RECS certificates as well as its consequences and proposes some guiding principles how RECS certificates may or may not be used within product LCAs and the eco-balances of companies.

During the last years, countries with large shares of hydroelectric power exported significant amounts of RECS certificates to other European countries. These RECS certificates are used to lower the carbon footprint of electricity purchased by companies and electricity providers. Thus the exports are compensated by imports of the same amounts of non renewable electricity qualities. This leads to substantially higher carbon intensities of the electricity mix of exporting countries.

We therefore recommend to disregard independently traded RECS certificates in product and service LCA as long as the LCI of national electricity mixes is based on international statistics disregarding RECS trade. If RECS certificates are linked to the production and delivery of renewable electricity, we recommend to include the respective share of renewables in the electricity mix.

LC01B-2

Using water markets and consequential LCA to assess indirect impacts from water use

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Using water can incur direct impacts on human health from water deprivation for domestic use or agriculture. However, these impacts do not occur in regions where economic resources are sufficient to allow the deprived users to turn towards technology to meet their needs. On the other hand, this technology leads to burden shifting that should be captured in a comprehensive assessment of water use in LCA. This paper proposes a consequential framework and model using water markets and marginal technology in order to assess LCA indirect impacts from water consumption and degradation, by identifying marginal processes and including their life cycle inventory.

Adaptation occurs when a given type of water withdrawn, defined by its source and quality, is constrained. The assessment whether a water type is constrained or not is performed through a scarcity parameter, with the underlying assumption that an unconstrained resource is not scarce. The market concept, applied to water, is then used for each water type to consider all sources for this water type. These include available water of this type, treated water from a lower quality source of water, desalinated water, and imported water. Additional processes are associated with each of these source of water.

Results consist of quality-specific marginal water source available for 808 hydro-economic cells worldwide, as defined in Boulay et al, resulting in 6464 (808 cells x 8 water types) marginal processes which can then serve as input to the inventory and be assessed by any impact assessment methodology. Preliminary results are plotted on a world map giving additional energy demand incurred by the marginal processes for a 1000 m3 of good quality surface water use for each 808 hydro-economic cells.

This paper presents a novel approach assessing indirect impacts from water use due to compensation scenarios. It is especially relevant for developed countries facing water scarcity and/or poor water quality by overcoming current methodological limitations that solely consider direct impacts on human health from water deprivation. This model is based on a consequential approach, identifying the unconstrained marginal water source and treatment processes required to compensate the deprivation from water use.

LC01B-3

Modelling of biogenic CO2 fluxes in LCA and their integration with the global C cycle

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In Life Cycle Assessment (LCA), the same characterization factors are conventionally applied irrespective of when the emissions occur (the same importance is given to emissions in the past, present and future). This accounting paradigm makes bioenergy systems climate neutral as long as they are carbon neutral (the same amount of CO2 released from combustion is sequestered by growing trees). When the analysis is constrained by specific time boundaries the validity of this convention becomes shaky.

We stress the fact that the equivalency between C and climate neutrality in bioenergy LCA ignores the temporal gap between the emission (usually at a single point in time) and the sequestration flux, which is usually distributed over many years. Neglecting these dynamics has an influence on the final climate impact assessment. The challenge for LCA practitioners is to model these fluxes and calculate the resulting climate impacts with unit-based indicators that be included in LCA case studies.

In this work, we elaborate on this issue using relatively simple mathematical methods which provide simulations of the carbon flow dynamics of biomass systems. Probability distribution functions are used to include in LCA the dynamic profiles of CO2 emissions and removals associated with biomass management for bioenergy and wooden products. Following an approach based on Impulse Response Functions, CO2 atmospheric profiles are calculated together with the respective changes in radiative forcing. Results show the importance of using emission and removal functions rather than single pulses or linear amortization procedures, which generally overestimate the climate impact of CO2 emissions, especially in presence of short time horizons and for relatively wide time-distributed emissions.

This method is flexible and can provide outcomes that are suitable to be routinely applied in LCA. Following this, we believe that a debate should be open in the LCA community to properly address timing of emissions and revisit the climate metrics used in the assessment. Besides normalized metrics as GWP, absolute metrics showing how the impacts change over time are preferable and can provide additional insights on the effective mitigation potentials of the system under study. The approach presented here deserves future research for a possible use in accounting of emissions from harvested wood products and in processing data by emission inventory experts within the Kyoto protocol and its successor.

LC01B-4

Evaluating the greenhouse gas emissions of retrofitting the existing Irish housing stock - A combination of process analysis and input-output analysis

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Historically, in Ireland focus has been on the domestic greenhouse gas emissions to the neglect of the emissions attributable to imports in retrofitting the existing Irish housing stock. This paper reports on a life cycle study of energy efficient retrofit options for the existing Irish housing stock with a focus on the significance of greenhouse gas emissions due to imports in order to depict the true total emissions attributable to retrofitting the existing Irish housing stock. While typical commercial LCA software tool was used to assess environmental impacts due to imported goods, domestic generated emissions including those due to imported products induced by addition of energy inputs were evaluated using input-output analysis. Results show that imports caused about 50% of the total greenhouse gas emissions. Although operational phase consumption and emissions was much greater than any other phase, there was a wide variation in the impacts on this balance across the retrofit options. Using these results an appraisal is provided of the benefits of utilizing process-based hybrid LCA to provide greater information to aid policy making on the life cycle performance of existing dwellings.

LC01B-5

A framework for prospective hybrid life-cycle assessment and its application to energy technologies

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Life-cycle assessment and input-output analysis literature abounds with case studies that have been developed at a particular place and a particular time in the past, being as many photographic representations of specific systems. In this literature body, analyses addressing future situations in which products will be produced, utilized and disposed are rare. Some are based upon optimization and equilibrium models. With the remarkable exception of the NEEDS project, future life-cycle inventory or input-output databases are not available. There is nonetheless an ever-increasing need for a life-cycle capable scenario modelling framework. We propose here a procedure to adapt life-cycle assessment (LCA) databases and multi-regional economic input-output (MRIO) tables to future years (up to 2050) according to various external parameters that have been identified as central in determining the environmental impacts of the overall economy. The EXIOPOL project has recently produced a highly detailed input-output model; we utilize this model to build a life-cycle based framework for scenario modelling. A combination of existing prospective studies, including International Energy Agency's are used to produce a consistent hybrid model of the world economy which can serve as the background for prospective life-cycle assessment studies. We present the model development and provide some basic comparisons of the scenarios implemented. Finally, in order to exemplify the use of such a prospective hybrid LCA model, we apply it to the integrated environmental assessment of selected low-carbon electricity production technologies.

LC02 - Developments in life cycle sustainability assessment

LC02A-1

UNEP/SETAC approach for a life cycle sustainability assessment of products - just in time

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Context

Every day, unsustainable patterns of consumption and production methods as well as population growth challenge the resilience of the planet to support human activities. At the same time, inequalities between and within societies remain high - leaving billions with unmet basic human needs and a disproportionate vulnerability to global environmental change. To counteract this trend, UNEP and SETAC (Society of Environmental Toxicology and Chemistry) have worked together through the UNEP/SETAC Life Cycle Initiative (Initiative) to develop the current work Towards a Life Cycle Sustainability Assessment (LCSA). A key objective of the Initiative is to help extend life cycle assessment (LCA) methods and practices. This approach towards a LCSA bases on ISO 14040/44, ISO 26000 and acknowledges the developments of number of international initiatives and experiences about sustainability assessment. This publication is a natural step in UNEP's work, which has in the past decade focused on developing the 10-Year Framework of Programmes for Sustainable Development and which is now also focusing on economic sustainability through the UNEP Green Economy Initiative.

Scope

This publication describes life cycle techniques that can measure sustainability and allow LCA to support decision-making toward more sustainable product and process systems. An (Environmental) LCA looks at potential impacts to the environment as a result of the extraction of resources, transportation, production, recycling and use and discarding of products; life cycle costing (LCC) is used to assess the cost implications of this life cycle; and social life cycle assessment (S-LCA) examines their social consequences. However, in order to get the 'whole picture', it is vital to extend current life cycle thinking to encompass all three pillars of sustainability: (i) environmental, (ii) economic and (iii) social. This publication shows how all three techniques - which all share similar methodological frameworks and aims - can be combined to make the move towards an overarching LCSA possible.

Conclusion

LCSA has significant potential to be used by enterprises, governments, agencies for international cooperation and other organizations in society (such as consumers' associations) in their efforts to produce and consume more sustainable. Still more research and applications are needed, but its application is already feasible and encouraged to speed the learning curve of the society.

LC02A-2

Challenges in implementing Life Cycle Sustainability Assessment (LCSA) and in an LCSA-based decision-making

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Life Cycle Sustainability Assessment (LCSA) has been proposed to be a combination of three assessments: environmental, economic and social (incl. socio-economic). In this way LCSA tries to make a bridge between the traditional environmentally oriented and generic life cycle assessment (LCA) and the more site- and time-specific sustainability assessment (SA), which takes into account all three generally accepted pillars of sustainability (economic, social and environmental). In the process of trying to bridge and to draw from the strong characteristics of SA and LCA, LCSA encounters its own challenges.

LC02A-3

Sustainability and human labour: how can LCA answer?

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In LCA the intrinsic dependence of productions on human labour (HL) is usually disregarded, without providing any clear argument. Apparently, HL is not considered to be related to and affected by, changes to the functional unit. Nevertheless, HL could be seen as the common numeraire among the three pillars of sustainability: environment, economy and society. This research aims at investigating the relationship between HL and LCA and at developing an operational framework to include HL in LCA. We defined three HL types (qualified worker, technician, manual worker). A comparative LCIA of the HLs types was carried out using an environmentally extended input-output model of EU-27. Afterwards, ten agri-food and industrial LCAs case studies were modified for hybrid LCAs, adding HL inputs to existing LCIs. The LCIA comparison showed that a qualified worker always generates impacts greater than other workers. The impact of average HL is higher in EU countries with the highest household expenditures' budget. Within the case studies, the HL significantly contributes to the total impact for several categories (e.g. fossil and ozone depletion up to 16% and 20%, respectively). We argue that an eco-profile of HL should always be added to LCI models that entail a significant direct human contribution: adding inputs of HL to the LCI of a product or process can improve its accuracy. This could enable to establish a common framework (same LCI) to account for HL under the three pillars of the life cycle sustainability assessment. So far, additional cost and social/organizational data of HL might be integrated in LCIs to provide useful information and an added value for more comprehensive assessment of the real life cycle cost (e.g. through addition of salaries and wages to the Life Cycle Costing (LCC) analysis) or social quality factors of labour (e.g. through implementation of further labour impact categories in the Social Life Cycle Assessment - SLCA) in a production chain. The proposed methodology can be used for a future implementation of HL at a level of unit process in LCA. By using a hybrid approach, possible double counting with non-human (i.e. machine driven) labour is easily avoided. However, humans are not machineries and they are driven by flows of information, knowledge, educational and cultural experience, and so forth. These are essential items for our future sustainable development but how to integrate them into human labour LCI profiles remains an open task.

LC02A-4

What life cycle sustainability assessment does and does not for new and innovative technologies

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Performing a sustainability assessment of new and innovative technology is a complex task, as showed by the definition itself, which refers to two big issues: Technologies

and Sustainability. Technologies can be classified in many ways, depending on the different typologies, development levels, effects and impacts on sectors, territories, markets, etc. The relation with sustainability is twofold, because technology can be considered both a cause of many environmental problems and a key to solve them. In fact, technologies are today considered the main agent of the present industrial, economical and social evolution and the main cause of the high speed of the present changes. As far as sustainability and sustainable development are concerned, they are very controversial and disputed at scientific and social level. Indeed, the sustainable development cannot be considered simply a goal, rather a social process where shared sustainability principles are considered as starting point for assessing decisions through an interactive learning process.

Being sustainability a global concept, inevitably calls for a system-wide analysis, a perspective that is at the core of the life cycle approach. A framework for life cycle sustainability analysis has been proposed, namely LCSA, which requires the application of LCA, Life Cycle Costing (LCC) and Social Life Cycle Assessment (S-LCA) under specific consistency requirements. The framework has been applied to the assessment of an innovative technology, in order to test its applicability, and it turned out to be a challenging task, mainly in relation to the following aspects: use of identical system boundaries; data availability and their significance; functional unit definition, especially in the case of S-LCA, and scenarios vs. product analysis. On the other side, the framework showed also its strengths in forcing practitioners in thinking about the different options, and thus in detecting important aspects that at first sight could be considered negligible. However, we suggest supporting the application of the present LCSA with other methods and tools, able to take into account also aspects like the different ethical values, risk elements and the concept of social acceptance. Thus, LCSA can learn from the field of Technology Assessment in the way in which the problem is dealt with: the technology is at the core of the analysis, but the infrastructure and the organisation around it are equally important ingredients.

LC02A-5

Best Available System (BAS) for municipal solid waste management in developing Asian countries

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Fast growing mega cities in developing Asian countries are faced with municipal solid waste management (MSWM) related problems due to rapid urbanization, industrialization and population growth. Proper waste management with energy and material recovery prolongs the life of existing landfills, contributes in providing renewable energy and reduces pollution.

Although the need of shifting from landfilling to more environmentally sound solutions of solid waste management system is obvious, developing Asian countries have their own way of thinking, lifestyle, culture, budget related issues and concerns that reflects on their social and economy priorities in their decision making. For example, the fact that many scavengers lives depend on collecting and reselling plastic waste from the landfill to private recycling plants may interfere with the calorific value requirement of an incineration plant.

This study attempts to take into account the social and economy related considerations in Life Cycle Assessment (LCA) by using an integrated, survey based methodology called Environmental Load Point (ELP) developed in Nagata laboratory of Waseda University. ELP has broadened indicators categorized in 9 impact categories, which are energy depletion, global warming, ozone layer depletion, acid precipitation, resource consumption, air pollution, ocean and water pollution, waste disposal, and ecosystem influence. The ELP result is weighted with text mining factors from the national newspapers to find out the importance of each impact categories in the concerned country. Additionally, Life Cycle Cost (LCC) is combined with ELP results to give cost estimation of the constructed scenarios. This combination is called Best Available System (BAS) for MSWM. ELP represents the social considerations while LCC represents the economy factor of sustainability.

Different scenarios are constructed in this study with different portion of waste being recycled and incinerated, considering the composition of local waste and number of employment opportunity of scavenger in the new systems. BAS result may help decision makers to determine how far they should compensate with the lost of job market as well as taking a better MSWM technology with higher confidence because it has incorporated the ecology, economy, and social concerns. Moreover, text mining result is a reflection of the national concerns and priorities, thus it increases agreeability among the related community.

LC02A-6

Towards comparative life cycle sustainability assessment of road marking systems

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Public purchase decisions must cope with shrinking budgets at one hand and increasing demand for environmentally friendly products on the other. This study looks at both economic and environmental impacts of road markings considering the whole life cycle from manufacturing to disposal. For the first time all four major binder-based raw material options are evaluated that can be considered to equip a road section with road markings that provide high visibility even at wet nights for a period of ten years. For the accuracy of the study an external expert panel has reviewed the assessment.

Social aspects are considered in a second step. It is not trivial to quantify these.

LC02B-1

Evaluating multiple dimensions of sustainability in the case of bioenergy production based on multi-criteria analysis and life cycle assessment

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Environmental impacts of products and services have been traditionally the main focus in life cycle assessment (LCA). Recently, also other dimensions of sustainability including economic, social and even cultural aspects have been received more and more attention in addition to ecological impacts. Multi-criteria analysis (MCA) is a toolbox of methods that evaluate the properties of decision alternatives with respect to multiple, usually conflicting decision criteria.

First, this presentation discusses how MCA can be utilized in LCA when evaluating the sustainability of products and services. The most important benefits of MCA are connected to integration of impact assessments not measured directly in commensurable units and incorporation of subjective preferences into the assessment framework. In general, the performance of decision alternatives depends on the weights given to various decision elements of the decision hierarchy and the performance of the decision alternatives with respect to the lowest level elements. Different life cycle stages capture the life cycle of the production alternatives from raw material and utilization to end use.

Second, this presentation demonstrate the empirical results of forest biomass based bioenergy production in Eastern Finland that was the topic of recent research project funded by Finnish Funding Agency of Technology and Innovation. The project carried out the comparison of the alternative production chains in the methodological framework described above. The analyzed production chains were as follows: (a) Local heat entrepreneurship based on forest chips (Eno energy cooperative). (b) Wood pellets produced in Finland and distributed to domestic and global markets (Ilomantsi pellet plant). (c) Direct peat combustion in large combined heat and power (CHP) plants (Fortum CHP in Joensuu). (d) Biodiesel produced from both forest biomass and peat (Varkaus experimental plant).

The empirical results of the multi-dimensional comparison of the production chains including economic, environmental, social and cultural sustainability as well as the overall performance when all dimensions are taken into account simultaneously are presented. In addition, the methodological gaps and future development needed are discussed.

LC02B-2

Reducing the carbon footprint of the Swiss energy system: which options are most sustainable?

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One of the main objectives of the research project CARMA (Carbon Management in power generation) is the comparative evaluation of potential options for the reduction of Greenhouse Gas (GHG) emissions from the energy system in Switzerland within the framework of future energy scenarios. This assessment is carried out by applying Multi-Criteria Decision Analysis (MCDA) methodology. The MCDA approach allows considering a comprehensive set of performance indicators addressing environmental, economic and social aspects in decision making and therefore taking into account all three pillars of sustainability; besides these objective criteria, it also allows considering subjective stakeholder preferences, which are used for weighting of the indicators. These preferences are established by a purpose built interactive web interface. The combination and aggregation of performance indicators and preference profiles results in a single performance index for each mitigation option, which can be used for a ranking of these alternatives.

The evaluated GHG reduction options cover the most important economic sectors in terms of GHG emissions: the residential sector, traffic, power generation, and industry. The individual options are evaluated compared to a kind of "Business-As-Usual" (BAU) reference scenario until year 2050. This BAU scenario assumes a moderate and steady economic growth as well as a slightly increasing population in Switzerland going along with an increase in passenger and freight transport as well as the habitable surface area per capita.

Depending on the indicator weighting profile used for the MCDA - i.e. on the subjective preferences - the comprehensive evaluation of GHG reduction options can lead to different results compared to the most frequently used purely economic perspective. While specific measures are very attractive from an economic long-term perspective, they can lack individual acceptance, or can be associated with high investment costs as a major obstacle to their implementation. Showing the advantages and disadvantages of the evaluated options together with their ranking depending on subjective weighting profiles in a transparent way can facilitate decision processes and help in understanding barriers in the implementation of effective GHG reduction strategies.

LC02B-3

Evaluating sustainability as Environmental performance of the regional energy systems

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LC02B-4

Integrating life cycle analysis, human health and financial risk assessment for the evaluation of contaminated site remediation

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When evaluating remediation technologies for contaminated soil and groundwater, the beneficial effect of the remediation, namely cleaner soil and groundwater, are mostly emphasized without consideration of the environmental and social impact of the remediation activities themselves. Nevertheless, practitioners and decision makers can rely on a broad range of decision tools that can help them to achieve a better balance between economic, social and environmental health aspects of contaminated land remediation. A holistic approach for the management of contaminated land should ideally include an assessment of the environmental risk of the contamination, an assessment of the environmental, social and health impact of the remediation process and a cost-benefit analysis of the remediation project.

A life cycle framework, including a life cycle management (LCM) approach structuring environmental activities and life cycle analysis (LCA) for a quantitative examination, can be helpful for the selection of site remediation options with minimum impact on the ecosystem and human health. Besides addressing the environmental impact of the remediation activities for, attention should also be paid to the consequence of reintroducing a remediated site into the economy.

Moreover, certain soil remediation technologies, especially the more 'gentle' remediation technologies are characterized with a lot of uncertainty with regard to the time frame in which the final remediation goals will be achieved. Unexpected situations can result in an increase of the costs of the remediation project. Therefore, there is a need for practical tools that help practitioners in choosing the correct technology that will not only be effective but also will minimize the financial risk associated with the cleanup. In the present study, several methods that can be used to estimate the environmental, financial and health impact of a soil remediation process were compared. The case studies worked out in this paper want to provide a basis for a more sound selection of soil remediation technologies based on human health, environmental impact and financial risk criteria.

LC02B-5

Developments in Social Life Cycle Assessment (S-LCA) for Life Cycle Sustainability Assessment (LCSA) - application to the construction and demolition sector in France

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The results of a project aiming at developing a methodology for sustainability analysis dedicated to infrastructures and buildings construction projects will be presented. This research project was carried out in collaboration with ADEME (French agency of environment and energy control), University of technology of Troyes, the Industrial Ecology Club (guild) of Aube and Eiffage (French public works firm). The project is based on a specific case-study concerning the construction of a part of the ring road of Troyes city (Aube, France) where alternative construction materials and techniques, such as secondary raw materials and local natural resources, have been used. A sustainability LCA framework was designed, built on environmental and social LCA methodologies to assess the environmental and social efficiency of such construction projects. It was applied to the case study to compare its global performance with a similar case which would have been conducted in "business as usual" conditions. An environmental LCA was conducted, based on existing international standards. At the same time, a S-LCA methodology was developed on the basis of UNEP-SETAC Guidelines and applied to the French sector of building and construction, to assess social impacts supported by the system's stakeholders. Results showed a high contribution of natural mineral resources use in the total environmental impact, due to extraction and transportation. It was also demonstrated that the energy necessary for asphalt production was much higher than the one for its implementation. Environmental impacts are thus highly dependent on asphalt production and natural mineral resources' quantities and origin. Regarding social impacts, specific impacts categories, indicators and data were searched for and an impacts screening was conducted. Based on sectorial data, it focuses on two stakeholders' categories: workers and local communities. Final results highlight the fact that S-LCA needs strong methodological developments to address intra Europe comparative case-studies and to produce methodologies for system definition, data inventory, impacts characterization, reference databases and specific indicators.

LC02B-6

Life cycle costing of farm milk production - cost assessment of environmental impact mitigation strategies

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Agriculture is a significant contributor to environmental impacts. A study by the University of Arkansas [1] showed that 70% of the carbon footprint of US milk occurs at or before the farm gate. Agriculture is also one of the main contributors to water use and land use, as shown by an ongoing comprehensive milk life cycle study (University of Michigan, 2011). The goal of this presentation is to add costs to the LCA study to determine the impact of the farm milk production system on both the environment and production costs using the same boundaries.

Results and discussion

Three case studies were carried out to compare scenarios from a cost and environmental viewpoint.

The first case study describes a change of manure management system to a digester. For a pilot farm located in upstate New York, the global breakdown of the different processes was assessed both in terms of carbon footprint and production costs. Change in GHG impacts and costs are assessed in parallel. Discussion on sources of revenues depending on farm state's location are also discussed.

The second case study compares the cost and environmental impacts of the production of corn, which is one of the major dairy feeds. The costs and impacts (water and land use) are quantified in 3 cases in a sample US state: rainfed, gravity irrigation, and pivot irrigation. This case study provides perspective on how best to represent the costs and revenues. Trade-offs between yield and environmental impacts are also discussed.

The third case study focuses on energy saving on the farm, for a pilot farm in upstate New York. It analyzes the breakeven point, both in term of greenhouse gas emissions as well as in terms of costs.

Conclusion

This study couples environmental impacts and costs, providing a general approach to improve the environmental footprint of milk production on a large range of impacts (GHG, water use, land use) with a measure of the financial costs or benefits of production changes.

LC03 - Increasing scientific and policy understanding through meta-analysis of life cycle assessments

LC03-1

Clarifying estimates of life cycle greenhouse gas emissions from electricity generation technologies: the LCA harmonization project

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Thousands of LCAs of electricity generation technologies have been published. Quality of these studies has varied considerably, as have reported estimates of life cycle GHG emissions. We have completed a systematic review of this literature that included: exhaustive identification and collection of English language LCAs of electricity generation technologies with no bias toward publication type (journal article, report, conference paper, thesis); and multiple, independent reviews of each candidate reference by an interdisciplinary review team that met regularly to ensure consistent screening based on quality of LCA methods, completeness of reporting and modern relevance of evaluated technology.

Estimates varied mainly owing to differences in assumptions of key parameters. It is possible to adjust previously published LCA results to use more commensurate input assumptions and system boundaries, a process we call 'harmonization.' We harmonized estimates of life cycle GHG emissions for six categories of electricity generation technologies: coal, natural gas, nuclear, wind, photovoltaics (crystalline silicon and thin film), and concentrating solar power. While this process is not a formal sensitivity analysis, it does suggest which are the key parameters influencing life cycle GHG emissions for a given technology, such as thermal efficiency for combustion systems, solar resource for solar technologies, and capacity factor for wind turbines, amongst others. Harmonized estimates of life cycle GHG emissions compared to published estimates generally reveal a significant reduction in range and interquartile range from harmonization and often, but not always, an unchanged estimate of central tendency (median). In this way, harmonization is shown to be a method that can reduce variability and clarify central tendency of estimates of life cycle GHG emissions of electricity generation technologies in ways that are useful for policymakers and analysts. Given tight harmonized distributions of a large number of estimates, it is concluded that new LCAs of many technologies (e.g., onshore wind, pulverized coal) are not necessary for the purposes of broad policymaking and first-order analyses, while knowledge gaps or uncertainty remain issues for several other technologies (e.g., natural gas, deep offshore wind).

LC03-2

Meta-analysis of life cycle analysis studies on electricity generation with carbon capture and storage

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Currently, electricity generation contributes to 40% of global carbon dioxide (CO₂) emissions. Carbon capture and storage (CCS) technology is widely recognized as an

appropriate option to achieve ambitious CO₂ reduction targets. In the last decade, numerous life cycle assessments (LCA) on environmental impacts of electricity generation with CCS have been conducted. This meta-analysis comprises fifteen LCAs of the three CCS technology routes (post-combustion, oxyfuel, pre-combustion) for greenhouse gas reduction for different regions (Europe, United States, Japan, global), different fuels (hard coal, lignite, natural gas), and different time horizons (between present and 2050). The goal of this meta-analysis is to provide a structured overview of assumptions and methodological choices made and, where possible, their effects on the outcomes. The study answers three key questions: 1) Is LCA an appropriate method to evaluate the environmental effects of CCS, and with what validity or limitations?; 2) Is it possible to draw general conclusions regarding the environmental performance of CCS power plants compared to power plants without CCS from the existing LCAs?; 3) Do certain trends arise across the different capture routes or fuels used?

All LCAs show reduction in global warming potential but an increase in other impact categories, regardless of capture technology, time horizon, or fuel considered. Three parameter sets have a significant impact on the results: 1) power plant efficiency and energy penalty of the capture process, 2) CO₂ capture efficiency and purity, and 3) fuel origin and composition. However, the normalization indicates only a small impact from CCS power plants on total global environmental impacts.

The meta-analysis proves that LCA is a helpful tool to investigate the environmental consequences associated with CCS. Differences in the underlying assumptions of the LCAs as well as methodological shortcomings yield in heterogeneity of results. A sophisticated and common understanding of the most important technological parameters is necessary to draw a clearer picture of both single CCS techniques and comparisons across techniques. Therefore, it is essential that LCAs include well documented parameters and describe uncertainties and assumptions precisely. There also remains a wide field of subjects and CO₂ capture technologies of 2nd generation (like membranes) that have not been covered yet.

LC03-3

Simplified life cycle approach: GHG variability assessment for onshore wind electricity based on Monte-Carlo simulations

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The environmental impacts of electricity production systems have been widely assessed over the past years with many published LCAs in the literature. In the special case of greenhouses gases (GHG) from wind power electricity, the LCA results variability observed is very high, for example ranging from 2 to 81 g CO₂eq/kWh in a literature review performed by the IPCC in 2011. Such result might lead policy makers to consider LCA as an inconclusive method. For environmental impacts from electricity generation this is a sensitive issue.

There is a need for a more comprehensive approach to assess the GHG variability so as to define generic results which meet a general consensus. Different attempts have been initiated in order to address this problematic, the use of meta-analyses in LCA being one of them.

The main objective of this paper is to build a representative model of onshore wind turbines (WT) to assess environmental performances with a simplified life cycle approach.

A first methodology to generate simplified models for WT environmental performances has been designed (reported in the special issue of the Journal of Industrial Ecology) and is now improved with a better identification of the GHG variability assessment. Variability of GHG performances of onshore wind turbines, generated for a representative sample, is assessed through the running of Monte-Carlo simulations to identify the key parameters having the biggest influence on the results. Based on these Monte-Carlo simulations, we plotted GHG performances distributions for two key identified parameters: the WT life time and the annual average wind speed. A set of generic GHG performances curves has been defined as a function of these key parameters. Results are ranging from 2.7 to 119.7g CO₂eq/kWh, a range which is comparable to the mentioned IPCC literature review. These results can be adjusted as a function of either one or both key parameters. This methodology will be applied later for all types of electricity generation systems to generate simplified life cycle approaches.

LC03-4

What can meta-regression analysis tell us about variations in life cycle assessment (LCA) results for greenhouse gas (GHG) emissions estimated for advanced biofuels?

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This paper presents a systematic literature review of LCA studies of advanced biofuels (i.e. biomass-based fuels produced from lignocellulosic materials and microalgae).

The main factors influencing LCA study results are characterized and estimated using a meta-regression analysis in order to clarify conclusions about their environmental performances. LCA has been widely applied to calculate the Global Warming Potential (GWP) is an impact indicator for GHG emissions) associated with biofuel life cycle.

Those LCA results can vary significantly depending on various factors: assumptions at biomass production step (N₂O emission estimations, inclusion of direct and indirect Land Use Change-LUC), data for biomass conversion into biofuel and general LCA methodological choices (system boundaries, coproduct impact accounting, etc.).

Here, we propose an alternative approach to previous narrative surveys of biofuel LCA studies using the meta-regression analysis methodology to describe and synthesize existing estimates of the GWP of advanced biofuels. It allows to compare and/or combine outcomes of different individual empirical studies with and without similar characteristics that can be controlled for. A database has been built containing a vector of previous studies estimates of GWP (GHG emission indicator expressed in mass of equivalent CO₂ per megajoule of biofuel) that has been chosen as the dependent variable of the model since GHG emissions reduction is the most important environmental criteria in regulatory texts, and a vector of explanatory variables. These are factors that can influence LCA results and some study characteristics. Advanced biofuel LCA results from peer reviewed articles, research reports (grey literature) and regulatory texts (RFS and RED) are included in the database (43 LCA studies, 585 estimates). Preliminary results show, *ceteris paribus*, that the mean life cycle GWP associated with 1 MJ of ethanol, BTL and algae fuel are 27, 21 and 83 gCO₂eq respectively (RED fossil fuel reference value is 83.8 gCO₂eq/MJ). The analysis shows that GWP estimates are higher in studies that take into account estimate uncertainties, LUC, infrastructures in system boundaries and lower in studies accounting for other impact indicators than only GHG emissions. Moreover, our results indicate that regulatory texts provide lower GHG emissions estimates than peer reviewed studies and that estimates from European studies are statistically higher than American ones.

LC03-5

Sources of variation in life cycle assessments of desktop computers

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Life cycle assessment (LCA) studies of desktop Personal Computers (PCs) are analyzed to assess environmental impact of PCs, and to explain inconsistencies and disagreements across existing studies. Impacts, characterized in this work in terms of primary energy demand and global warming potential, are decomposed into inventory components and impact per component in order to expose such inconsistencies. Additional information from related studies, especially regarding use-phase energy consumption, helps interpret the LCA results. The weight of evidence strongly suggests that for primary energy demand and contribution to climate change, the use phase is the dominant life cycle phase; manufacturing impacts are smaller but substantial, and impacts due to product transportation and impacts due to end-of-life activities are much smaller. Each of the few LCA studies that report manufacturing impacts as being greater than use-phase impacts make unrealistically low assumptions regarding use-phase energy consumption. Estimates of manufacturing impacts, especially those related to printed circuit boards and integrated circuits, are highly uncertain and variable; such estimates are very difficult to evaluate, and more systematic research is needed to reduce these uncertainties. The type of computer analyzed, such as low-power light desktop, or high-power workstation, may dominate the total impact; future studies should therefore base their estimates on a large sample to smooth out this variation, or explicitly restrict the analysis to a specific type of computer.

This abstract is based on a journal paper currently in press with the Journal of Industrial Ecology, due to be published in early 2012 in a special issue on meta-analyses of life cycle assessments.

LC04 - Latest developments in uncertainty management - adding value to LCA studies

LC04A-1

Quantification of uncertainty and spatial variability of characterisation factors in the new global LCIA method IMPACT World+

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IMPACT World+, a new LCIA method representing an update of the IMPACT 2002+ LCIA method, was developed as a regionalised methodology covering the entire global scale, and including uncertainty information encompassing spatial variability and model uncertainty. Up to now uncertainties were only quantified in life cycle inventory databases, notablyecoinvent. IMPACT World+ now provides quantitative uncertainty estimates for each characterisation factor (CF) and thus allows for a complete uncertainty analysis for an entire LCA study. This presentation introduces the approaches used to estimate, quantify, and combine uncertainty and spatial variability for CFs in each impact category. The uncertainty and spatial variability are quantified and kept separately, allowing for differences in spatial variability of CFs in different spatial scales (e.g. watersheds for water use impacts, biomes for land use impacts, and urban/rural archetypes for respiratory impacts, or soil and water archetypes for metal toxic impacts compared to a global or continental average CF. This results in two semi-quantitative pedigree-matrices, per impact category respectively representing uncertainty and variability. Assigning a GSD2 to each of the five uncertainty/variability classes ranging from "no" to "very high" uncertainty/variability allows translating the qualitative into quantitative information. The resulting GSD2 values for the identified main coefficients that compose the CF, e.g. for human health iF, dose-response, and severity factor, can then be combined into a total GSD2 for each CF. A pragmatic and operational solution was required to provide estimates for characterisation factors that count from a handful in some impact categories such as Eutrophication to up to thousands for human health or ecotoxicity. IMPACT World+ is the first LCIA method to provide

quantitative uncertainty for each CF using an estimation framework that is consistent with life cycle inventory uncertainty estimates from e.g. ecoinvent. Given the various types and sources of uncertainty contributing to the overall uncertainty of a CF, the estimates provided are not covering all uncertainty sources and types, and might have a somewhat limited accuracy. They are however an important operational starting point to consistently apply uncertainty analysis in LCA, ultimately allowing to quantify the confidence in LCA results and hence the related conclusions and decisions.

LC04A-2

Quantification of uncertainty of characterisation factors due to spatial variability

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Except for a minority of foreground production sites, elementary flow geographic location is only known to the country resolution. More precision is needed to pick the right spatialized characterisation factors (s-CF) from a set. Countries are usually covered by several s-CF, and their value might span over several orders of magnitude. So far, this source of variability has not been quantified. This paper presents a general method quantifying the uncertainty created by the dichotomy in spatial resolution between inventory and impact assessment.

For each country, a generic CF (g-CF) has to be calculated with the s-CF covering it. Two elements are necessary to build the g-CF: the probability density function (PDF) of each s-CF, and a set of weighting factors. The weighting set provides a likelihood of emission on the zone covered by each s-CF, an information lacking in the generic unit processes. The average and standard deviation of the g-CF is not calculated directly from the weighted s-CF. Instead, the PDF of the g-CF is built by adding the weighted PDF of each s-CF. The average value of the g-CF can be calculated from the PDF and used in deterministic calculations. The standard deviation could be useful if the resulting PDF can be approximated by a normal or lognormal. Otherwise, the full PDF should be reported and used directly in a Monte Carlo simulation.

Finally, the coefficient of variation (CV = average/standard deviation) of the g-CF can be compared to the CV of the s-CF. The CV of the g-CF will be higher, and this augmentation of uncertainty reflects the spatial variability that cannot be taken into account, due to the lack of spatial information in the generic unit processes.

The impact category chosen to demonstrate this methodology is water use on human health. The spatial delineation of this method is the intersection of countries and watersheds, resulting in 808 cells. The likelihood of water use in each cell is based on data from the Watergap model. China is covered by 20 watersheds. The corresponding 20 s-CF are lognormally distributed with CV between 0.6071 and 0.6218. After adding their weighted PDFs, the resulting g-CF is bimodal, with a CV of 7.63. This means that if a use of good quality water occurs somewhere in China, without further information on the watershed of consumption, it will be characterized with a g-CF of a value potentially much greater or lower than with the right s-CF, and roughly 10 times more uncertain.

LC04A-3

Matrix-based sensitivity and uncertainty assessment for evaluating human intake of pesticide residues in food

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Multiple pathways contribute to human exposure towards pesticides: inhalation after air emissions, ingestion after soil and water emissions and, most importantly, ingestion of directly sprayed food crops. For the latter, we developed a dynamic multicrop model for assessing pesticide residues in food crops and subsequent human intake. However, full uncertainty analysis is still lacking. We already identified half-lives in plants and time between substance application and crop harvest playing a crucial role, but other aspects may also be important.

We designed a simple but accurate regression model with time from application to harvest, half-life in plants, residence times in the environment and key substance properties as influential input variables. Additional influences are identified by assessing relative sensitivities of a wide range of input variables, thereby addressing parameter correlations and defining geometric standard deviations for all relevant input variables as a function of their base uncertainty and spatial/temporal variability.

We studied the uncertainty propagation of the model output by applying a new approach of calculating the overall output uncertainty as a function of the matrix of relative sensitivities of input variables and the covariance matrix expressing their correlations. Model output, i.e. human intake fractions from consumption of food crops treated with pesticides, showed highest relative sensitivities across substances to half-lives in plants and on plant surfaces as well as to the time between substance application and crop harvest. Total output sensitivity is a function of the crop species and is highest for leafy vegetables (lettuce) and fruit trees (apple). We also studied the variability across pesticides, from which we derive crop-specific regression models predicting residues in food crops across pesticides within a factor of 10 of those calculated with the complex model as a function of only a handful of input variables. These simplified models are adequate to assess direct residues for multimedia models used for risk and impact assessment and, hence, enable the user to calculate direct pesticide residues by only providing a very limited set of input information.

LC04A-4

Uncertainty classification and implementation in life cycle impact assessment: application to freshwater ecotoxicity of pesticide application to Maize in The Netherlands

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How to deal with uncertainty has become a key challenge for integrated assessments. As yet, the application of an uncertainty analysis is not common practice in life cycle assessments. A proper analysis will be facilitated when it is clear which types of uncertainties exist. The aim of this research is to set up a framework to classify levels of uncertainty in life cycle impact assessment (LCIA) and demonstrate the practicability of the framework with a case study example on freshwater ecotoxicity caused by pesticide application in The Netherlands.

Three levels of uncertainty were distinguished. (i) Statistical uncertainty, arising from measurement errors, analytical imprecision, and limited sample size; (ii) Decision rule uncertainty, caused by ambiguity or controversy about how to quantify or compare social objectives; (iii) Model uncertainty, defined as uncertainty about the relations and mechanisms being studied. To quantify statistical uncertainty, uncertainty distributions need to be derived for input parameters and propagated to output uncertainties, e.g. via Monte Carlo simulation. Decision rule uncertainty and model uncertainty can both be made operational with the help of a choice analysis.

A case study that addresses the application of pesticides to maize in the Netherlands was performed to show the application of the various uncertainties for freshwater ecotoxicity. Statistical uncertainty in freshwater ecotoxicity damage scores was quantified by performing a Monte Carlo analysis with chemical-specific input parameters set as uncertain. Decision rule uncertainty was determined by identifying choices in the damage quantification, i.e. availability of toxicity data, choice of effect factor model (linear or non-linear), and the damage factor (modeling up to midpoint or to endpoint). Model uncertainty was quantified by including and excluding pesticide transformation products.

Results showed that damage can vary substantially depending on the value choices made. Parameter uncertainty can increase to a large extent when a more accurate model is applied. Going from midpoint to endpoint applying an uncertain damage factor decreases the damage slightly, but hardly changes uncertainty. Only when transformation products are included, going from midpoint to endpoint increases median damage clearly.

LC04A-5

Data quality metrics in Life Cycle Impact Assessments: application to a case study of cellulosic biofuel

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Although guidelines exist for assessing data quality in life cycle impact assessment (e.g., ISO-1404), they are seldom implemented satisfactorily in practice. A need therefore exists for transparent, consistent, and credible data quality metrics that facilitate the prioritization of data needs and enhance the reliability of LCIA. Monte Carlo (MC) methods, such as sensitivity and uncertainty analysis are used to identify the most influential inputs and to understand how input uncertainty (variability and lack of knowledge) is propagated through to the calculation of output metrics. However, MC methods are rarely applied in LCIA. Here we demonstrate the application of sensitivity analyses coupled with a data quality evaluation scheme, to assess uncertainty in output metrics in our LCA "Carbon Tool", which quantifies the environmental and human health impacts associated with national-scale cellulosic biofuel production. Programmed in Excel, the Carbon Tool estimates environmental and health impacts associated with biomass, biorefining, and transportation stages of biofuel production. Specific output metrics include: greenhouse gas emissions (fossil CO₂ and GWP100), total energy, and human health impacts associated with criteria air pollutant emissions. We use the Spearman rank correlation coefficient to identify influential variables for each output metric, with specific application to Miscanthus ethanol production. We then evaluate the uncertainty in influential system variables, using a qualitative data quality evaluation scheme adapted from Junnila and Horvath (2003). Of the eight variables evaluated, Combined Heat and Power (CHP) penetration was the most influential system variable on the total energy and fossil CO₂ output metric. However, the "CHP penetration" input ranks low in terms of the geographical correlation and overall representativeness, and is based on assumptions (low rating for data acquisition). For human health impacts, specifically those for PM emissions, the three most influential system variables are associated with truck and rail distance traveled. But, the data for these inputs rank low in terms of temporal, and geographic coverage and are based primarily on assumptions (medium rating for data acquisition). We find that our data quality framework, coupled with MC methods, can help prioritize data collection efforts to focus on those inputs that significantly influence the outcome metrics, and have low data quality ranking.

LC04A-6

Uncertainty analysis in macro-level life cycle assessment

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Developments in life cycle assessment (LCA) have led to new life cycle thinking applications such as the macro LCA approach (M-LCA), an extension of consequential LCA (C-LCA) that implements elements of prospective LCA in order to model medium- or long-term environmental impacts related to global economic perturbations caused by major changes occurring in multiple life cycles. In M-LCA, economic impacts are based on the GTAP model, an economic general equilibrium model (GEM), which provides the production variations for each economic sector in each region of the world in response to a given economic change. The environmental impacts of each regional economic sector are then computed according to the LCA methodology. As uncertainty related to GEMs and LCAs is recognized as significant, the uncertainty associated with M-LCA is expected to be as well. For this reason, an uncertainty analysis was conducted on an M-LCA comparison of two European Union (EU) energy policies (business as usual vs. bioenergy). Uncertain exogenous variables and internal parameters used by GTAP were combined in order to develop 27 scenarios that were run in GTAP to define a tree of potential economic consequences caused by each EU policy under the assumption of each scenario. Environmental impact uncertainty for the 27 scenarios was then assessed using a Monte-Carlo (MC) analysis. Results of the uncertainty analysis show the M-LCA comparison of EU policies is not sensibly affected by uncertainty for climate change and natural resources while the comparison is more uncertain for human health and ecosystems. The uncertainty analysis also highlights that several sources of uncertainty in M-LCA cannot be assessed due to lack of information on data uncertainty (external data used for the linkage of GTAP and LCA databases and forecasts used to model the evolution of temporal variables) and the difficulty in comparing GTAP results with historic data. Additionally, it appears MC analysis is not adequate to study uncertainty propagation in complex models such as M-LCA. Indeed, due to the significant quantity of uncertain parameters, the time required to conduct the MC simulations makes this approach unfeasible without extensive computational resources. Therefore, the development of another approach based on an uncertainty management method more suitable for large models, like Gaussian quadrature or Fourier transformations, would improve the management of uncertainty in M-LCA.

LC04B-1

Technological, geographical, time-related uncertainty measurements for textile spinning and weaving processes

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Life Cycle Assessment (LCA) is very dependent on the quality, relevance and reliability of the Life Cycle Inventory (LCI) data sets selected by the LCA-practitioner. When modeling a system, the representativeness of the LCI data set is complemented by the appropriateness of the data set in the context of the specific system. The appropriateness characterizes, in how far a data set in a system model represents the truly required process or product. According to the ILCD handbook [1], the use of not fully appropriate data is justifiable only if this is not relevantly changing the overall LCIA results compared to using fully representative data; otherwise the lower achieved representativeness shall be documented in the report. But when the required data is missing the practitioner has limited possibilities to evaluate any differences between the required data and the available data.

This paper aims at evaluating the appropriateness of different LCA-practitioner choices regarding the geographical, technological and time related representativeness in the modeling of a product life cycle. The examples are taken from two technologies from the same industry domain that behave differently and require different LCA methodologies.

First, different LCI data sets for spinning and weaving processes in the textile industry are inventoried. The precision, completeness, representativeness and methodological consistency of these LCI are briefly described. Then, the following data quality tools: gravity analysis and sensitivity analysis [2] are applied at the assigned LCI results. The gravity analysis reveals the main contributors to indicator scores. The sensitivity analysis measures the change in the indicator results for induced changes in LCI results. Practically, the LCI results for different geographical situations, for different technologies (time related or not) and for different scenarios (average world scenario and worst case scenario) are compared.

LC04B-2

A comparison of uncertainty propagation methods in an LCA study

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Several methodologies can be used for defining and propagating uncertainties affecting model input data in LCA studies, according to the nature of available information. Probability theory is the most commonly used and consists in defining uncertainties as single probability distributions and then propagating them through stochastic modelling or analytically. For cases where available information does not justify the use of single probability distributions for representing uncertainty, possibility theory provides an alternative method which consist in defining uncertainties as fuzzy sets.

The objective of this paper is to illustrate the fundamental differences between these approaches using on the one hand classical stochastic modelling, fuzzy calculus, and finally a hybrid method which combines both approaches. In this last method some parameters are represented by single probability distributions (because they are justified by available data) while others are represented by fuzzy sets (because available data is incomplete and/or imprecise). The joint propagation of these different modes of uncertainty representation was performed using the independent random set method proposed by [1].

The case study investigates the benefits of sending the organic fraction of household waste to anaerobic digestion instead of incinerating it, in Danish conditions. For each chosen parameters, data was gathered through a literature review to define the uncertainties underlying each parameter, using either a single probability distribution representation, or else a fuzzy-set representation.

Comparison between the three propagation methods illustrates the very conservative nature of the purely fuzzy calculation, of which the result of the stochastic calculation is but one representative among others. Results of the hybrid calculation on the other hand are more precise than the fuzzy calculation but of course less precise than the purely stochastic result which assumes that single probability distributions are perfectly known for all input parameters.

In real-world situations of LCAs, available data are typically of different natures. Therefore the proposed method can serve to jointly propagate the different types of uncertainties in the LCA.

[1] Baudrit, C., Dubois, D., Guyonnet, D., 2006. Joint propagation and exploitation of probabilistic and possibilistic information in risk assessment models. IEEE Transactions on Fuzzy Systems, 14 (5), 593-608.

LC04B-3

The impact of processing natural resources on uncertainties in life cycle assessment

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Natural resources are often connected to variable compositions. Ore from mining operations seldom provide the same ore concentration of their production everyday and the further processing has to deal with it. The same effect is visible in renewable material flows like wood, grass, and crops for example. By processing these materials the process has to be either adapted to the changing quality of input material or it is run on average level. An average process control level may have an inefficient effect on the process quality.

This presentation will concentrate on the question: How to run a material and energy efficient production process with less environmental impact? Our study case is the production of pig feed in a commercial feed processing plant in Northern Germany.

Our aim is to relate the energy consumption not only to the process steps but to the various recipes. By identifying the carbon footprint for each recipe we are going to optimize the energy input into the process without influencing the product quality. This is our highest challenge, since the quality of the end product cannot be changed due to the animals demand.

This can be achieved by monitoring the process by online measuring systems and developing an expert system that is able to control the process by using fuzzy rules. The definition of the fuzzy rules are dependent on the identified uncertainties within the process. During the handling of renewable materials uncertainties can be found in their composition due to the fact that we are handling natural resources with varying properties like their water content for example.

The uncertainties in feed processing can be divided into:

- uncertainties that can be influenced by the process control (adjusting the water content by adding water)
- and uncertainties that can not be influenced by the process control (chemical composition)

The development of the expert system of the production process (case study pig feed) is performed according to uncertainty management. Uncertainties are present at any time in the process and has therefore taken into account during the decision making process. In the end it will be possible to simulate the production process with varying parameters to evaluate the result of the simulation. This helps in assessing the process set up and in logistics question (what is the best cyclic order of the recipes to consume the least energy?).

LC04B-4

Introducing an uncertainty analysis methodology, in an international carbon footprint accounting for decision making, Renault Group case study

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Climate change and energy dependence are major challenges for the transport industry. Tackling these requires major decisions and therefore to integrate Greenhouse Gases

(GHG) management in companies. The two first actions to go through are accounting over the complete life cycle and involving all the employees and key stakeholders in the progress. Due to the breadth of the approach, it is of utmost importance to evaluate uncertainty in order to ensure the best decision-making. The hybrid product/organisation accounting method is based on the product Global Warming Potential calculated and reviewed according to ISO LCA norms. LCA often neglect employee mobility and workplace behaviour. Therefore these items are added according to the GhG Protocol. Interpretation of the results is crucial and a specific uncertainty analysis method has been developed. Uncertainty is analyzed separately for Physical Input (PI) data and Emission Factors (EF). The pedigree matrix takes into account reliability, completeness, and temporal, geographical and technological representativeness of data according to five different quality levels. Accuracy is increased by introducing a specific factor for the availability of Physical Input primary data versus secondary. Finally Emission Factor quality are specific of industrial activity. The uncertainty factors are used together to evaluate the global uncertainty.

The majority of the carbon footprint (85%) calculations are in the “good” level, while 10% are of “middle” uncertainty and finally 5% are evaluated as “very good”. When making a decision, it is important to allocate budget toward the most important topics. This uncertainty approach enables deciders to choose under the best conditions. The risk factor can be considered in decisions, helping us to evaluate and improve the inventory each year. All uncertainty factors are based on Renault expert judgment and should be challenged by external expert (eg. Quality of background LCI database). Implementing Carbon Footprint accounting is a major step towards an Environmental Footprint management by introducing the life cycle perspective and involving the whole company. Nevertheless such a wide perimeter implies a complex calculation protocol and the uncertainty calculation will have a huge importance to tackle the complexity of this management and implement the best decisions. Finally, the authors stress that even if uncertainties exit, these shall not be a barrier for taking action.

LC04B-5

Uncertainty and variability in the carbon footprint of U.S. coal-fired power production

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The overall carbon footprint of power generation can be calculated using life cycle assessment (LCA). For most LCAs, average conditions are used to estimate the environmental impacts of a process or product. However, regional and technological differences in product manufacture, use, and disposal may cause a specific life cycle impact (e.g. GHG emissions) to be lower or higher than that of an “average” life cycle. Moreover, uncertainty in process data used to model life cycle stages may affect the accuracy and precision of life cycle impact assessments. Whereas uncertainty is defined as lack of knowledge and can potentially be reduced by additional research, variability is an inherent characteristic of supply chains and the environment and cannot be reduced.

The aim of this study is to quantify the contributions of uncertainty and variability to the range of the carbon footprint associated with coal-fired electricity production in the U.S. We quantified the effects of spatial and technological variability upon the life cycle emissions e.g. distances between coal mines and the power plants they supply and power plant efficiency. We also characterized parameter uncertainty, e.g. the methane emission associated with a coal basin. The LCA included three stages: mining, transport and power generation, and utilized a functional unit of 1 kWh of electricity generated at the plant. Parameter uncertainty was quantified via Monte Carlo simulation, whereas variability was taken into account via the efficiencies of power plants and coal transportation receipts reported by the U.S. EIA.

We present the individual carbon footprints for U.S. power plants with uncertainty ranges. Our results show that the variability in electricity plant efficiencies particularly lead to a large overall variance of the life cycle emissions of coal power. Results indicate that improvements in inventory data and assessment parameterization reduce the variance of the life cycle emissions. The results can be used to assist decision-makers in ranking the carbon footprints of coal plants and facilitate the comparison of coal power emissions with other power generating technologies on a statistical basis.

LC04B-6

A protocol for approaching uncertainties in life-cycle inventories Monte Carlo analysis - a practical example using aquaculture feeds

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Although LCI values often are presented in absolute numbers, uncertainty and variability are common traits of both foreground and background data. Uncertainties result from limited temporal, spatial and technological coverage of both economic and environmental flows, with a direct influence on results. In LCIs of food production system, however, the influence of variability (ontological uncertainty) may be increasingly predominant as production is governed by natural fluctuations (e.g. yields). Outcomes of LCA results describing the same production system may therefore vary with up to an order of magnitude.

Aquaculture feeds are here used as a practical example to demonstrate the proposed approach to uncertainties in LCIs. The modelling includes foreground data collected from feed mills in Asia and literature sources, while background data derive from the ecoinvent v 2.2 database. Each processes within the system boundary was approached using a standardised protocol to assign mean, standard deviation and distribution to most economic and environmental flows. Each of these variables were based either on primary data, weighted averages derived from a meta-analysis or a numeral unit spread assessment (NUSAP) pedigree. The inventories were later simulated using a Monte Carlo analysis to generate confidence intervals for individual flows.

Using relevant up-to-date inventories is crucial for achieving accurate results and taking uncertainty into account is the only way to justify these results. We, therefore, herein present a practical way on how to consistently source and analyse inventory data using a mix of real values, weighted averages and NUSAP estimations. This allows for a wide range of data sourcing and a constructive strategy for highlighting areas of great uncertainty and identifying data gaps.

LC05 - Monetisation for weighting and aggregation in Life Cycle Impact Assessment and Cost-Benefit-Assessment

LC05-1

Development of national average weighting factors in LIME2 - Visualization of the variability of external cost using statistical analysis

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Recently several methods of economic evaluation have been published in the scientific literature increasing the credibility and the review process. Examples of more recent methods are methods based on monetary valuation of endpoints (Itsubo et al., 2004; Weidema, 2009), the Ecotax method based on a monetary valuation of midpoints (Finnveden et al., 2006), and panel methods for midpoints (Soares et al., 2006; Huppes et al., 2007). Methodological aspects of weighting methods are also reviewed and discussed by (Ahlroth et al., 2011; Mettier and Hofstetter, 2005).

The results are not only easy to understand but can also be used for cost-benefit analysis. While the best way to allocate economic values to environmental impacts such as a loss of health or a decline in biodiversity is still in the development stage and has not yet been fully established.

This study summarized a result of development of national average weighting factors with visualizing of the variability of external cost using statistical analysis. Conjoint analysis was applied to weight across several safeguard subjects and random parameter logit model was adopted to measure the confidence interval of external cost.

LC05-2

Environmental damage cost factors per functional unit of transport activities, heat & electricity generation in Germany

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For decision support, results of LCA often have to be weighted to allow aggregation of impacts and evaluation or ranking of alternatives. Impacts can be expressed in monetary terms to facilitate appropriate taxing or subsidies, and cost-benefit-analysis, etc. If impacts on human health and the environment are expressed in monetary terms they are called environmental damage costs (EDC). The share which is not internalised into economic decisions is called external costs. Starting in the 1995, within the ExternE (External Costs of Energy) project-series methods for estimating EDC have been developed. The methodology has been further improved in several EU-projects. In 2011, within a project for the German Federal Environment Agency, cost factors have been re-calculated for different transport activities, heat & electricity supply in Germany including operation and up- and downstream processes, also outside Germany. A large amount of results is available providing differentiation between energy carriers and technologies for heat and electricity. Moreover, with regard to transport activities different energy carriers, modes and different technologies have been assessed. Results are available at www.ExternE.info.

The updated and extended impact pathway approach provides results which are more reliable and conclusive as it combines site specific assessments, as far as possible, but also includes average values for different source characteristics and regions.

The uncertainties due to spatial variability inherent in many LCIA methodologies are transparent because country specific values are further differentiated into sub-regions, urban and rural environment, and different heights of releases. The results can be used to support decision making, perform cost-benefit-analysis and test more simplified approaches in the future.

LC05-3

Comparative LCA of potable water production plants using economic valuation

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In potable water production, the question of finding a fair trade-off between the water quality increase, from raw water to at least the potable water standard, and the total effort to be spent to reach that objective is very common despite not trivial. Few comparative LCAs of potable water plants have been published, mostly disregarding the water quality increase in the functional unit and therefore obtained erroneous conclusions. Water quality gain (WQ) can be evaluated by using water quality indicators as the Quality Valuation System for Water (SEQ-EAU in French), further aggregated into a single score through a statistical approach. Regarding the total effort spent to reach the gain (TE), monetisation of LCIA results (Stepwise2006 and Eco-costs2007) is indeed a very effective and practical approach, as compared to traditional single scores like ReCiPe, to obtain a single numeraire, which can be further combined to the total operational costs of the water treatment plants to be fully understandable by investors and decision makers. In the framework of an international research project, we developed a novel performance index (PI), defined as the ratio WQ/TE, which allows to properly compare alternative potable water production plants. Statistical tests are used to get a confidence interval for the PI of each plant but also for the difference of the PIs between plants. The PI was applied on two existing treatment plants managed by Suez Environnement in France. The plant comparison using the PI calculated with ReCiPe is not conclusive, since the confidence interval of the difference of the average PIs includes zero (too high uncertainties). The same evaluation with monetized scores, augmented by operational costs, gives a clear preference for one of the two site. Further research is ongoing to add infrastructures data in the LCIA calculations and to implement LCIA uncertainties into the performance index.

LC05-4

Development of an integrated indicator for land use based on ecosystem services

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In life cycle impact assessment (LCIA), potential impacts due to land occupation are calculated with the equation: $I_{occ,i} = \text{surface} * \text{time}_{occ} * \Delta Q_i$. Currently, the only i indicator for the land quality loss ΔQ_i is related to terrestrial biodiversity (PDF.m².year) and is not representative of all impacts, which are described by the European Commission (2010). Recently, an improved land use method has been developed with the UNEP/SETAC Life Cycle Initiative project (LULCIA, 2008-2011). This method relates land use to six new indicators: biotic production (BPP), erosion regulation (ERP), fresh water regulation (FWRP), mechanical and physicochemical water purification (WPP) and carbon sequestration (CSP) potentials, which represent provision and regulation services, as defined in the Millenium Ecosystem Assessment (2005).

While this development improves LCIA methodology, it also reduces the decision support system capacity of LCA, providing seven midpoints for land use alone.

This project aims to develop a new method (factors in \$/indicator unit) using economic valuation as a common thread to convert the new indicators to endpoints representing ecosystem services loss (potential damage costs). This conversion also allows aggregating into a single indicator. The values of BPP and CSP are respectively estimated with productivity loss and carbon tax. The other regulation services values are estimated through current compensation costs, as they are considered essential (conservative approach). As a local impact category by nature, spatial variability and the availability of the compensation systems are taken into account. Economic valuation assumptions will be discussed. Finally, this method brings a new level of interpretation and potentially allows LCA to assess other impacts related to land use, such as aesthetics and recreational aspects.

This method will be applied on a case study involving the comparison of production locations for bio-based polymers.

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LC05-5

How to correct price for monetising non-renewable resource consumption?

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Monetising aims at reflecting how human well-being is affected by an activity. This paper focuses on the impact category “Non-renewable resource consumption” (mineral and fossil). The cost for the society of consuming such resources corresponds to the well-being loss due to the increased resource scarcity in the future as a consequence of its consumption today. It can be assessed through the opportunity cost, defined as the welfare loss (the cost) of being deprived of an extra unit of resource for its most valuable use, i.e. the one providing most utility. In a perfect market, the price is optimal as it allows maximising the total welfare provided by the resource (optimal resource allocation). In a more realistic market, the question is: “Does the actual market price need to be corrected for deriving the cost for the society of consuming resources?” The methodological approach developed is based on the following analysis:

- the market price is driven away from the optimal price since, as generally admitted, private discounting rate is higher than social discount rate.

- Other main drivers in market pricing have similar mechanisms in actual and perfect markets and, furthermore, these drivers are better assessed by the market than by a modeller

The developed monetisation factors for non-renewable resource consumption consist hence in using trend market prices multiplied by a factor correcting for the excessive myopia of the market (too high discount rate). The correction factor is calculated using Hotelling's rule to re-discount the projected future opportunity cost at exhaust to a current social cost. For this purpose, market and social discounting rate values are assumed as well as the number of years before the last unit is consumed. A sensitivity analysis on these parameters is carried out. The approach allows obtaining either the whole cost for the society or only the external part.

Main limits are that (i) the correction factor is common to all resources while resources could be classified into different families (ii) the factor is obtained by assuming a partial equilibrium economic model (i.e. effects of potential future internalisation are not taken into account).

In practice, when assessing for example various end-of-life scenarios for electric and electronic waste, the total monetised impacts obtained for all impact categories are often dominated by the contribution due to resource depletion (mineral and fossil).

LC06 - Life cycle management (LCM): Success factors and barriers

LC06-1

Barriers and success factors in the use of LCA and ecodesign tools at Nestlé

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Nestlé, the World's leading Nutrition, Health and Wellness Company, is committed to environmentally sustainable business practices. We assess the environmental impacts of our products using LCA and ecodesign tools. While LCA has been shown to be useful for claims and communication of the environmental performance of our products to third parties and the public, the timeline and cost of conventional LCA is not practical for a widespread application throughout the company.

PIQET, a packaging ecodesign tool, is being used to assess the environmental performance of packaging systems. Its low cost and rapid results generation allow for it to be systematically applied throughout all packaging innovation & renovation projects at Nestlé, creating knowledge on environmental impacts of our packaging and promoting innovation to develop new packaging systems with reduced environmental impacts.

Building on the success factors of PIQET (pre-defined goal & scope and inventory database, simple to use, fast result generation), we have developed a new ecodesign tool that will take into account the entire life cycle of a food product, including the production of ingredients, processing, packaging, distribution, consumer use, and end of life. To further accelerate the use of such a tool, a link with the company recipe and trial management system has been established. By selecting an existing recipe or trial of a new product from the management system, the ingredients specifications, quantities, and the corresponding processing steps can automatically be uploaded to the ecodesign tool, significantly reducing the manual work required to complete the assessment. By integrating the outcomes of the ecodesign assessment into the stage-gate process for product innovation, a systematic evaluation and optimization of the environmental impacts of all products can be achieved.

For claims and communication of tool results to the public, however, an independent peer-review process according to ISO 14040 & 14044 will still be required. To simplify this process, independently established, internationally agreed LCA and ecodesign methodologies (e.g. the EU Food Sustainable Consumption & Production Roundtable) are used. Furthermore, transparent, independently generated LCI data is incorporated into the ecodesign tool. This reduces the possibility for tool users to introduce bias into their studies and increases the reliability and credibility of the ecodesign tool.

LC06-2

Mainstreaming life cycle management: using a sector based and regional approach in Northern France in the textile, seafood, packaging and mechanical sectors

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Whilst **Life Cycle Management** is becoming commonplace in larger corporations, or forward thinking governments, it is far from mainstream. To achieve sustainable production and consumption patterns, LCM needs to be taken up by whole supply chains that include many **small and medium enterprises**. These businesses typically lack the financial capacity or human resources to implement LC tools on their own, and are wary of working with support organisations outside of their sector or local area.

In Northern France, the **textile, seafood, packaging and mechanical** sectors are developing strategic action plans to integrate Life Cycle Approaches (including ecodesign and product environmental labelling) into businesses, education and research organisations. Each study is led by a competitiveness clusters or technical centre working within the sector.

The paper will present results of the benchmark of life cycle initiatives and tools relevant to each sector, and assessment of the existing capacity of businesses, education bodies and research centres in the region. Focus will be given to how potential actions are prioritised through engagement with key stakeholders (such as businesses, universities and professional federations) and how this inclusive process maximises with success factors and helps overcome barriers to implementation.

The four action plans are developed in parallel, enabling the sectors to identify cross cutting actions. The paper will explain how this process is managed through a **network of "Life Cycle Champions"**. Champions have been trained in the eight sectors; and meet regularly to exchange experiences in implementing life cycle approaches and identify cross cutting projects. The 4 other sectors will undertake the strategic action planning process in a second "wave", to incorporate learnings from the first process.

This innovative approach to mainstreaming LCM leverages sectorial and regional networks to help overcome barriers to implementation. From a business perspective, integration with existing professional organisations means that SMEs access advice and tools through organisations that they already know and trust. Working with several sectors in parallel through the Life Cycle Champion network encourages a multidisciplinary approach, essential to improving decision making across entire supply chains.

LC06-3

Driving proactively the sustainability agenda for the European detergents and maintenance products industry, via the A.I.S.E. Charter for Sustainable Cleaning

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A.I.S.E., the International Association for Soaps, Detergents and Maintenance Products, has a long tradition of proactive work towards sustainable production and consumption. Its main overall scheme is the A.I.S.E. Charter for Sustainable Cleaning, encouraging the adoption of sustainability management practices at all stages of the product life cycle; launched in 2004 in all EU countries plus Norway, Iceland and Switzerland, the Charter covers all products categories of the detergent, cleaning and maintenance products' industry.

Participating companies report regularly progress on Key Performance Indicators, leading to the publication of an annual sustainability report.

As at November 2011, commitment to the Charter is a success with 160 companies committed, covering approximately 85% of the total industry's production output;

An update of the Charter has been rolled out in summer 2010, introducing a product dimension to the existing Charter requirements. This fits very well with the overall objectives of the European Commission SCP/SIP Action Plan, and particularly the Ecodesign Directive.

LC06-4

Life Cycle Assessment, from an evaluation tool to a collaborative eco-design enabler for electric vehicles

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The automotive industry is facing major challenges and the stress is strong in order to reduce production cost and usage value within the capacity of the nature to support the mobility growth.

This goal will be achievable only when a co-innovative product design will be set up, involving the whole value chain [1]. This is an ambitious target and this paper will show how life cycle assessment evaluation tool could be a major contributor to bring the various parties altogether.

Several example are identified, from the more obvious which is the critical review during which the practionner will open his work to Research Institutes / Universities or Environmental Non-governmental Organizations. Regarding the impact assessment, reliability can be worked with Research Institutes when comprehension can be studied with Customers. Concerning Inventories employees and suppliers will work hand in hand and finally for the scope definition, this will be a good opportunity to involve the customers in order to define the relevant functional unit. This last point is particularly true when speaking of the electric vehicle, a new technology and potentially a major change in people habits of buying and consuming their mobility.

One result example is the definition of the functional unit for electric vehicles LCA [4]. The main difficulty [5] is to define a function equivalent between thermal and electric vehicles. The ILCD handbook [2] recommends a functional unit define by four items: What, How much, How long and in What way. The reason for any doubt take place in the last item, in what way. This question was studied under two approach, one "product centric" based on functional analysis and one "people centric" based on customer (emotional) needs. Then we can enlight the main differences between the thermal vehicle strengths - reassurance and aesthetic - versus electric vehicles ones - environmental friendly and fun-. Therefore, equivalent functional units shall add a reassurance dimension to the electric vehicle functional unit such as an easy access to a "long autonomy range" vehicle when needed.

This experience on the electric vehicle shows also that LCA can be a very useful tool to bring the stakeholders (unusual ones as NGOs as well) around the table with a positive and constructive scientific approach.

LC06-5

LCT in the floor-covering industry: the strategy of Tarkett

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Tarkett is world-wide leader in innovative flooring and sports surface products, providing integrated solutions to professionals and end-users. Despite its long time concern about environmental issues, actions remained not harmonized so far. The main tool reflecting environmental performances of Tarkett activities and products was the regular publication of five environmental "Key Performance Indicators" on global production. The need for a comprehensive sustainable development strategy to be integrated in the daily practice has emerged and Tarkett is now engaged with a multifold challenge. To tackle this endeavor, Tarkett has chosen the support of the Public Research Centre Henri Tudor. Life-Cycle Thinking (LCT) has been identified as main approach, since the products are at the core of the environmental strategy system. Tarkett deals with a wide range of market segments, 30 worldwide production sites, 8 families of products, and therefore a wide range of raw materials. The implementation of LCA as a fact based practice for process and product environmental improvement has therefore become a priority. Although LCA is used as an eco-design driver and a strategic tool, the company is aware that this tool is not sufficient at its actual level of development to address some specific issues. Therefore Tarkett is engaged in complementary development studies and processes. The adopted approach is a progressive integration of LCA as a decision-making tool in a coherent way for all Tarkett's branches. First Tarkett acquired knowledge on LCA, through training sessions in Europe and the US. Then, systematic LCAs of product families have been realized. Dedicated IT software is also being developed to facilitate the access to LCA models and allow Tarkett professionals to run ecodesign simulations. The positive feedback from this test integration phase convinced Tarkett of the ability and reliability of LCT to drive the implementation of environmental strategy at the decision making level. Further development phase is now launched; the vision is to combine the complementary tools already used by Tarkett in the LCT process, in order to set a robust decision making based on eco-innovation criteria.

LC06-6

Promoting use of life cycle management in Finnish companies - challenges, benefits and suggestions for future value networks

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Life cycle thinking (LCT) and life cycle assessment (LCA) along with other life cycle (LC) methods are important tools in assessing environmental impacts of products and services, and to support environmental decision making in companies. Use of those methods can also help companies to find out business opportunities by taking precautionary actions. This study presents the main drivers and barriers for companies to apply LCT, LCA and other LC methods. We also suggest a roadmap for the promotion of these methods in companies and their value networks. We first assessed the methodological challenges of the LCT, LCA and other LC methods from the company perspective. The applicability of those tools was assessed in several case studies. The assessments were based on the data from literature and companies. Several stakeholder workshops were organized to get practical views on the use of LC methods. The current and future use of LC methods in companies was also studied using an internet questionnaire and thematic interviews. They revealed that the companies can coarsely be categorised in four main company types regarding their knowledge, resources and potential to utilize life cycle methods in practice. The findings were supported by the case studies. Three company types were named as 'interested outsiders', 'learners' and 'forerunners'. Additionally a large group of companies are not aware of the use of life cycle methods and their potential benefits. The main drivers for the environmental management of companies included legislation requirements, improvement of cost-efficiency and customer requirements, among others. A large number of

companies are not familiar with life cycle methods and their potential benefits. Additionally, especially of small and medium-sized enterprises (SMEs), often have no temporal, human and economic resources to learn and educate themselves on the benefits of using LCT and LCA. The main challenge related to wide use of LCT and LC methods in companies is probably related to the communication problem between the LC researchers and companies, and therefore much more practical approach need to be used when spreading LCT in wide scale. The next step in promoting the use of LCT and LC methods in decision making in practice will be a pilot project, in which all the actors within a region, value-chain or sector will be introduced to work with each others in to a networking process, including special type of training for SMEs.

LC07 - The UNEP-SETAC Life Cycle Initiative: a decade of supporting the global LCA community

LC07-1

Scientific legacy of the UNEP-SETAC Life Cycle Initiative: contributions and significance

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The UNEP-SETAC Life Cycle Initiative is a joint venture formed in 2002, among several purposes, to foster the development, validation, and dissemination of methods, tools and practices for lifecycle assessment and management. Over its ten year history the Initiative has conducted dozens of projects which have contributed to the efficiency, effectiveness, and rigor of life cycle approaches on a global scale. This presentation will address those contributions from the viewpoint of meeting critical needs for maintaining and enhancing the quality of the science underpinning life cycle approaches. It will also place those developments in the context of emerging research and practice from academic, government and business organizations, providing a perspective on the significance of the Initiative's contributions to the science of LCA.

Technical work products of the Initiative generally fall into three broad categories - consolidation and cohesion of life cycle inventory and impact methods (particularly with a view towards how those may support social and sustainability assessments), development of practice guidance and support tools for data and databases, and creation or validation of models for various life cycle impact categories. Instances of these developments will be described along with commentary on their consequences for the LCA practitioner community.

LC07-2

Top 10 points about life cycle every government decision maker should know

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The 10 bullets that government officials should remember about Life Cycle Approaches when considering sustainability decisions is a contribution of the UNEP/SETAC Life Cycle Initiative to the discussions for the next UN World Summit for Sustainable Development.

The Initiative has successfully responded to the call from governments for a life cycle economy as stated in the Malmö Declaration and contributed to the development of a 10-year frame work of programs on Sustainable Consumption and Production (SCP).

"To bring science-based life cycle approaches into practice worldwide" is the Mission of the Initiative and 3 objectives were established to achieve it: enhancing the global scope of life cycle data and methodologies through research and innovation; facilitating the use of life cycle approaches worldwide by business, governments and the general public; and expanding capabilities worldwide to apply and to improve life cycle approaches through education and training.

The 10 bullets were disseminated for the first time amongst government representatives during the High-Level CSD Intercessional Meeting on SCP on 2011 as:

- Every product causes environmental impacts in its supply chain, during its use and in its disposal
- To improve and preserve the world, the different impacts of products must be identified and quantified
- Life cycle assessment is the only standardized international tool for identifying and quantifying impacts of products
- By identifying impacts, they can be understood and managed by companies and governments
- Environmental impacts affect ecosystems in many different ways
- Ultimately, all environmental impacts affect humans
- Companies and the decision of governments must be made based on which impacts are most important and which should be addressed first
- Importance of impacts depends on your perspective
- All life is connected, how those connections are managed is up to you
- When making sustainability decisions, you must consider the entire life cycle including trade-offs

While the first five bullets introduce key concepts the sixth bullet highlights the strong linkage between the environment and human beings. The last four bullets compell the reader to realize the responsibility of his/her decisions. The conclusion of the presentation sheds light on role of the Initiative to further contribute to government and private sectors at a very timely moment when the new mission for the next years of the Initiative is being shaped.

LC07-3

Strategizing capacity development for life cycle management - cases from Vietnam and Malaysia

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The promotion of life cycle management in companies in developing countries has to build upon capacity to perform and interpret life cycle assessments. Drawing on two

case studies: one on pangasius aquaculture in Vietnam and the second on palm oil production in Malaysia, the paper identifies - in these two different national contexts - current capacity constraints and future options for strategizing efforts, which target transition towards sustainable production and consumption. Both cases point to the need for capacity to collect valid and reliable data. New life cycle inventories (LCIs) have to be built to reflect real processes in the main sectors of the national economy. While subsidiaries of foreign owned companies take the lead in adopting a life cycle approach in environmental management as a result of corporate policies, the large sector of medium and small scale companies have few or no incentives in their national context at a time when export markets introduce conditionality concerning sustainable production practices. The case studies also show that most examples of LCA practiced primarily seem to be motivated by concerns about the public image of a particular product in the market place. The most significant driver currently seems to occur, when a vital product in the export profile of a developing country is contested on the export markets for its environmental impacts. In conclusion, to move beyond the awareness of life cycle thinking and the communication of 'greener' brands, the linear model of rolling-out and implementing LCA practices in developing countries, which basically is derived from requirements of the LCA methodology itself, needs revision. Establishing knowledge and data resources, facilitating communication and training, providing limited support for application, and creating international networks for research definitely contributes to capacity development. However, a coherent strategy - drawing on the paradigm shift initiated by UNDP in 2002 and subsequent research on the concept of capacity development - is needed to identify options for interventions for direct stakeholders, for entities in the enabling institutions, for foreign investors, and for regulatory authorities in economic and industrial policies and foreign trade relations.

LC07-4

Water use in life cycle assessment and water footprinting: outputs and prospects of the working group WULCA

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Water use has been largely neglected in LCA of the first decades. While some water use data has been collected in inventories, environmental impacts related to use and consumption of this vital resource have not been addressed until 5 years ago. At this time different research groups have started to develop methods to quantify impacts, and the UNEP-SETAC life cycle initiative has established the working group for water use in life cycle assessment (WULCA).

The group is organized by a core of researchers and practitioners actively contributing to the development of the field, while a larger group is involved in rounds of feedbacks and invited to meetings in order to allow input from various stakeholders in a very vast and dynamic field.

The deliverable of the group was to compile a consistent framework for addressing the environmental impacts related to the various types of water use and areas of protection affected. Another challenge was to establish a common terminology. As a result, the core of the group published a scientific article which builds the basis for future research in this area. In a second phase, the group focused on comparing different available methods, comparing their scientific quality, completeness and applicability on order to provide guidance to LCA practitioners and other interested people about the status of inventory data and impact assessment methods. A scientific paper will be submitted in December 2011.

The working group is also involved in the standardization process of water footprinting, cooperating with research partners and practitioners outside the LCA community in order to contribute to a standard that is compliant with the LCA perspective.

Future action points are the harmonization of existing LCA methods and inventories. Building up on the methodological review, quantitative comparison of different approaches targeting similar impacts are performed to derive estimates of robustness, while research of missing impact pathways is fostered. Inventory data collection has

been of lower priorities so far, however, with increasing demand for both robust water use information and related impact assessment, the supply of representative water use data it becomes a relevant issue, especially due to the high spatial variability of water use and related impacts.

LC07-5

Value of a Life Cycle approach in evaluating the environmental impacts of packaging for food and beverage applications

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An articulation of the benefits of the life cycle approach to design, manufacturing, use and end of life management of packaging for food applications is important to further examination of the role of packaging within the nexus between security and safety of packaging in food and beverages and environmental protection. Key items of focus for the research and analysis included, but not limited to:

- What is the value of a life cycle approach for beverage and food products and packaging?
- What is the value of including all life cycle stages in evaluating the packaging/food systems to reduce overall life cycle impacts?
- What is the value of including multiple impacts in evaluating the packaging/food systems to reduce overall life cycle impacts?
- What is the value of including the food and/or beverage into an evaluation of the packaging life cycle impacts?
- What characteristics of future LCA studies should be considered when evaluating the food/packaging life cycle?
- Examples of how the waste management hierarchy and LCA results interface/connect

This presentation will present the results of study to examine the Value of a Life Cycle Approach in Evaluating the Environmental Impacts of Packaging for Food and Beverage Applications.

RA01 - Applying models to risk assessments: from the organism to the ecosystem level

RA01-1

How do adverse effects on individual endpoints translate to effects at the population level?

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Toxicity of chemicals is measured on many individual endpoints: feeding, growth, reproduction, and survival. The sensitivity of these endpoints of model species to chemicals is used to gauge the negative effect these chemicals will have on higher levels of biological organization such as populations. However, when using different individual endpoints to estimate risk at the population level, it is important to know the relative sensitivity of populations to changes in different endpoints. For example, is a 20% reduction in feeding more harmful to a population than a 20% reduction in reproduction? In part, these types of questions have been addressed via "elasticity analyses" using matrix models. Using elasticity analysis, the sensitivity of population growth rate to changes in different generalized modes of action (reduction in survival, reproduction, or growth) is calculated. While this provides a useful estimate of relative sensitivity of one important aspect of populations, intrinsic growth rate alone does not define the dynamics of populations. Most population's dynamics are driven in some way via an interaction with their prey species, and a change in the dynamics of individuals may alter this interaction in a way not described by the population growth rate.

We overcome this problem by using a method which considers the interaction of the population and its prey and their dynamics in time by using an individual-based model (IBM). Specifically we use the recently developed framework DEB-IBM (Martin et al. in press). The DEB-IBM framework is an implementation of the Dynamic Energy Budget (DEB) theory in an individual-based modeling context designed to extrapolate from individuals to populations. In this framework DEB provides the rules for how environmental conditions (food, temperature, toxicants) are translated into individual performance (growth, reproduction). DEB theory is generic, as differences between species are described via different parameter values within the same model framework. Using DEB-IBM (<http://cream-itn.eu/projects/wp-1/daphnia-2/deb-ibm>), we parameterize a model for *Daphnia magna* with individual level data and validate the model under control and exposure conditions at the population level. We then use this to predict the sensitivity of populations to reductions in various measures of individual performance (feeding, maintenance costs, reproduction, and survival) due to hypothetical chemical stressors in dynamic environments.

RA01-2

Exploring the consequences of spatial heterogeneity in pesticide exposure using an individual-based model of collembolan populations

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Suitable habitat for soil organisms may be scarce, thus leading to locally high population densities, because soil, being more static than water or air, is heterogeneous: physical conditions often vary widely on a scale of a few centimetres. Moreover, toxic chemicals are likely to be unevenly distributed in the soil as well. To explore the consequences of these heterogeneities for the population dynamics of soil invertebrates, we developed a spatially explicit individual-based model of the collembolan *Folsomia candida*.

In the model, individuals are assumed to sense and avoid contaminated habitat with a certain probability, which depends on contamination level: avoidance of toxicant influences the feeding behaviour of the organisms, and this in turn affects all the other biological processes. Model rules and parameters are based on data from the literature; for toxicity, only data from standard laboratory tests (survival, reproduction and avoidance) are used. The model has been parameterized and evaluated using Pattern Oriented Modelling (POM): in POM, models are required to simultaneously reproduce a diverse set of patterns.

The model has been run with two simulation scenarios where polluted area and concentrations of toxicant (CuSO₄) are the same, but spatial arrangement and connections between clear patches are different. In both cases the presence of patches of suitable habitat allows the population to grow, although the size is reduced with respect to the control. At the lowest concentration, the organisms cannot sense the toxicant and therefore do not avoid the contaminated areas: for this reason toxic effects are much less than proportional to the concentration. From the comparison of the two scenarios, it appears also that when the population is more affected during the spring peak, it is then more resistant during the fall peak, and vice versa.

To conclude, a combination of spatial heterogeneity and stressors can lead to unexpected effects of toxicants at the population level. Individual-based models help disentangle and understand these effects and therefore add ecological realism to environmental risk assessment of chemicals.

RA01-3

A comparison of deterministic and stochastic matrix population models to evaluate ecological risk of chemicals

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Environmental management decisions regarding toxic chemicals are mainly based on measurements on individuals, e.g., mortality and fertility. However, the aim of environmental management is, most often, to protect populations, not individuals. The necessary extrapolation from individuals to populations introduces a large degree of uncertainty in the risk assessment process. Presently, this uncertainty is dealt with by using fixed safety factors that are set to be protective in the majority of the cases. One method to reduce this uncertainty may be to use population models. However, before such models can be used routinely in ecological risk assessment of chemicals, the types of models to use must be determined as well as the level of model complexity necessary to provide an accurate risk assessment for different management scenarios. The objective of the present study was to determine whether simple deterministic matrix population models can be used as a first tier in risk assessment of a chemical, or if more complex models that include environmental stochasticity are needed. Matrix population models for two species of fish (eelpout and perch) were used in combination with dose-response data for a metal mixture, 2,3,7,8-tetrachlorodibenzo-p-dioxin, and tributyltin. From the deterministic models, the maximum acceptable exposure was determined as the level where the population growth rate (λ) is equal to one, which means that the population size is stable. For the stochastic model, the 5th percentile for $\lambda=1$ was used, which means that there is 5% probability for population decline. The results were compared to traditional risk ratios, based on individual level responses, and safety factors (in accordance with European Union guidance documents). Although the results of the stochastic models are more informative with regards to probability for adverse ecological effects, the results of the deterministic models clearly reduced uncertainty compared to the traditional risk ratios. This means that simple deterministic models, in combination with appropriate safety factors, can reduce the risk for over- as well as underprotective risk assessments.

RA01-4

Stochastic density dependent matrix model to extrapolate effects of toxicants from laboratory tests to population-level effects: case study on *Folsomia candida* exposed to cadmium

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Ecological risks of toxic chemicals are usually assessed on the basis of individual responses, such as survival, reproduction or growth. These data could be more effectively exploited if they served to predict effects at higher levels of ecological organization. This can be achieved by means of mathematical and simulation models. When extrapolating from the individual to the population level, environmental stochasticity and other key ecological processes should be taken into account to assure more precise and unbiased results. This study is aimed at developing a general model framework for extrapolating laboratory based individual-level effects of toxicants to populations in the field by considering environmental fluctuations and effects of density-dependence. The practical applicability of the model was illustrated for a *Folsomia candida* population exposed to cadmium and influenced by seasonal variation of temperature and density dependence.

The general structure of the model was based on an age-structured Leslie matrix. The effects of fluctuating temperature were linked with vital rates through a series of projection matrices for a range of temperature intervals. Individual-level effects of chemicals were based on classic deterministic dose-response relationships for survival and reproduction. Density dependence function is included in the stochastic matrix model directly by developing a logistic equation analogous to unstructured population model.

The technique outlined here allowed us to include effects of environmental fluctuations and density dependence in matrix population models. This can enhance the capability of matrix models to extrapolate individual-level effects of chemicals to the population level. The developed model is generic and can be adopted for any study with few modifications. The data inputs required for the model are based on the standard OECD test endpoints and annual climate data required for the study area under consideration. In the simulations the population growth rate at constant or mean annual temperatures are always greater than the stochastic population growth rate at the same contamination levels. Our model shows that neglecting density dependence in populations and variability of environmental conditions may seriously underestimate extinction probabilities in toxicant-exposed populations. Whether density dependence aggravates or counterbalances effects of toxicants on populations may depend on whether $\lambda > 1$ or $\lambda < 1$.

RA01-5

Applying an AQUATOX Aquatic Ecosystem Model with FOCUS exposure scenario to assess pesticide effects

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Aquatic ecosystem modeling can complement empirical approaches to pesticide risk assessment by allowing efficient evaluation of indirect effects mediated through species food-web interactions, testing of multiple exposure scenarios, and assessment of potential long-term effects and recovery times. In a companion poster presentation, we report on the development and calibration of an aquatic ecosystem model based in AQUATOX (version 3.0) for evaluating the potential direct and indirect ecological effects of HerbicideX (hypothetical name) on freshwater systems. We report here on the expansion and application of the calibrated model to: 1) assess potential community-level effects of HerbicideX in a generalized stream characteristic of agricultural areas of northern Europe, 2) demonstrate dose-responsiveness of the AQUATOX stream model, and 3) estimate recovery times of the biological community and key species for cases (if any) where HerbicideX causes significant effects.

The R1 Stream Scenario of FOCUS (2001) was used as the basis for the site characterization and parameterization of physical and chemical elements of the stream model. Nutrient loading similar to HerbicideX experimental mesocosms was used. A fish species (three-spined stickleback) and a dragonfly species (common club-tail) were added to the previously calibrated model, which already had phytoplankton, periphyton, zooplankton, benthic and epiphytic macroinvertebrates, and macrophytes. Immigration and emigration (drift) terms were also parameterized. Information on toxicity and environmental fate of HerbicideX was obtained mainly from the Draft Assessment Report developed by the responsible Rapporteur Member State. An annual time series of exposure concentrations for HerbicideX was developed from a TOXSWA fate model based on the R1 Stream Scenario (i.e., a realistic worst-case scenario). Almost all model species (or groups) showed negligible differences (<3 percent) between control and HerbicideX-exposed cases based on the FOCUS (2001) R1 Stream Scenario, including model species or groups of phytoplankton, macrophyte, zooplankton, benthic/epiphytic invertebrates, and fish. Overall, the behavior of the mesocosm-mimic and generalized stream models follows an expected pattern for aquatic systems, with model species responding to physical-chemical conditions and trophic interactions in ecologically realistic ways.

RA01-6

An ecosystem model for risk assessment of aquatic environments impacted by endocrine disrupters

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Endocrine disruption has been measured in many aquatic environments across the world but the consequences on the whole ecosystem are still unclear. Experimental approaches for characterizing the ecological impact of such disturbances are costly and time-consuming. Therefore, ecological models are currently being developed to support risk assessors in their decisions. Ecosystem models are required for assessing effects at high levels of organization, but the need for extensive calibration for a specific ecosystem limits their application in ecological risk assessment. This study aims at providing risk managers with an ecosystem model able to predict critical changes in aquatic environments impacted by endocrine disrupters. The objective is to find a compromise between the data available and the complexity required for the model. The ecosystem model is developed with field data obtained from a multi-year whole-ecosystem study performed at the Experimental Lake Area (ELA, Ontario, Canada): (i) two years of reference data (ii) three years of exposure to environmentally-relevant concentrations of the synthetic hormone 17 α -ethinylestradiol (EE2) and (iii) five years of recovery data. EE2 was chosen because it is one of the most widespread and potent endocrine disrupters. Indeed, the fathead minnow population collapsed after the second year of EE2 additions and endocrine disruption was observed in the other fish species as well. The developed ecosystem model considers direct effects of EE2 on fish species but also the consequences on the whole ecosystem through ecological interactions i.e. feeding and competition relationships. The model is based on simplified equations of the AQUATOX model (USEPA, 2002) with additional equations for endocrine disruption. Three fish classes are used (juveniles, females, and males) with a variable representing the reproductive ability associated with each class. The physical dynamics of the lake (stratification and mixing) was successfully modelled as well as the dynamics of zoo- and phytoplankton naturally present in the experimental lake. This ecosystem model was developed with a focus on EE2-endpoints reliable for risk assessment and will be validated with data from other whole-ecosystem studies performed at the ELA. Further model development will also allow for predicting the percentage of intersex fish, males, and females.

RA02 - Approaches for comparative hazard and risk assessment of chemicals

RA02-1

PRiME - an approach and web-based tool for the comparative risk assessment of pesticides

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Most approaches for the comparative assessment of pesticides are hazard-based. They typically score combinations of fate and toxicity endpoints from standard laboratory tests. Application rates may or may not factor in this comparison. The best approaches derive exposure estimates through models, as regulatory authorities typically do. However, even those approaches that attempt to combine toxicity and exposure into an actual measure of risk, fail in a number of key areas: 1) Adequately addressing inter-species differences in toxicological susceptibility, a key source of uncertainty; 2) Including local conditions such as rainfall, proximity to water bodies and soil type to produce a context-specific risk score; 3) Adjusting risk scores for application methodology; 4) Calibrating the estimated risk score against documented field impacts. The Pesticide Risk Mitigation Engine (PRiME) was designed to address all of these key areas. It was developed with the support of a Conservation Innovation Grant administered by the U.S. Department of Agriculture's National Resource Conservation Service as well as with time and resource contributions from numerous partners in the agricultural and food industry sectors. PRiME uses acute and other toxicity endpoints from both regulatory and non-regulatory tests on a wide range of species. Species-sensitivity distributions are used to derive taxon-specific endpoints. Fate and runoff models are run to assess likely environmental exposures and risk scores are calculated for a number of environmental and human receptors across a selection of pesticides. Most of those scores are estimated probabilities of harm or undesirable effect. Risk is presented on a low/moderate/high scale for final output to the user. Risk score indices designed to date are described. Others (e.g. pollinators) are in development.

RA02-2

An approach for environmental risk assessment of pharmaceuticals by using fuzzy AHP

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Pharmaceuticals in the environment is one of the mostly studied topics for three decades. There are significant amount of studies that are effect directed and do not indicate conclusive results on risks caused by pharmaceuticals. Moreover, everyday new pharmaceuticals are released to markets and it is difficult to predict environmental effects of them. Risk is the combination of likelihood (RL) and probability (RP) of an event and the strength of the results (RS) of this event. Fuzzy analytic hierarchy process (FAHP)

provides to organize these criteria systematically and conclude priority numbers for the risk management activities. Risk magnitude (RM) can be inferred by assessing expert opinions instead of strict formulations. Fuzzy inference (FI) systems are used for complex matrices that cannot be regulated or formulated. It is aimed to propose an approach for ERA of pharmaceuticals based on FAHP and FI system as an objective and sensitive method in order to overcome uncertainties and compensate data gap in literature. A separate hierarchy was developed for RL, RS and RP in order to get the scores of AHP for inference of RM. Characteristics of the pharmaceuticals and environment under evaluation were considered as the subfactors of the RL in order to assess fate/transport of the pharmaceutical in the environment and possible exposure pathways for the ecosystem. Effect assessment is conducted by using RS hierarchy including subfactors of ecotoxicological effects of pharmaceuticals and environmental characteristics that contribute the magnitude of these effects. RP hierarchy contains the sources of the pharmaceuticals and factors related with treatability indicating the possibility of release to environment. AHP scores of these factors were combined by using fuzzy inference (intersection and union operations) based on expert opinions and RM for the case evaluated is achieved. Risk assessment of pharmaceuticals in the environment by using AHP provide to consider all of the factors contribute to risk both related with pharmaceuticals and environment in terms of risk components that are RL, RS and RP. FI method enables to use expert opinions instead of ampic formulations which is beneficial for the complex assessments that are affected by numerous factors and cannot be completely formulated like ERA. Proposed approach reduces uncertainties and subjectiveness and provides more rigid RM that can be used as a guide for the risk management attributes.

RA02-3

Exposure hazard assessment: lessons learned from cyclic volatile methyl siloxanes

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Two cyclic volatile methyl siloxanes, octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5), have recently been subjected to close scrutiny of their exposure hazard by the European Union. These chemicals have unusual physical chemical properties which place them outside of the domain of well-studied organic chemicals. It is thus not surprising that difficulties were encountered when existing methodologies for exposure hazard assessment were applied to these chemicals. Here we explore these difficulties with the aim of extracting the lessons that can be learned from these chemicals. The D4 and D5 cases illustrate nicely that persistence should be assessed for individual media, but they also make it clear that the assessment should be conducted in a multimedia manner for each medium. A further lesson is that it can be necessary to consider differences in the standard of care to be applied to different environments in the context of exposure hazard assessment. Considering bioaccumulation, these chemicals make it clear that the TMF cannot be applied as a gold standard of bioaccumulation assessment to all chemicals. As with persistence assessment, a multimedia perspective in the assessment of bioaccumulation is also useful. Finally, we argue that persistence should have a clear priority over bioaccumulation in exposure hazard assessment.

RA02-4

A new methodology for PBT prioritization of chemical inventories

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A new PBT prioritization methodology has been developed in view of regulatory requirements for quantitative assessment of persistence, bioaccumulation potential and toxicity of chemicals. CATALOGIC/OASIS and EPI Suite QSAR models for calculating P, B and T endpoints have been used. The methodology is implemented in a software system for computerized PBT classification of large chemical inventories.

The developed methodology introduces fate/hazard classification of chemicals based on regulatory thresholds for identifying PBT substances. Thresholds corresponding to the variation of respective test procedure results are further introduced defining uncertainty bright lines around the central threshold values. The model applicability domain of each used QSAR model is incorporated in the scheme in order to evaluate confidence of obtained estimates.

The developed approach introduces a two-stage PBT classification scheme - primary classification of parent chemicals and secondary classification of stable degradants identified by OASIS biodegradation models. Single P, B and T screening results and their associated confidence are used in order to assign parent chemicals to a priority class. The introduced secondary classification based on stable degradants provides an in-depth PBT profile. Following a more conservative approach of applying the prioritization scheme, identification of a hazardous stable degradant is considered grounds for inclusion of the parent chemical in a higher priority class.

RA02-5

Comparative risk assessment of arsenic trioxide and its substitutes for occupational exposure in Murano (Venice, Italy) artistic glass production

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Arsenic trioxide is included in the Candidate List of Substances of Very High Concern (SVHC) and soon it could be subject to authorization under the REACH regulation, because of its properties of carcinogenicity, mutagenicity and toxicity for reproduction (CMR substance). In the district of Murano artistic glass (Venice, Italy), arsenic trioxide has been used since centuries as additive for refining and bleaching melting glass. It is estimated that about 8 tons per year of arsenic trioxide are currently used in Murano glassworks. Small particles of arsenic powder can be easily inhaled by workers; furthermore, when melting the glass mixture at high temperatures, arsenic toxic fumes are released. To reduce the occupational health risks of glass production, the use of suitable substitutes for replacing arsenic trioxide is being explored and cerium oxide and blast furnace slag are among the most promising candidates. A comparative study has been performed with the aim of evaluating the occupational health risks of substitute substances in comparison with arsenic trioxide. Specifically, the objectives of this study are: i. to assess arsenic trioxide health hazard in comparison with cerium oxide and blast furnace slag; ii. to develop for all production phases suitable exposure scenarios for workers, representative of a typical glassworks; iii. to quantify inhalation and dermal contact exposure to arsenic trioxide and its substitutes through the application of different occupational exposure models (i.e., ECETOC worker tool, MEASE and ART) and to compare models' performance for inorganic substances in the selected exposure scenarios; iv. to estimate and compare health risks for glass workers associated to inhalation and dermal contact for the substances of interest. The comparative risk assessment procedure proves to be effective in demonstrating that the use of cerium oxide and blast furnace slag would reduce the health risks associated to occupational exposure. Cerium oxide and blast furnace slag are therefore confirmed as interesting candidates to replace arsenic trioxide in artistic glass production.

RA02-6

Comparative hazard and risk assessment of flame retardants (Prototypical Case Study): ENFIRO

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Several brominated flame retardants (BFRs) have unintended negative effects on the environment and human health. Some of them show a strong bioaccumulation in aquatic and terrestrial food chains, some are very persistent, and some show serious toxicological effects such as endocrine disruption. During the last decade, an increasing number of reports have presented evidence of these negative effects caused by BFRs. A number of BFRs (in particular polybrominated diphenyl ethers (PBDE's), hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBP-A)) can be found in increasing concentrations in the human food chain, human tissues and breast milk [1-4]. Less toxic alternatives appear to be available already but comprehensive information on their possible toxicological effects are lacking. The European Commission-funded project ENFIRO investigates a prototypical case study on substitution options for some BFRs and compares the hazard, risk, fire performance and application of the alternatives versus the BFRs. In addition, a comparative life cycle assessment is carried out. The current paper address the ENFIRO approach, and discuss and explore different comparative (hazard) assessment approaches for halogen free flame retardants (HFFRs) and BFRs, as guidance documents are still lacking on comparative assessments. In conclusion, the US EPA and Green Screen PBT thresholds were more suitable in comparative hazard assessments due to larger number of threshold categories compared to REACH. The challenge in a substitution study is not only to perform a comparative hazard and risk assessment, but also to integrate additional highly important factors, in the case of flame retardants these are fire performance, technical application, life cycle, and costing aspects. The use of decision support systems for this integration was explored.

RA03 - Are Environmental Specimen Banks ready to face future challenges of environmental chemistry and regulatory toxicology?

RA03-1

A fish tissue archive for monitoring chemical pollution in UK rivers. How it operates and its application to EU priority substances

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The UK Fish Tissue Archive

In 2007 CEH and the Environment Agency in England and Wales (EA) began to build a fish tissue archive by collecting 10 fish (generally roach) per year from a number of river sites and storing them long term at -80°C to provide a resource for future retrospective monitoring of pollutants.

Measuring pollutants in biota is often preferable to water because fish and other biota accumulate chemicals both from the water and the food chain over their lifetime,

making the values less variable, more relevant to the potential threat to flora or fauna, or easier to measure because the concentrations are higher.

Environmental Quality Standards (EQS)

EU Directive 2008/105/EC lists freshwater EQS for over 30 substances, but it only includes an (optional) biota standard for 3 (article 3, 2a) requiring member states that use this option to “apply, for mercury and its compounds, an EQS of 20 µg/kg, and/or for hexachlorobenzene, an EQS of 10 µg/kg, and/or for hexachlorobutadiene, an EQS of 55 µg/kg, these EQS being for prey tissue (wet weight), choosing the most appropriate indicator from among fish, molluscs, crustaceans and other biota”

Results

We analysed some of the fish from the UK Fish Tissue Archive for these three substances and found:

Mercury, which was used in many industrial and agricultural applications in the past and is still released as a trace component of fossil fuels and some electrical components was analysed in roach. About half the samples exceeded the EU EQS of 20 µg/kg (median: 23 µg/kg), but even the maximum of 68 µg/kg is about a factor of 10 below the EU food limit of 500 or 1000 µg/kg depending on fish species.

Hexachlorobenzene, a fungicide which is no longer used in the EU, was below the EQS of 10 µg/kg in all fish analysed. Eels had higher concentrations with a median of 1.8 and a maximum of 6.4 µg/kg compared to 0.9 and 4.5 µg/kg in roach.

Hexachlorobutadiene which was in the past used as a solvent in polymer production and as a fungicide and seed dressing and is still generated in small amounts as a by-product, was not detectable in the majority of samples.

Conclusions

- The samples stored in the Fish Tissue Archive are well suited for monitoring of priority substances, especially those for which an EQS has already been set.
- Mercury levels are of some concern in English rivers.
- As the Fish Archive grows it will become possible to determine temporal and spatial trends of these and other substances

RA03-2

Environmental monitoring data: support for an effectiveness assessment and a success control under REACH

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Environmental specimen banks support competent authorities in the assessment of chemicals. Together with national and EU-wide monitoring systems they can give information for the identification of new substances of regulatory concern. Beyond the assessment of individual chemicals, monitoring data can also be used to develop indicators to evaluate the implementation of different chemical regulations by providing information on the impact of chemicals to human health and different compartments of the environment.

By June 2012, the European Commission has to present a first evaluation of the effectiveness of the REACH regulation. Human biomonitoring and environmental monitoring programmes may provide valuable data to underpin this evaluation. In the EU there is a variety of environmental monitoring activities which have been established for various reasons. They are - in different degrees - suitable to indicate the implementation of REACH. A research project - funded by the German Environment Protection Agency (UBA) - analyzed the available monitoring programs (including environmental specimen banks) to identify those who can contribute to evaluate the effectiveness and success of different REACH tasks, starting from specific tasks related to specific parts of REACH (e.g. registration) up to tasks related to the effectiveness evaluation of REACH as a whole.

In order to determine appropriate indicators and methods to evaluate the impact of the European chemicals legislation REACH, a detailed and complete review on ongoing environmental monitoring activities has been conducted. Furthermore, a guidance document for the use of environmental monitoring data with a view to the evaluation of chemicals has been developed and presented. It includes both the company's self-monitoring as well as government monitoring and important regulatory focal points under REACH. Exemplary case sheets on selected chemicals were documented for the monitoring / identification of substances of concern, thereby using the Environmental Specimen Bank (ESB).

Future challenges in regulatory risk assessment go beyond time trends of individual substances. Effectiveness assessments of regulations such as REACH require indicators for the total environmental burden due to the wide dispersive uses of a large number of substances - and its effect on biodiversity. Environmental specimen banks can support such assessments and the elaboration of adequate indicators.

RA03-3

Mercury stable isotopes in seabird eggs from the NIST Marine Environmental Specimen Bank reflect a gradient from terrestrial geogenic to oceanic mercury reservoirs

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The Seabird Tissue Archival and Monitoring Project (STAMP) is a collaborative effort run by the U.S. Fish and Wildlife Service Alaska Maritime National Wildlife Refuge (USFWS-AMNWR) and the National Institute of Standards and Technology (NIST) as a systematic, long-term program to monitor persistent organic pollutants (POPs), mercury, and other contaminants in Alaska's marine environments using seabird eggs. Eggs are collected and processed using established protocols and shipped to NIST's Marine Environmental Specimen Bank (MESB) at the Hollings Marine Laboratory in Charleston, South Carolina, for long-term (decadal) cryogenic storage. To date, over 1600 clutches of eggs have been collected across 1000's of kilometers of Alaska's marine environment. This systematic and broad scale effort has provided a valuable archive of samples to select from for environmental contaminant research. Elevated mercury concentrations ([Hg]) were found in Alaskan murre (*Uria* spp.) eggs from the coastal embayment of Norton Sound relative to insular colonies in the northern Bering Sea-Bering Strait region. Stable isotopes of Hg, carbon (C), and nitrogen (N) were measured in the eggs to investigate the source of this enrichment. Lower $\delta^{13}\text{C}$ values in Norton Sound eggs (-23.3 to -20.0 ‰) relative to eggs from offshore colonies (-20.9 to -18.7 ‰) indicated that a significant terrestrial C source was associated with the elevated [Hg] in Norton Sound, implicating the Yukon River and smaller Seward Peninsula watersheds as the likely Hg source. The increasing [Hg] gradient extending inshore was accompanied by strong decreasing gradients of $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ in eggs, indicating lower degrees of mass dependent (MDF) and mass independent Hg fractionation (MIF) (respectively) in the Norton Sound food web. Negative or zero MDF and MIF signatures are typical of geological Hg sources, which suggests murre in Norton Sound integrated Hg from a more recent geological origin that has experienced a relatively limited extent of aquatic fractionation relative to more oceanic colonies. The association of low $\delta^{202}\text{Hg}$ and $\Delta^{199}\text{Hg}$ with elevated [Hg] and terrestrial $\delta^{13}\text{C}$ values suggested that Hg stable isotopes in murre eggs effectively differentiated terrestrial/geogenic Hg sources from oceanic reservoirs.

RA03-4

Temporal trends in dioxins and dl-PCBs from Baltic herring (*Clupea harengus*)

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The unintentional release of dioxins (dibenzo-p-dioxin (PCDD), dibenzofuran (PCDF), and dioxin like PCBs (dl-PCB)) into the environment was at its peak prior to the 1980s [1]. Since then, extensive measures have been taken to reduce dioxin emissions within the EU e.g., the Helsinki Convention (1974, 1992), the Stockholm Convention on Persistent Organic Pollutants (POPs), the plan for integrated pollution prevention and control (IPPC) [3]. However, dioxin concentrations continue to be higher than expected in Baltic fatty fish. A large proportion of fish caught in the Baltic Sea exceed the limit for marketing fish within the EU [1] (4 pg WHO05-TEQ/g ww ($\Sigma\text{PCDDs}+\text{PCDFs}$); 8 pg WHO05-TEQ/g ww ($\Sigma\text{PCDDs}+\text{PCDFs}+\text{dlPCBs}$)) [2] and thus, ongoing environmental monitoring occurs in many Baltic countries.

Within the Swedish National Monitoring Programme, Baltic herring, *Clupea harengus*, a fatty fish consumed by humans, are analysed yearly for dioxin concentrations. As dioxins are lipophilic, fatty fish tend to bioaccumulate greater concentrations of these contaminants compared to lean fish species. Here, yearly monitoring data of herring from three sites on the east and one site on the west coast of Sweden are presented. The aim of this work is to monitor and detect temporal trends and changes in dioxin concentrations over time in a fish species of importance for human consumption and top marine predators. Results are used to inform policy and regulation makers so dioxin guidelines can be adjusted accordingly.

RA03-5

Temporal trends in human exposure to fluorosurfactants and related chemicals in two cities from Germany

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A total of 420 samples collected from the period of 1981-2009 and from two cities in Germany: Münster (n = 10 samples (5 male and 5 female) per year from 1981-2009 (no sample for 1994), for a total of 270 samples) and Halle (n = 10 samples (5 male and 5 female) per year from 1995-2009, for a total of 150 samples), were analyzed for a suite of fluorinated analytes. The specific targets include current and legacy commercial fluorinated surfactants, such as the polyfluoroalkyl phosphoric acid diesters (diPAPs), the N-ethylperfluorooctanesulfonamidoethanol-based polyfluoroalkyl phosphoric acid diester (SAmPAP), and the perfluoroalkyl acids (PFAAs). Both the diPAPs and various PFAAs, that include PFOA and PFOS, have been detected in human blood. Among the 420 samples analyzed here, the perfluorosulfonates (PFSAs: i.e., C6 (0.053 - 3.83 ng/g)

and C8 (0.318 - 79.0 ng/g)), perfluorooctanesulfonamidoacetates (N-MeFOSAA (<0.0031 - 8.11 ng/g), N-EtFOSAA (0.0058 - 9.00 ng/g), FOSAA (<0.0011 - 8.25 ng/g)), and the perfluorocarboxylates (PFCAs: i.e., C8 (0.176 - 31.7 ng/g), C9 (0.020 - 2.70 ng/g), C10 (0.020 - 0.880 ng/g), C11 (0.003 - 0.555 ng/g) were detected in over 80% of the samples. The long-chain PFCAs (C12 (<0.0017 - 0.056 ng/g) and C14 (<0.0017 - 0.049 ng/g) PFCAs), however, were only detected in 20% of the samples. Temporal trends can be observed for some of the analytes: PFOS concentrations peaked in 1986 (~ 30 ng/g) and reached a plateau before they began to decrease starting in the year 2000 until 2009 (~ 4 ng/g) in Münster samples. A similar decline in PFOS concentrations (1995-2009) was also observed for samples from Halle. The temporal trend observed here for human PFOS contamination mirrors industrial production patterns, with the post-2000 decline in PFOS sera concentrations occurring concurrently with the phase-out of PFOS and related chemicals starting in 2000. A total of 320 samples were analyzed for DiPAPs and SAmPAP, although quantifications had been done only for 6:2/6:2 and 8:2/8:2 DiPAPs due to the limited availability of analytical standards. Further confirmation using matrix matched calibration curve and standard addition will be carried out for 4:2/4:2, 10:2/10:2, and SAmPAPs. The 6:2/6:2 (<0.00048 - 0.762 ng/g) and 8:2/8:2 (<0.0004 - 0.285 ng/g) diPAPs were detected in 46% and 32% of the samples, respectively. No distinct was observed for 8:2/8:2 DiPAP, however, an increasing trend could be observed for 6:2/6:2 DiPAP after year 2000.

RA03-6

New challenges for environmental specimen bank applications - banking for marine mammal health research

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For 33 years the National Institute of Standards and Technology (NIST) has been banking environmental and biological specimens emphasizing long-term storage under cryogenic conditions to minimize sample degradation over time, use of special materials and procedures for specimen collection and handling, to minimize the possibility of introducing an artifact into the sample and thus biasing the analytical results, and well documented and published standard protocols and procedures covering all steps in sampling and banking including collection procedures, sample handling and processing, specimen transport, bank specimen log-in and tracking procedures, and continuous recording of specimen storage conditions over time. Presently, the banking program of NIST is centered at its Marine Environmental Specimen Bank (Marine ESB) that is located at the Hollings Marine Laboratory, Charleston, South Carolina, USA. The greatest number of specimens in this bank with the widest geographic coverage is marine mammal tissues. These tissues are collected and banked primarily for retrospective studies of contaminants in these aquatic animals. The banking of marine mammal tissues began in 1992 as part of the National Oceanic and Atmospheric Administration's (NOAA's) Marine Mammal Health and Stranding Response Program (MMHSRP). In 2003, NIST began collaborating with organizations and researchers on the U.S. east coast in studies to assess the health of bottlenose dolphins, *Tursiops truncatus*. This health assessment and banking approach is being expanded to other marine mammal species and to other regions of the US. Based on the experience gained through the bottlenose dolphin health assessment project and an ongoing marine mammal studies related to the recent Gulf of Mexico oil spill incident, NIST is working with NOAA and its collaborating partners to establish the marine mammal specimen bank as a major resource of samples for integrative animal health research. This expansion will be emphasizing the banking of specimens for wildlife disease studies, determining exposure to biotoxins, and developing health biomarkers, in addition to determining contaminant exposures. This expansion is requiring additional banking expertise, identification of additional kinds of matrices to bank, and the storage conditions required for long-term banking. These and other aspects of the expansion will be discussed.

RA04 - Established and emerging footprints - striving towards a valid and comprehensive support for decision-making processes

RA04A-1

Midpoint and endpoint indicators for global scale terrestrial acidification: a dilemma for decision-making

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For some impact categories, such as climate change, the link between midpoint and endpoint indicators is based on a proportionality factor, which adds relevance, but does not add further discrimination between emitted pollutants. However, for other impact categories, such as acidification, all three modeling steps: fate, sensitivity and effect, add discrimination between chemicals in addition to relevance. Whether to make a decision based on a midpoint or endpoint indicator is an old debate. This paper aims at contributing to this discussion by showing for the first time, the lack of correlation between midpoint and endpoint indicators for acidification, and in light of these results, further discuss the relevance of using midpoint and/or endpoint indicators and the implication for decision makers. Midpoint CFs [(mol [GREEKX] L⁻¹) [GREEKX] ha [GREEKX] (kg S or N emitted)⁻¹ [GREEKX] yr] results from the multiplication and subsequent summation of an atmospheric fate factor (FF) and a soil sensitivity factor (SF). Endpoint CFs [ha [GREEKX] (kg S or N emitted⁻¹) [GREEKX] yr] were obtained by adding a biome specific vegetation effect factor (EF) to the midpoint CF. CFs were obtained at a worldwide 2°x2.5° (latitude x longitude). FF [(kg S or N deposited) [GREEKX] yr⁻¹ [GREEKX] (kg S or N emitted⁻¹) [GREEKX] yr] describes the atmospheric impact pathway from the emission location *i* of pollutant *p* to the corresponding deposition in the receiving soil *j*. SF [(mol [GREEKX] L⁻¹) [GREEKX] (kg S or N deposited)⁻¹ [GREEKX] yr [GREEKX] ha] translates the change in soil pH according to a change in emission. Changes in the vegetation EF [(mol [GREEKX] L⁻¹)⁻¹] is a function of biome specific coefficients. The midpoint CF quantifies the concentration of H⁺ ions (evaluated through pH) and assumes that any change in pH will have consequences on the receiving environment while the endpoint CF evaluates the decrease in species richness of the biome vegetation related to this change in pH. Comparison of results between the midpoint and the endpoint assessment showed the importance of introducing a further modelling step to highlight biomes having vegetation with lower resistance to pH change. They also showed a medium-low correlation (R² = 0.60) between midpoint and endpoint indicators. This work thus raises questions about which indicator is more relevant for terrestrial acidification and/or should be used since a further modelling step from midpoint to endpoint is no longer synonym of proportionality between the elementary flows and thus LCA conclusions can be inverted.

RA04A-2

Global life cycle impact assessment on marine eutrophication

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As part of the ongoing EU FP7 project LC-Impact (www.lc-impact.eu) new life cycle impact assessment (LCIA) methods are going to be developed and tested on industry cases. Among the life cycle assessment (LCA) impact categories in focus are aquatic eutrophication. As related to especially the marine environment very few and restricted attempts have yet been done on trying to include eutrophication in LCA. The aim of LC-Impact is to develop both a global and a spatial (and temporal) differentiated model, as both central fate processes, sensitivities of receiving environments (e.g. differences in limiting nutrient and variations in this over the year) and the resulting damage can show important spatial variations. Both midpoint and endpoint (damage) modeling are to be included and the aim is to base the damage modeling on dose-response curves expressing the correlation between the (increase in) nutrient concentration and the potentially affected fraction of species in the marine ecosystem. This paper presents the first draft on the midpoint model for global marine eutrophication due to nitrogen emissions for the emission sources "fertilizer in agriculture", "manure in agriculture", "N-fixation in agriculture", "air emissions of NH₃", air emissions of NO_x and "water emissions" including direct sewage water emissions and emissions after sewage treatment.

RA04A-3

Life-cycle Assessment of biofuels for transportation: understanding the effects of scale

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Several types of biofuels are being used around the world, with typically different biomass feedstocks and pathways of production. Biofuels substitute for gasoline or diesel in transportation. The largest scale conversion of biomass into biofuel is currently taking place in the United States, supplying about 7% of the fuel market by volume. Corn ethanol carries a number of environmental problems. Instead, alternative biomass feedstocks are being considered at a scale not seen ever before. We quantify the effects of scale on the production and use of ethanol. In contrast to the majority of extant research, which focuses on a unit of transportation biofuel but ignores the economic, industrial, and environmental feasibility of delivering billions of units to the final customers, we address the availability of land, the needed agricultural practices, refining operations, and storage and distribution of biofuels at industrial scale. We also study the environmental and human health effects of combusting ethanol in vehicles on large scale. We summarize the experiences and environmental effects of large-scale biofuel production and use in Europe and Brazil, and contrast them to the proposed 40 billion liter biofuel federal mandate by the year 2020 in the United States, partially to be met by conversion of cellulosic matter into ethanol. The crops of choice will be grasses, *Miscanthus x giganteus* and switchgrass, and crop residues such as corn stover. Life-cycle assessment is used as the framework to study the effects of scale throughout the entire life-cycle of biofuels. Preliminary results indicate that growing biomass for fuel at an industrial scale presents a number of unique challenges as well as opportunities. We show that economies of scale make a decisive difference between industrial-scale and lower-scale ("boutique") biofuels. For the United States, we show that making 40 billion liters of biofuels by a deadline only 9 years away requires careful environmental, technical, and economic planning. We present scenarios for land conversion, trying to answer the question where and on what kind of land will biomass of this scale be grown. We quantify soil organic carbon emissions from massive biomass cultivation, and the need of crops for water, fertilizer and biocides. We assess the greenhouse gas, water consumption, and criteria air emissions of converting cellulosic biomass into ethanol at industrial scale.

RA04A-4

Accounting for greenhouse-gas emissions in LCA from the degradation of chemicals in the environment

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Chemicals are often released into to the environment at their end of life. As they degrade, emissions of CO₂ and other greenhouse gases (GHG) occur, which are often excluded from LCA studies. This work addresses this methodological gap by presenting a method to account for (GHG) emissions from degradation, namely carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O). In addition, carbon accounting rules for biogenic and fossil carbon are discussed and several options are presented that provide a consistent framework for LCA studies.

A Level III fugacity model is proposed to estimate the multimedia distribution and degradation of chemicals released to the environment. The environmental distribution, along with emission factors and assumptions for each compartment (air, water, soil, sediments) is used to calculate emissions of CO₂, CH₄ and N₂O following initial release of the chemical to either the air, water or soil compartments. The method is described and applied to nine organic chemicals. GHG emissions representing the end-of-life stage of these nine chemicals are shown to be significant when compared with the corresponding cradle-to-gate emissions, particularly for those situations where there is no prior waste treatment phase, such as in a wastewater treatment plant. Chemical composition is shown to be important and for three of the nine chemicals and release scenarios the degradation emissions exceeded the cradle to gate phase. For chemicals released directly to water, the total GHG emissions are comparable to those expected if the chemicals are treated in a wastewater treatment plant, including the emissions from energy use of the latter.

In terms of carbon accounting, we show the need to account for biogenic carbon sequestration if a chemical does not degrade at its end of life, as well as the need to correct the Global Warming Potential (GWP) of methane, depending on whether it is of biogenic or fossil origin.

In conclusion, the method presented enables the accounting of GHG emissions that are currently neglected in many LCA and carbon footprint studies. The model has been parameterized for a generic environmental scenario but it can be adapted for more site specific conditions. The main limitation and source of uncertainty is the potential lack of chemical-specific data required to model environmental fate of a chemical. Also, the method uses generic assumptions and emission factors, that might not be appropriate to describe local conditions.

RA04A-5

Water footprint and life cycle assessment frameworks: synergies and hurdles

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Water footprinting has come a long way in the past 10 years, from simple water volume inventories, to scarcity assessment, to damage oriented LCIA modelling of several impact pathways up to endpoints. With this increase of methodological developments and applications, the scientific and industrial communities are now debating about the ultimate meaning(s) of a water footprint. This paper aims to evaluate the synergies and hurdles between the so far developed water footprint approaches, with the traditional LCIA midpoint-endpoint framework. We therefore propose to demystify: a) Standalone versus full life cycle assessment water footprint; b) Single indicator versus multiple indicators; c) Midpoint versus endpoint assessment; d) Impacts of water use versus impacts on the water resource and e) Availability of water resource versus water pollution. This discussion is illustrated with a simplified example of aluminium production, where available methods are compared and synergies and contradictions are presented. The review by Kounina et al. identified several methods for impact assessment in each of the areas of protection: human health, ecosystems and resources. However, these solely assess the potential impacts related to water availability (impacts from water use). Potential impacts from pollution of the resource water (impacts on the water resource) are considered by the traditional LCIA models of, for example, aquatic ecotoxicity, eutrophication, etc. These two distinct concepts can however lead to common endpoints and we propose to group them into three aggregation level: I) a Water Availability Footprint (WAF), a standalone method which address availability issues from water use, including lowered availability from pollution (but no other impact related to water pollution), II) a Water Footprint Assessment (WFA) as standalone, which includes all impacts of an activity on the water resource or III) as part of an LCA methodology. The results of the three aggregation levels applied to the aluminium illustrative example show that the WFA profile is fully compatible to the more comprehensive LCA profile, the impact on water resource being a fraction of each LCIA damages on human health, ecosystem quality and resources. The WFA profile, can be further disaggregated putting in perspective the relative contribution of each impact pathway to the overall impacts on the water resource within each area of protection.

RA04A-6

Chemical footprint from point sources in Sweden

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This study has identified a method to quantify the chemical footprint from point sources with data from the E-PRTR (European Pollutant Release and Transfer Register) together with USEtox. USEtox is often used in Life Cycle Impact Assessment.

There is an EU regulation since a few years saying that industrial facilities (e.g. industries) emitting over certain thresholds have to report to a register, E-PRTR, annually. The industrial facilities covers 65 economic activities within the following 9 industrial sectors: energy, production and processing of metals, mineral industry, chemical industry, waste and waste water management, paper and wood production and processing, intensive livestock production and aquaculture, animal and vegetable products from the food and beverage sector, and other activities. Data is provided in the register for 91 pollutants falling under the following 7 groups: greenhouse gases, other gases, heavy metals, pesticides, chlorinated organic substances, other organic substances and inorganic substances. Data was collected from the EEA website, where all E-PRTR data is available for all EU27 countries. The emissions were listed by amount to water and air respectively.

A calculation on the impact is made using the USEtox method as implemented in SimaPro. The USEtox model is an environmental model for characterization of human and ecotoxic impacts in Life Cycle Impact Assessment and for comparative assessment and ranking of chemicals according to their inherent hazard characteristics. In this study the impacts human toxicity (cancer and non-cancer) and ecotoxicity are included. The calculations are performed in SimaPro. The results are given in CTU (comparative toxic units).

The results are for the year 2008. Sweden has delivered emissions for 53 substances, some to air or water and some to both. The total emission to air is very much larger than the total emission to water. The expected results are: an aggregated measure of the toxicity from the sources included in E-PRTR, a calculation on contribution to toxicity by different industry sectors, information on each substance's contribution to toxicity, identification of the most important substances. For these four the results are divided in contribution to human toxicity and to ecotoxicity. This study makes it possible to discuss how useful this method is to quantify the chemical footprint by using data from E-PRTR and the USEtox method.

RA04B-1

Probabilistic environmental hazard assessment of implementing green chemistry property design guidelines to reduce acute and chronic aquatic toxicity

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One of the Twelve Principles of Green Chemistry emphasizes the need to synthesize safer chemicals. These chemicals should be capable of performing their desired function but would be designed to elicit minimal toxicity. In support of this effort, two recent studies by Voutchkova et al have explored the relationships between chemical properties and acute or chronic toxicity as measured through standardized OECD and EPA protocols. Mechanistically-rationalized guidelines were derived resulting in two practical design guidelines: acute and chronic toxicity could be minimized if compounds had an octanol-water partition coefficient (log Pow) below 2 and HOMO-LUMO gap (dE) greater than 9 eV. In this study, we examined the potential utility and effectiveness of these design guidelines to reduce acute and chronic aquatic toxicity of common industrial chemicals using probabilistic environmental hazard assessment (PEHA). Chemical Toxicity Distributions (CTDs), a type of PEHA modelling approach, are ideal for predicting the probability of encountering compounds or toxicological thresholds when information is lacking for new chemicals. CTDs were performed: 1. to predict the likelihood of encountering industrial chemicals exceeding established US EPA thresholds of standardized acute and chronic toxicity to algae, cladoceran and fish models; 2. to predict the likelihood of exceeding these thresholds if chemical safety guidelines were followed; and 3. to examine acute and chronically toxic chemicals, chemical classes and modes of action of chemicals that may not be "designed out" by chemical property guidelines. For example, in the absence of chemical design guidelines, our model predicts that 14.5% of chemicals would be classified by the EPA as being of "High Level of Concern" for acute toxicity (LC50 of 0-1 mg/L) to the fathead minnow. However, if log Pow and dE guidelines were employed during chemical development, only 3.3% of industrial chemicals are predicted to be of High acute toxicity concern to the fathead minnow. If the two green chemistry design guidelines put forth by Voutchkova et al are employed, the present study predicts that reduced acute toxicity to the fathead minnow could be achieved for over 10% for industrial chemicals classified with High acute toxicity.

RA04B-2

Biodiversity footprinting - quo vadis?

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Biodiversity loss, and its potential impacts on human welfare, are an increasing environmental concern, and should be assessed in product assessments, in order to provide

adequate support to decision-makers. Likewise, there is an upcoming trend to quantify environmental impacts of products in terms of so-called footprints, which should address biodiversity effects in addition to the conventional carbon and water footprints. However, biodiversity is a multifaceted concept with strong variation in time and space, which makes it difficult to assess. Some current methods are based on the unit “potentially disappeared fraction of species” (PDF), but the scale (e.g. biodiversity loss at the local, regional or global level) and choice of which species should be protected are not consistently defined across impact categories. Therefore, impacts to biodiversity at local, regional or global scales are often aggregated, leading to misinterpretations of LCA results. Here, we attempt to provide a framework to guide methodological choice. Clarifying why we are concerned about biodiversity loss is the first step towards determining what we should be measuring. We distinguish between valuing biodiversity due to intrinsic value or due to its utility for mankind. For the first, enough primary habitat needs to be conserved and the human-modified landscapes need to be managed to avoid global extinction of species. The utility can be manifold and the concept of ecosystem services could serve as a starting point that helps to structure, classify, and measure different functions that ecosystems provide for humans. We also highlight value judgments involved in assessment frameworks, indicators and weighing schemes. To assess cause-effect chains of biodiversity loss, multivariate models, meta-analysis of literature of cause-effects from field surveys, extrapolation of lab experiments or expert opinion can be used.

RA04B-3

Valuating ecosystem goods and services in LCIA

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In LCIA models, resource assessment is centred on the notion of scarcity: depletion is evaluated using a static perspective by comparing a resource's rate of abstraction versus the available stock and its rate of regeneration, or by quantifying the marginal extraction cost. As a result, a limited number of resources (essentially fossil fuels and minerals) is included: ecosystem goods and services (EGS) are basically ignored, despite their crucial role. Dynamic accounting is a prerequisite to consistently embrace the actual pressure of human activity on renewable resources, their marginal rate of regeneration and the interdependence of their production patterns. Hence, ecological modelling can be a way to build a consistent and practical framework of characterization of EGS in LCA. The GUMBO model details the internal behaviour of the global natural system (geobiosphere) and its interactions with the anthroposphere; it is adjusted with key metrics measured during the 20th century to reflect global dynamics. The model delivers the economic values of human, social, built and natural capitals (i.e. EGS) according to user-defined scenarios for the societal mankind during the 21st century. The use of monetary units enables direct comparisons among 'capitals' and is a strong vector to communicating results. However, it drives to a utilitarian, end-user perception of EGS' usefulness, disregarding the role of indirect EGS and the past natural 'investment' to this capital. Therefore, other metrics developed in the field of sustainability indicators may be tested, such as emergy. Acknowledged to be fruitful EGS accounting in LCA, the emergy method provides a measure of the global-scale geobiosphere work with a common physical numeraire (equivalent joules of solar energy, seJ). We expect GUMBO to improve consistency in the emergy evaluation of global natural processes and resulting Unit Emergy Values (UEV) of EGSs, which can be used to obtain complementary (physical) information to the (economic) characterization of EGSs in LCA. Although regionalization of this framework remains unclear, it is expected to be highly representative at global scale and open the road to a comprehensive, ecology-oriented perspective in the evaluation of the natural capital and its use by human systems. The aim of this presentation is to illustrate the methodological approach and its preliminary outcomes.

RA04B-4

Exergetic footprint as indicator to assess the environmental sustainability of processes

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Exergy analysis has two key attributes for being used as an environmental indicator: first, given that the environment is used as a reference state, exergy is a measure of any thermodynamic deviation with respect to its normal state; second, it allows comparisons between inflows and outflows, regardless they are mass or energy streams, using the same physical units (exergy) for their analysis.

Besides, the exergetic footprint (ExF) bears in mind the exergy that still remains in products and highlights the use of exergy as a convenient unit of measure and comparison. In this paper, the (ExF) was used as a resource/waste accounting indicator for the environmental assessment of a production process. The proposed methodology was applied to a wood-based particleboard production process from a factory located in Galicia (NW Spain).

The study was proposed as a gate to gate analysis. The incoming streams were categorised as raw materials, secondary materials and energy sources, while the output streams were the generated waste and the produced particleboard. 1 m³ of produced particleboard was selected as functional unit. Raw material for the production process was wood from pine (75%), black poplar (10%), mixed wood (5%) and recycled wood waste (10%). Additionally, with the aim of assessing the influence of the use of recycled wood as raw material on the ExF, some scenarios of the production process were built considering and increasing ratio of recycled wood used until the complete substitution of the roundwood.

The total exergy consumption for the particleboard manufacturing process was determined as the sum of each input category exergy plus the exergy content of wastes, reaching a value of 17.56 GJ per functional unit. Meanwhile, to determine the ExF the exergy that still remains in the produced particleboard was also considered, and a value of 3.5 GJ per functional unit was obtained. For the scenarios built, CEx ranged from 17.76 to 15.73 GJ·m⁻³ particleboard and the ExF from 3.69 to 1.66 GJ · m⁻³, for 0 to 100% of recycled wood wastes employed.

The material resources, especially wood material, were the main contributors to CEx and ExF, as it had been identified by the ecological footprint in a previous work. The results suggest that a clear improvement in the sustainability of the process could be attained by increasing the feasibility of using different waste materials in the design of the particleboard.

RA04B-5

Thermodynamic resource indicators and footprint in LCA: a case study of titania in China

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LCA has standardized methods for assessing emission impacts but no comparable methods for the accounting or impact assessment of resource use. This study contributes to the existing research by offering a comprehensive comparison of different resource indicators, in particular those based on thermodynamics, and testing them in a case study of titania in China.

The system boundary for resource indicators and the case study is defined as a thermodynamic hierarchy at four levels. Seven resource indicators are applied. Four are thermodynamics based: cumulative energy demand (CED), solar energy demand (SED), cumulative exergy demand (CExD), and cumulative exergy extraction from the natural environment (CEENE), and three have different backgrounds: abiotic resource depletion potential (ADP), environmental priority strategies (EPS), and eco-indicator 99 (EI99). The foreground inventory data has been collected through on-site interviews and visits. The background inventory data are from the database ecoinvent v2.2. Characterizations factors are based on CML-IA database. Computations are with the CMLCA software.

Results show that the scores of resource indicators of the chloride route for titania system are lower than that of the sulphate route except in terms of SED. Within the four thermodynamic indicators for resources, CED, CExD, and CEENE have similar scores while their scores are five orders of magnitudes lower than the SED score. Atmospheric resources do not contribute to the SED or CEENE score. Land resources account for a negligible percentage to the SED score and a small percentage to the CEENE score. Non-renewable resources have a dominant contribution to all seven resource indicators. The global production of titania would account for 0.12% and 0.14% of the total anthropogenic non-renewable resource demand in terms of energy and exergy, respectively. The entropy footprint of global titania is 5.2 E+07 m².

We demonstrate the feasibility of thermodynamic resource indicators and recommend CEENE as the most appropriate one for accounting and characterizing resource use. As for the three non-thermodynamic resource indicators, they take the different resource issue as their key problem and have higher environmental relevance in terms of expressing the resource scarcity and depletion than the thermodynamic ones. As the conceptual basis for the several indicators differs fundamentally, different cases might well show more diverging outcomes.

RA04B-6

European guidelines for measuring the environmental footprint of products and organisations

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In close co-operation, DG Environment and the Joint Research Centre of the European Commission are developing European guidelines for measuring the Environmental Footprint of products (covering goods and services) and organisations.

The objective is to support decision-making processes in business and policy with a comprehensive multi-criteria set of indicators based on the robust and quality assured measurement of environmental performance.

The documents provide guidance on how to calculate a Product Environmental Footprint (PEF) and an Organisation Environmental Footprint (OEF), as well as how to create product category or sector specific requirements for use in Product Footprint Category Rules (PFCRs) or Organisation Footprint Sectorial Rules (OFSRs).

Each requirement in the PEF and OEF guidelines has been chosen taking into consideration the recommendations of existing environmental accounting methods. Although existing methods align on some of the guidance they provide, discrepancies and/or lack of clarity remain on a number of important decision points, which significantly reduces the consistency and comparability of the results. In some cases, it was necessary to go beyond the level of guidance provided in existing documents. An example is the coverage of impact categories and the models used for calculation.

For the selection of relevant impact categories, a “default list” of 14 midpoint Life Cycle Impact Assessment (LCIA) categories is provided together with models and characterisation factors. The default list is based on the International Reference Life Cycle Data System (ILCD) Handbook recommendations for LCIA. If it can be demonstrated that a certain impact category is not relevant for the product or organisation under study, it can be neglected. If it can be demonstrated that other impacts are relevant or that for a specific impact category a better model is available, this information can be provided under “additional environmental information”. However, for comparability reasons, the impacts have to be calculated as “baseline” according to the model from the default list. In the creation of the guidelines, the right balance had to be found between different objectives. For instance, to ensure a more robust and reproducible decision support compared to existing approaches, comparability was often given priority over flexibility. A balance also had to be found between latest scientific developments and practicability and required efforts.

RA05 - Classification and risk assessment of metals and inorganic substances

RA05-1

Speciation in the transformation/dissolution examination of tungsten and antimony metal and compounds

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Speciation is held to be a key factor in the human health and environmental effects of metals in solution. In this study, we present the results of the examination at pH 6 and 8.5 of the T/D (Transformation/Dissolution) characteristics of W (tungsten) metal and several of its compounds, measuring the concentrations of total dissolved W and the tungstate ion, WO_4^{2-} . We have also determined the T/D behaviour of Sb (antimony) metal and several of its compounds with respect to total dissolved Sb, and for the 1 mg/L loadings, the speciation of dissolved Sb(III) and Sb(V). We show how the T/D data have been used to derive UN GHS (United Nations Globally Harmonized System of Hazard Classification) or EU CLP (Classification, Labelling and Packaging) outcomes. We used high performance liquid chromatography and inductively-coupled plasma mass spectrometry to analyse for the corresponding dissolved species. For the W compounds examined, the T/D data revealed that all dissolved W existed as WO_4^{2-} . $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$, ammonium para- and meta-tungstate were readily and completely soluble, yielding measured W concentrations of about 67 mg/L, and WO_4^{2-} concentrations in the range 75 to 93 mg/L with 100 mg/L loadings, which correspond to all W dissolved as WO_4^{2-} . Compared to an acute ERV (Ecotoxicity Reference Value) of 31 mg/L, their 168-hr T/D concentrations would classify $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$, APT, AMT, WO_3 and $\text{WO}_{2.9}$ as GHS Acute 3, but would not classify them under the EU CLP scheme. W metal and tungsten carbide would not classify under either scheme. Regarding $\text{NaSb}(\text{OH})_6$ and Sb_2O_3 , Sb dissolved entirely as Sb(V) and Sb(III), which are its respective valences in these compounds. With Sb metal, Sb dissolution was primarily as Sb(III). For Sb_2S_3 , the speciation data suggested a significant degree of Sb(III) oxidation to Sb(V) over 28 days. For NaSbO_3 , Sb dissolved as Sb(V), since antimony is pentavalent in this compound. For $\text{Sb}_2(\text{C}_2\text{H}_3\text{O}_2)_3$, the $\text{C}_2\text{H}_3\text{O}_2^{2-}$ ligand appeared to stabilize Sb(III) in solution, with only a moderate amount of oxidation. Similar comments apply to the CH_3COO^- ligand in $\text{Sb}(\text{CH}_3\text{COO})_3$, particularly at pH 6. On the other hand, Sb(III) released from SbCl_3 was readily oxidized to Sb(V). With pentavalent Sb in Sb_2O_3 , the speciation data indicated an initial small amount of Sb(III) that oxidized to Sb(V) over the 28 days. A comparison of the T/D data with the 6.9 mg/L acute ERV for dissolved Sb revealed that none of Sb metal and its compounds would classify under the EU CLP.

RA05-2

Transformation/dissolution of copper alloys: comparison of two anti-abrasion devices for massive sample testing

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In view of deriving the environmental classification of copper alloys, the potential for metal releases from a range of copper alloys was assessed using the OECD Transformation/Dissolution (TD) test (2001).

Metal releases from metals in massive and powder forms are a function of the surface area exposed to the media. Consequently, to assess comparative metal releases from copper alloys, there is a need to standardize the surface area exposed and to preserve the integrity of the surface as well as the corrosion and passivation layers formed. The aim of this study was to define a protocol suitable to TD testing of massive forms of copper alloys.

Accordingly, two anti-abrasion devices were designed to protect the integrity of the alloy samples. The first device involves two polypropylene (pp) wheels attached to the ends of an alloy wire sample, to avoid metal contact with the vessel walls. The second device is an epoxy jacket mounted around the sample; in this case the alloy surface exposed to the media was polished to assure a known and homogeneous surface quality and avoid effects of corrosion and passivation, proper of sample aging under uncontrolled environmental conditions.

The non-abrasion devices were applied to nine different copper/zinc and copper/tin alloys and the samples were subjected to 7 days TD at pH 6.

The T/D results of samples embedded in epoxy resin showed lower experimental noise compared to those for the polypropylene wheels and is therefore considered as a more appropriate methodology for transformation/dissolution testing of massive forms.

Microscopy studies of samples surface concluded that defects in the alloy surface during the wire production were responsible for the higher variability in the T/D results of the polypropylene wheels protocol, compared to the epoxy polished mounted sample protocol.

The surface-specific metal releases from the alloy samples, using both anti-abrasion protocols, usually increase linearly with time, with faster dissolution rates for lead and zinc and lower rates for copper and nickel. The dissolution kinetics of lead in three samples, demonstrate a decrease in the metal release ratio as the tests progress, suggesting a depletion of lead from the alloy surface. For copper, the main component of the alloys, the dissolved copper concentrations measured in the alloy were between 0.04 and 2.05 $\mu\text{g}/\text{mm}^2$, compared with the values observed from pure copper samples (0.14 to 0.41 $\mu\text{g}/\text{mm}^2$).

RA05-3

Transformation/dissolution of copper concentrates: effect of mineral composition on metal solubility

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In view of deriving the environmental classification of copper concentrates, being complex, heterogeneous and variable inorganic substances, this study aims at understanding the relation between the elemental/mineralogical composition of some selected copper concentrates and the Transformation/Dissolution behaviour of these concentrates.

The total elemental composition of five copper concentrates, obtained from different copper ore bodies and assessed demonstrated the presence of Cu, Fe, Zn, Pb, Cd, Co, As in various concentrations. The mineralogy of the samples, determined by X-ray diffraction and microscopy studies, revealed the presence various copper minerals: Bornite, Chalcocite, Chalcopyrite, Covellite, Digenite, Enargite and Tennantite.

The release rates of Cu, Pb, As, Zn, Co and Cd from the five copper concentrates and the 7 minerals identified in the copper concentrates were subsequently assessed, during short term (7 days) Transformation/Dissolution tests (TD) at pH 6 and a loading of 100 mg/L, in accordance to the OECD Transformation/Dissolution protocol (TDp) and the standard operating procedure.

The results demonstrate that during TD, especially copper, lead, zinc and arsenic and sometimes cobalt and cadmium are released from the concentrates. Relatively lower release rates were observed for copper and arsenic (0.2-4%) and the highest release rates were observed for lead (7- 35%). The detailed kinetics from the T/D tests show that the metal release rates generally decrease as a function of time.

Copper releases from copper minerals are lowest for Chalcopyrite (0.3% copper release during 7 days TD) and highest for Chalcocite (5 % copper release during 7 days TD). From the data, it can be concluded that it will not be possible to derive one release or one environmental classification entry applicable to all copper concentrates. Comparison of release data among concentrates and their mineralogy clearly indicate that the metal release rates depend on the elemental and mineral composition of the copper concentrate. The results further indicate that, as demonstrated for copper, that the metal releases from copper concentrates can be predicted from the mineral composition and the mineral- specific metal releases. The releases of lead remain difficult to interpret.

RA05-4

Metal classification using a unit world model: assessing removal rates from the water column and remobilization from sediment with TICKET-UWM

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European Union (EU) regulations pertaining to Classification, Labelling, and Packaging (CLP) of chemical substances follow the United Nations Globally Harmonized System (UN GHS). Under this system, speciation, partitioning, and precipitation are recognized as critical elements in metal hazard classification. Guidance on environmental transformation of metals provided in the EU CLP guidance document includes a provision for demonstrating removal from the water column to assess the “persistence” or lack of degradation of metals. In analogy to organic chemicals, “rapid degradation” for metals requires greater than 70% removal within 28 days. However, unlike organic chemicals, where removal from the water column occurs via degradation, metal removal occurs through changes in speciation (partitioning and precipitation) followed by sedimentation which transfers metal to the sediment. Therefore, in line with the GHS guidance, “rapid degradation” for metals requires one to demonstrate not only rapid loss from the water column, but also limited remobilisation potential from sediment.

A unit world model for metals in lakes, TICKET-UWM, has been developed that considers key processes affecting metal transport, fate, and toxicity including complexation by aqueous inorganic and organic ligands (e.g., DOC), adsorption to particulate organic carbon (POC), binding to biological receptors (biotic ligands), and transport of dissolved metals and solids between the water column and sediment. The TICKET-UWM was used to assess the rate at which metals (Cu, Pb, Zn, Ni, Co, and Cd) are removed from the water column in a generalized lake system through partitioning and settling. The model was also used to assess metal speciation changes in the sediment and the potential for metal remobilization from sediment.

Model results indicate that, in most cases tested, greater than 70% of the metal added to the water column was removed within 28 days. Results also suggest the potential for remobilization of metals is limited, particularly when acid volatile sulfide (AVS) is present to precipitate metals as metal sulfides.

RA05-5

A BLM probabilistic approach to integrate variability in the derivation of Water Quality Criteria at regional and local scales

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The biotic ligand model (BLM) is one of the more promising models allowing to derive Water Quality Criteria (WQC) based on true bioavailable fractions of metals.

However, several physico-chemical parameters must be assigned for running BLMs (i.e. pH, major cations, DOC) and the operational use of BLMs can suffer several flaws because of:

(i) the lack of comprehensive data. National monitoring programs of water bodies were indeed not originally designed for BLMs application and, as a consequence, some datasets can be incomplete;

(ii) natural variability. Rivers and lakes can indeed show great spatial and temporal variations. So far however, only single values were generally considered for describing variables of concern, and the high natural variability with time and along a river watershed was ignored.

The main objective of this study was then to investigate the potential of probabilistic approaches able to capture the uncertainty of variables of concern and the incompleteness of datasets for BLMs application on large scales. The methodology was tested on the Loire river watershed for copper, where different spatial scenarios were considered.

Data needed for running the selected BLM model were extracted from the database freely put available on the web by national Water Management Agencies. Considering all data available for pH, DOC and Ca concentration, normal Probability Density Functions (PDFs) were fitted.

Data available for copper showed that a significant fraction of dissolved concentrations (about 50%) were below the limit of detection. In order to impute realistic values to these non-detects, 'distributional' methods were used. In addition, probabilistic risk assessment was also conducted at local scale (i.e. considering data collected at a given monitoring station only). In such a case, the number of available data can be reduced dramatically and frequentist statistics can be more subject to criticism. To overcome the problem of data scarcity, a Bayesian approach was proposed.

Results showed that PNEC values calculated with or without probabilistic approaches significantly differ, e.g. if non-detects are ignored, the risk index is above one (situation at risk), while it is below one if a distributional approach is considered (situation at no risk). Similarly, in case of scarcity of data at local scale (i.e. at a given monitoring station), frequentist and Bayesian approaches led to results significantly different.

RA05-6

Accounting for both local aquatic community composition and bioavailability in setting local quality standards for metals

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Recent years have seen considerable developments in the ability to make water quality standards for trace metals more ecologically relevant by taking account of the effect of local water chemistry conditions on bioavailability. This prevents situations where a different level of risk is considered to acceptable at different sites due to changes in bioavailability which are not accounted for in the standard. We describe preliminary efforts to address an additional issue in the development of water quality standards which are specific to particular locations, by taking account of the composition of the local ecological community (the ultimate protection objective). This has occasionally been addressed through the use of field measurements to derive species sensitivity distributions (SSD) in sediments. An alternative approach, which combines a quality assessed ecotoxicity dataset with field measurements of the abundance of benthic macroinvertebrates to derive an SSD based on the community which is either expected to be present, in the absence of anthropogenic pressures, or the community which is present at the sites. Site specific standards are derived for zinc in an area impacted by historic mining activities. Site-specific targets for zinc, based on the macroinvertebrate ecology predicted or observed at a site, can be derived and can result in improved compliance compared to the use of both conventional and bioavailability-based EQS. In addition to zinc, the approach is likely to be applicable to other metals and possibly other types of chemical stressors (e.g. pesticides). However, the methodology for deriving site-specific targets requires additional development and validation before they can be robustly applied during surface water classification.

RA06 - Contaminated sediments in a changing environment

RA06-1

Bioavailability and beneficial use as primary demands for a management guidance of contaminated dredged sediments

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Management of contaminated sediments is a key issue in order to establish a sound development of the Baltic Sea and contributing to the EU Strategy for the Baltic Sea Region (EUSBSR). This issue is of utmost importance according to Helcom and addressed by the BSR funded project SMOCS (Sustainable Management of Contaminated Sediments). The main delivery of SMOCS comprises a guideline, including tools for assessment of sustainability, and decision support.

The purpose of this lecture is to present the draft guideline on sustainable management of contaminated sediments for dredging projects all around the Baltic Sea. This proposal is a result of the joint EU-project SMOCS under consideration of actual national and international regulations, integrating other projects as well as interests of stakeholders. For example, the participants of a workshop preferred a guidance document giving new ideas and an overview of options for dredged material management. Exact definitions for the condition of dredged material (when to call dredged material "clean", "contaminated" or "hazardous") and action levels were required.

Emphasis was given to fulfill political demands as to avoid waste production. Therefore it was primarily recommended to assess the feasibility of beneficial use of the sediments. Bioavailability concept for the derivation of sediment quality standards has a challenge to foster this approach, because not the total amount of contaminants is of importance rather than the biological available part. Such risk assessment based on laboratory or in situ studies meets the request of the European Water Framework Directive to develop a toxicity-based bioavailability model to estimate the risk of sediment-associated contaminants.

The purpose of an LCIA probably in combination with a Risk Assessment is to ensure that dredging activities are performed in an environmentally acceptable manner, use sound engineering techniques, which they are economically warranted and take sufficient consideration of long term effects. The choice of appropriate indicators is given in detail as part of a Decision Support Tool.

RA06-2

Natural attenuation in sediments

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Due to polluted groundwater in urban areas, sediments in urban water streams become polluted as well. This can cause pollution of surface waters. Natural attenuation of chlorinated hydrocarbons in sediment can be used to sustainably manage or remediate groundwater pollution. Due to the change in remediation procedures in the Netherlands from site specific remediation to area specific remediation, an approach based on natural attenuation will be increasingly considered. Provided that the risks of this approach can be controlled, natural attenuation processes in sediment can be a valuable contribution to area specific management and remediation plans.

However, there are still some knowledge gaps about these natural processes. Also research methods to quantify these processes are lacking. And the possible ecological effects in sediment or surface waters are still unknown.

From the knowledge gaps, research questions have been defined. How are the most important processes related and can these processes be modeled? Where does

biodegradation occur? How fast is this process? Can it be stimulated? Which techniques can be used for monitoring?

Six polluted sites in three cities in the Netherlands were sampled, including 1 reference sample per site. Samples were either frozen in liquid nitrogen or the individual sediment layers were sliced and sampled in the field. Chemical and molecular (Q PCR) analyses have been performed in order to characterize the sludge and sediment.

The first preliminary results show that in the top layer of the sediment, which contains the highest organic matter content, anaerobic conditions are present. In this layer the microbial activity is higher than in deeper sediment layers. The dechlorinating capacity in this layer is higher than in deeper layers. However, in the top layer, not only anaerobic organisms are present, but also aerobics and micro-aerophilics. This implies that microaerophilic conditions are present and chlorinated ethenes can be both anaerobically and aerobically be degraded. In the deeper layers the dechlorinating capacity is lower. This has implications for dredging management in this specific canal.

Dredging of the canal would, at least temporary, decrease the dechlorinating capacity. This could cause an increase in chlorinated ethenes in surface water and result in ecological risks during a certain period of time. The rest of the results will become available in December and January.

RA06-3

Assessing the impacts of climate change on the fate and transport of HCB and Cd in the Elbe River Basin

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Sediment-sorbed concentrations of Hexachlorobenzene (HCB) and Cadmium (Cd) in sections of the Elbe River basin significantly exceed maximum allowable concentrations established by the European Commission. Even though concentrations of both contaminants have decreased significantly over the past 15 years, levels remain elevated. In addition, previous studies have documented that downstream transport of contaminated sediment occurs primarily during high water events. Given anticipated climate-change induced changes in mean discharge, potentially including an increase in the magnitude of high and low water discharge events, this study seeks to evaluate the role of climate change in mediating the long-term fate and transport of HCB and Cd in the Elbe River basin. To better understand the transport of cohesive sediments and associated contaminants, a hydrodynamic and cohesive sediment transport model for a 230 km segment of the Middle Elbe River was developed. Of particular interest is contaminant transport to floodplains and retention time in the numerous groyne fields that line the banks of the Elbe River. Multiple climate change scenarios involving changes in the amplitude and phase of yearly streamflow were used to simulate contaminant transport throughout the Elbe River basin at 25 and 50 years in the future. Integration of modeling output with the results of recently collected (2010, 2011) sediment data enabled an enhanced understanding of current and potential future dynamics of HCB, Cd and cohesive sediment in the Elbe River. Modeling results, challenges simulating transport in groyne fields, techniques for resolving data gaps, and broad-scale suitability of the model to European river basins are discussed.

RA06-4

Dioxin-like activity of sediment samples from the Elbe River and soil samples from the Elbe associated flood area

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While the water quality of many European streams improved, sediments as sinks and secondary sources of persistent organic pollutants (POPs) are underestimated by the EG-Water Framework Directive. The aim of the present study was to compare the dioxin-like activity of Elbe River sediments and soils of the associated flood area.

Sediments from the year 2008 originated from Czech and German river sections and from the North Sea and were considered to reflect the present pollution of the Elbe River. In contrast, the soil samples of the Elbe associated flood area - taken after the Elbe flood of August 2002 - rather represented a remobilization potential of POPs during big floods. These samples originated from three transects, located downstream, upstream and close to the confluences of the Elbe tributaries Mulde and Saale. Hence, they also might indicate the influence of the tributaries on the contamination of the Elbe.

All freeze-dried samples were extracted by means of pressurized liquid extraction. Dioxin-like activities were determined via the EROD assay with RTL-W1 cells and in parallel via the H4IIE-luc assay with the eponymous transfected rat hepatoma cell line. All samples showed elevated dioxin-like effects, except two North Sea samples, showing no effects in the H4IIE-luc assay. Nevertheless, both bioassays gave a good correlation with Bio-TEQs between 1307 and 10462 pg/g dw. They identified the industrial city Lysa nad Labem as the highest contaminated site and revealed the floodplain soils to be equally contaminated like the Elbe sediments. But a flood influence could only be supposed due to the missing pre-flood data and a soil sampling depths that gave no information about freshly deposited matter during the flood 2002. An influence of the Elbe contamination through its tributaries could not be found. A comparison of Bio-TEQs with their respective Chem-TEQs for the sum of polychlorinated dibenzo-p-dioxins (PCDD/F) and dioxin-like polychlorinated biphenyls (DL PCB) was performed. The Chem-TEQs accounted between 0.1 % and 11.9 % for the observed Bio-TEQs. Lower percentages were found for sediments than for soils, indicating that PCDD/F and DL-PCB are more important for the floodplain contamination.

A multilayer fractionation of three selected transect samples, which eliminated the moderately persistent pollutants, gave clear evidence that the majority of dioxin-like activity in these samples was caused by non priority and non-persistent pollutants.

RA06-5

Can flood events affect rainbow trout? The biomarker-cascade after exposure to PAHs in sediment suspensions

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In context of the ongoing scientific discussion about the potential ecotoxicological impacts of flood events, it is important to understand the detailed mechanisms of contaminant uptake from suspended particles and related effects on aquatic biota. As part of the interdisciplinary project Floodsearch II, rainbow trout (*Oncorhynchus mykiss*) were exposed to suspensions of natural sediment from the River Rhine (Ehrenbreitstein Harbour). Prior to suspension, the sediment was spiked with the polycyclic aromatic hydrocarbons (PAH) pyrene, phenanthrene, chrysene, and benzo[a]pyrene at environmentally relevant concentrations (4.1, 5.0, 3.3 or 8.3 mg kg⁻¹ dry weight, respectively). A control treatment without addition of PAHs was also included in the experimental design. The experiment was conducted first at an average temperature of 24 °C and repeated at 12 °C. The nominal concentration of suspended solids was 10 g L⁻¹ in both experiments. After 0, 1, 2, 4, 6, 8 and 12 days of exposure, physicochemical parameters, concentrations of PAHs in suspended matter, as well as biomarkers of exposure in rainbow trout (biliary PAH metabolites, hepatic 7-ethoxyresorufin O-deethylase (EROD) activity and lipid peroxidation) were measured. Instrumental chemical analyses revealed that concentrations of pyrene and phenanthrene in suspended solids decreased over time, while no significant degradation was observed for chrysene and benzo[a]pyrene. Concentrations of biotransformation products of PAHs in bile of fish increased slightly in the treatment without addition of PAHs at 24 °C, while average levels increased to 166 µg ml⁻¹ for 1-hydroxypyrene (control value 4.6 µg ml⁻¹) and 17 µg ml⁻¹ for 1-hydroxyphenanthrene (control value 0.1 µg ml⁻¹) in the spiked treatment within two days, followed by a decrease. In the 12 °C experiment, uptake of PAHs was slower. With a latency of two days, the peak of metabolism in the 24 °C experiment was followed by a peak of lipid peroxidation that indicates oxidative stress caused by PAH metabolism. EROD was not significantly induced by the treatments. Significant differences were observed between the bioavailability of freshly spiked and field-aged PAH contamination. The results of this study indicate the importance to account for the temporal variability of biomarker responses in sediment suspension experiments to comprehensively assess the biological effects caused by particle-bound pollutants.

RA08 - Escape from the Ivory Tower - Environmental sciences should impact public and policy

RA08-1

The gap between scientific publication and the press - a case example

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Nanomaterials are of common interest for industry as promising novel technologies, but they are also of certain public concern due to lack of knowledge about their effects for health and environment. Results of our study on effects of carbon nanotubes (CNT) on green algae were published in 2011 in the peer reviewed journal *Environmental Science & Technology* [1]. The major findings of our study were that CNT inhibited growth of green algae, but not due to direct effects, like reduced photosynthetic activity of the green algae, but mainly due to indirect effects, namely shading of light in turbid test suspensions and agglomeration of the CNT with algal cells. After the scientific publication in fall 2011, we published a press release which surprisingly evoked a vast number of reactions in the media, the common public, the institutes involved, and a smaller number of reactions in the scientific community. The press discussed the results very diversely: Taking over our message to interpretations far away from the scientific facts presented in ES&T. Examples for headlines are (Translated from German): "Nanotubes as Growth Inhibitors - Toxicity Tests with Green Algae" [2], "Nanoparticles Identified as Potential Environmental Killers" [3], and "Nano-Waste Could Disturb Aquatic Life" [4].

The lessons learned from this case study are a) that news on topics of public concern such as toxicity of CNT and engineered nanomaterials are very closely monitored and instrumentalized both by the pro- and the anti-nano community for their respective intentions, and b) therefore, reactions on press releases concerning these topics have to be monitored closely, wrong quotations must be corrected and biased interpretations must be adjusted, in order to provide correct scientific information for the common public. [1] Schwab F, Bucheli TD, Lukhele LP, Magrez A, Nowack B, Sigg L, Knauer K. 2011. Are carbon nanotube effects on green algae caused by shading and agglomeration? *Environ Sci Technol* 45:6136-6144.

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RA08-2

Discourse of risk communication - taking the risk of communicating risks

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Risk communication, especially to the general public and end users of plant protection products, is an important challenge. Currently, much of the risk communication the general public receives is via the popular press, and risk managers face the challenge of presenting their decisions and their scientific basis in an understandable way. Therefore, we decided to explore the obstacles in risk communication, as done by expert risk assessors and managers. Using the discourse analysis framework we have studied perspectives of three stakeholder groups - regulators, industry representatives and academics across Europe.

We conducted 30 confidential interviews, which comprised 15 open-ended questions. A part of the interview guide was focused on communication of pesticide risk to the general public and the ideas experts in the field of risk assessment/management hold of the public perception of pesticides. We employed the key informant approach in recruiting our participants. They were first identified as key stakeholders in ecological risk assessment of pesticides and then sampled by means of a snowball sampling. The most visible motif we identified in the preliminary analysis was a strong disconnect between "emotions" and "science" in communicating risk. These two were seen as opposites, with "science-based" risk communication perceived as superior to the "emotion-based" communication. The latter was presented as detached from facts and related to political sensitivities.

Our results show that despite the attempt to polarise emotion-based and science-based communication, emotionally-charged language and reactions are always present in risk-related topics. Our preliminary findings suggest that there needs to be a balance of "emotions" and "science" in communicating risks - the discourse of risk is tightly related to emotional reactions. Moreover, studies into risk perception found that emotional reactions often play a more significant role in perceiving risks than our cognitive judgement. Whilst it is important to keep the process of risk assessment objective and science-based, the general public can be better informed from communicating risks with the costs and benefits clearly outlined, and emotional connotations are easy to understand. Emotionally-charged messages have been successfully used in health risk communication (e.g. fear appeal) and public awareness campaigns. It seems that risk assessors and managers can also learn from these examples.

RA08-3

Reach Regulation: communication behind the information needs

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Reach Regulation : Communication Behind the Information Needs.

The implementation of REACH aims a high level of protection of human health and the environment. Industry has the burden to prove the safety use of products and to ensure control of any risk. If the information to public is also one of the major goals of the regulation, the first steps of implementation show different ways to experience information diffusion.

These works were conducted in the framework of a research project « How to use REACH. How the stakeholders approach REACH through technical classes ». Discussion meetings have been organised with the parties who have designed the regulation and who are assuming the control of its implementation, but also people who are implementing REACH in professional organisations and private companies : Reach regulation designers, chemical producers, chemical industry trade-unions, state-member representative. Information on/into REACH, at this early stage (registration), is not a simple question and REACH proceeds by 'Learning by doing'. Information on REACH and products is circulating to some extent into topical focused forums (SIEF) and into chemical consortia, but it appears that different stakeholders don't share the same points of view (what kind of questions for which recipients?). Communication to public which is also an important objective of the regulation is far beyond what maybe expected at that stage. The next steps (evaluation, authorization) surely need to plan communication towards the public, outside from the closed and confined space of acting stakeholders.

RA08-4

Research findings and decision making: the case of renewable energy

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Scientific research can have a role in the promotion of more sustainable patterns of consumption and production because it can provide information aimed to raise awareness about the impacts of different behaviours and to support more sustainable choices from different kind of actors. The challenge posed to science in this context is to provide information that is effectively supporting for decision making processes at different scales and that can be easily understood by all the stakeholders involved in the process (policy makers, entrepreneurs and citizens). In recent years the attention of citizens on the issues of sustainability, environmental impacts and sustainable behaviour has grown considerably [1]. In parallel the demand for scientifically sound and transparent information upon which to base consumption choices and behaviours is growing among citizens.

Nonetheless, it is difficult to think that there may be a direct contact between those who do research and who makes the decisions: firstly, because in most cases there are no opportunities for direct contact (e.g. to citizens) and, secondly, because is necessary to translate the information resulting from scientific research in a language that is understandable. The lack of communication between science, policy and citizens communication can lead to not evidence-based decision making, lack of trust and unsustainable behaviour due to low level of information and awareness. One possible way of success in environmental communication could be represented by the presence of those intermediaries who have relationships with key stakeholders and are able to translate information for them so that they become understandable and translatable into action.

We present a discussion about wood use as a renewable energy source: communication of the main scientific findings about its sustainability and environmental impacts associated to it in support to decision making in energy planning and energy use.

RA08-5

Challenges of integrating science and people within a network of excellence

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A Network of Excellence (NoE) was recently formed in radioecology, a science concerned with the transport and effects of environmental radiation. The NoE was formed to combat several problems in radioecology that are not uncommon in many other disciplines: 1) a steadily decreasing funding base; 2) fragmentation of resources and infrastructures; 3) poor recruitment of young scientists; and 4) retirement of key personnel. Nine organizations from eight countries formed the NoE with goals of developing a sustainable, long-term integration of radioecology in Europe. The integration that was required by the scientists resembled, in the business world, a partial merger of divisions from within different organizations. We thus sought a business school that specialized in change management to help us with the integration. Our NoE became a special project for five of MBA students. This presentation highlights some of their findings. Foremost, the MBA team determined that to create a sustainable NoE, there had to be integration among the people. This included working towards a shared vision, understanding the cultural differences involved in the project, and sharing the same expectations about what the scientists as individuals and their respective organizations receive from the NoE. Studies from past attempts at mergers and acquisitions in the business world indicate that failure to integrate usually occurs because the fundamentals of change management are overlooked and/or taken for granted.

[NOTE to organizers: This platform session is intended for a special session organized by F. Brechignac and I. Linkov, (francois.brechignac@irsn.fr). I do not see it listed among the 'topics'. Although the subject of this presentation seems to fit perfectly with SETAC's conference theme of Integrating Science and People, I do not see where it might best fit among your topics. Below, I have listed F24....but welcome your suggestions as to other options. Thank you.]

RA08-6

Keep your boots muddy

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The German Federal Environment Agency runs a site for aquatic simulation in the very south of Berlin, which also includes a set of outdoor and indoor artificial pond and stream mesocosms (FSA) for ecotoxicological research (<http://www.umweltbundesamt.de/wasser-und-gewaesserschutz/fsa/>). Up to the present, 30 studies were carried out in that facility during the last 10 years. The results have been popularised via the official homepage, guided tours, conference posters, talks and sessions, scientific journals, magazines, newspapers, and TV features. In search for further means to reach a wider audience apart from ecotoxicological professionals and people interested in natural sciences, the idea of the artist Anne Rinn (www.anne-rinn.de) to stage an exhibition and to create a film entitled 'Keep Your Boots Muddy' was supported by the FSA team. Both exhibition and film pivot on the triangle nature-simulation-art with simulation trying to create artificial nature. The presentation will be the 10 minutes version of the German film with English subtitles.

RA09 - Focal species of birds and mammals and their ecological behaviour for refined risk assessments of plant protection products in Europe

RA09-1

Benefits of 'EFSA Risk Assessment for birds and mammals' guidance document for ecological refinements

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In order to gain EU Level approval of an active substance under Regulation (EC) 1107/2009, and previously EU Directive 91/414/EEC, it is necessary to assess the risk to birds and mammals. Since the early 2000's to 2009 the main guidance document used was the 'SANCO/4145 Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC', European Commission (2002) (hereafter referred to as SANCO/4145). However, in 2009 the European Food Safety Authority (EFSA) issued a new guidance document ('Risk Assessment for Birds and Mammals', EFSA (2009)) which aimed to update and improve bird and mammal risk assessment in the EU. Both guidance documents follow a tiered risk assessment whereby a first tier risk assessment seeks to address low risk situations. However, should a first tier assessment fail to be sufficient to demonstrate a low risk then it is necessary to refine the risk. There are various approaches outlined in both guidance documents including the use of ecological data. The presentation will outline the key differences between the two guidance documents and highlight the benefit of risk assessment methodology in EFSA (2009) in relation to ecological refinements. An analysis of a hypothetical case will demonstrate how the first tier risk assessment in EFSA (2009) provides valuable information for the refined assessment. An example will be given to show how improvements in the use of ecological data and uncertainty analysis will be used in the overall characterisation of the risk.

Improvements to first tier risk assessment methodology in EFSA (2009) result in a much more informative assessment. Further guidance is also given in conducting and interpreting ecological field studies and utilising the literature. It is hoped that this will aid the development of more robust risk assessments and consistency in evaluation. A further improvement is that it is necessary to conduct a risk characterisation and uncertainty analysis for every refined risk assessment. It is anticipated that this will improve communication of the risk as well as allowing risk assessors to make best use of the available information in a scientific manner.

The new EFSA guidance document will help applicants and risk assessors to produce reliable, robust and informative risk assessments. It provides more clarity at the first tier, for refined assessments and for the overall communication of the risk.

RA09-2

Risk assessment of birds and mammals exposed to plant protection products in the Nordic and Baltic countries

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Regulation EC 1107/2009 regarding placing of plant protection products on the market in EU entered into force 14 June 2011. A central aspect of the regulation is the principle of mutual recognition within zones with comparable conditions. To facilitate work-sharing within the Northern zone, the Nordic and Baltic countries have regular meetings to harmonize risk assessments. Agreements among the countries are compiled in a guidance document for the Northern zone which is available at the authorities' homepages.

To harmonize initial risk assessment of birds and mammals, the Northern zone has agreed on using the EFSA Guidance document (EFSA Journal 2009; 7(12):1438). If initial assessments indicate potential risks, refined risk assessments can be provided. Refinement options include which species that could be used (focal species) and the ecology of these species, e.g. proportion of diet obtained from treated area (PT) and composition of diet obtained from treated area (PD). Other refinement options consider the properties of the substance, e.g. degradation time in/on treated crops and weeds (foliage DT50).

To harmonize a refined zonal risk assessment, the Northern zone has agreed upon the following:

Default foliage DT50 used in initial risk assessment could be replaced if results from at least 4 study sites are available. If results from 4-10 sites are presented, the longest DT50-value should be used. If more than 10 values are available, the mean value can be used. Residue decline studies may only be used for refinement of DT50 if results are evaluated according to the EFSA guidance document.

Default PT (100%) could be replaced by referring to studies including, as a minimum, 10 individual animals. The 90th percentile PT value from these studies should be used in the refined risk assessment. Studies to refine the PT may only be used in the risk assessment if they are evaluated according to the EFSA guidance document.

Focal species and their PD and PT will be harmonized, as far as possible, for major crops within the zone. Such guidance will enhance uniform and agreed refined assessments and ease evaluation of registration reports. Our goal is that this guidance for refined risk assessment will be available in May 2012 when the SETAC conference is held.

Modelling (body burden or ecological models) are not considered appropriate for higher tier risk assessment until validated models and guidance are available.

RA09-3

Identification of bird focal species in France for refined risk assessments for plant protection products

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The refined risk assessment for birds and mammals might be based on different parameters such as measured residues in food items, and/or on behaviour and diet of focal species. The EFSA Opinion (2009) provides generic focal species for the major crops, but when further refinement is necessary, focal species defined in the opinion as "real species that actually occur in the crop when the pesticide is being used" should be identified. The National Museum of Natural History in France has got a huge data base containing registrations of bird presence on the territory, based on field observations made by a large network of naturalists. A methodology has been built to identify the focal species on several groups of crops by crossing the information issued from the Museum database, the repartition of crops in France, protected studies provided by industry, and a literature review. The outcome of this work, presented as a compiled document, contains identified focal species per groups of major crops, their behaviour and feeding habits.

RA09-4

Bird focal species in crops according the EFSA - how to find the right candidates

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According to EU Council Directive 91/414/EEC and Regulation 1107/2009, the effects of crop protection products on wild mammals and birds have to be assessed. For this reason crop-specific focal species candidates can be defined for different periods representing different BBCH growth stages. The total data base consists of 72 field studies of plant protection product producing companies who register their products within Europe (BASF, BCS, Cheminova, DOW, GOWAN, Makhteshim, Irvita, Isagro, Monsanto, Sharda and Syngenta). The studies have been conducted in eight different countries spread across member states in the central and southern registration zones of Europe. The total period during which the surveys of each study took place ranges from a minimum of two weeks to up to four months. Here we will present data of two crops (cereals and pome fruit), and the most important value calculated on the basis of the raw data is the frequency of occurrence per field (FOField), which denotes the number of fields where a species was recorded as percentage of the total number of fields regardless of the number of individuals observed. Furthermore it is necessary to consider parameters such as diet guild, feeding strata, body weight and food intake rate to ensure that a given focal species are representative to provide a robust risk assessment. The focal species selection methodology is discussed using cereals and pome fruit orchards in Europe as case studies.

Acknowledgement - The authors thank all the data owners within European Crop Protection Association for access to these data and the field workers for collecting the data in the field.

RA09-5

European distributions of farmland birds used as focal species for pesticide risk assessment

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Risk Assessment guidance for birds and mammals in Europe has been described by EFSA [1]. The process is tiered. To refine risk assessment it is necessary to define focal species in different crops at relevant growth stages appropriate for the use of the chemical applied. Focal species have been studied in 16 EFSA crop categories, mostly by Industry, in several EU member states. Here we present the data from field studies conducted by Industry. For most major crops the focal species have been determined in several MS's and this provides evidence of their distributions. Studies have not been measured in all crops in all Member States (MS) since there is room for extrapolation between zones and crops. The EU have established voluntary zonal work sharing for the registration and reregistration of plant protection products which have been described by SANCO/6896/2009 [2] stating it is reasonable to place MS in geographical zones (Zonal Approach) where there is greatest similarity in climate, crops and general conditions that may influence exposure to pesticides and hence the risk. The purpose of the presentation is to demonstrate how an evaluation of the distribution of focal species from these data can be done and to compare the distribution of focal species from these studies with the distributions for the same species provided by the European Birds Atlas database. To illustrate this approach, one crop example (cereals) will be used. The approach developed may allow extrapolation in line with the Zonal Approach, such that MS risk managers can have confidence in using refined exposure data for a single set of focal species applied to a crop within a zone or even across zones.

RA09-6

Small mammal communities in agricultural landscape in central Europe: review of long-term field data

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Little is known about general small mammal communities in agricultural landscape in central Europe. Most published data represent only a few months' data in a specific habitat type focussing on a small region. This presentation will review data from several studies performed in the last decades in agricultural landscape across different regions in Germany and the Czech Republic. Data on the distribution of small mammal species in agricultural landscape including cropped fields, meadows and adjacent field structures like hedgerows and woodland are presented. The results of three data sets in comparison will focus on general conclusions and may help to interpret the spatial and temporal composition and distribution of small mammal communities in agricultural landscape in central Europe.

RA10 - Global Mercury: Bridging science and policy

RA10-1

Mercury emissions in large Hg-polluted floodplain areas in Germany are an underestimated problem: challenges for reliable risk assessments and implications for authorities

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Environmental pollution by mercury is a world-wide problem. Particularly floodplain ecosystems are frequently affected. One example is the Elbe River in Germany and its catchment areas; large amounts of Hg from a range of anthropogenic and geogenic sources have been accumulated in the soils of these floodplains. They serve as sink for Hg originating from the surface water of adjacent river. Today, the vastly elevated Hg contents of the floodplain soils at the Elbe River often exceed even the action values of the German Soil Conservation Law. This is especially important as Hg polluted areas at the Elbe River achieve several hundred square kilometres. Thus, authorities are coerced by law to conduct an appropriate risk assessment and to implement practical actions to eliminate or reduce environmental problems. A reliable risk assessment particularly with view to organisms (vegetation as green fodder and hay production, grazing and wild animals) to avoid the transfer of Hg into the human food chain, requires an authentic determination of Hg fluxes and their dynamics since gaseous emissions from soil to atmosphere are an important pathway of Hg. However, reliable estimates of Hg fluxes from the highly polluted floodplain soils at the Elbe River and its tributaries, and its influencing factors are scarce. For this purpose, we have developed a new method to determine mercury emissions from soils at various sites. Our objectives were i) to quantify seasonal variations of total gaseous mercury (TGM) fluxes for floodplain soils at the Elbe River, ii) to provide insights into physico-chemical processes regulating these TGM fluxes, and iii) to quantify the impacts of the controlling factors soil temperature and soil water content on Hg volatilization from a typical contaminated floodplain soil within soil microcosm experiments under various controlled temperature and moisture conditions. Our study provides insight into TGM emissions from highly Hg-polluted floodplain soils in Germany and that those emissions are an underestimated problem. Current needs for reliable risk assessments, the induced implications for authorities, and future challenges will be discussed. The presented data will contribute to a better understanding of seasonal dynamics of Hg fluxes and its controlling factors. This presentation should be of large interest for a wide international audience, such as environmental scientists and managers, applied ecologists, and authorities.

RA10-2

Mercury in the Mediterranean: status and mass balance

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An interesting feature of mercury biogeochemistry in the Mediterranean is that several fish species from the Mediterranean show higher concentrations of Hg than same fish species in the Atlantic ocean, although the concentrations of total mercury in the open waters of both oceans are similar. Elevated Hg levels have been noted in environmental matrices from the Mediterranean regions adjacent to known mercury anomalies, yet, the data do not clearly indicate that the effects of these anomalies have been transmitted to open waters or to lower trophic level species living in these waters. Recent studies indicated that the main source of MeHg in organisms in the coastal areas is related to methylation in sediments, while net mercury methylation in the open ocean occurs in the water column and is linked to organic matter regeneration promoted by the presence of small-sized nano- and picophytoplankton, that dominate under oligotrophic conditions. Relatively large portion of mercury in waters is present as dissolved gaseous mercury (DGM), originating from photochemical, biologically mediated mechanisms and/or diffusion from deeper layer either due to biological and/or to tectonic activity which is typical of the Mediterranean region.

Recent studies on distribution and cycling of mercury and a mass balance were implemented. It has been shown that exchange with the atmosphere is the most important source/sink of mercury for the water compartment. Measurements have shown that the evasion of Hg varied between the different seasons with the highest evasion during the autumn and an estimate of yearly evasion from the Mediterranean Sea surface was estimated to range between 250-350 kMol/yr. Two important zones of MeHg productivity are reported in the Mediterranean Sea: one at the bottom of the euphotic layer and the other at the oxygen minimum in the thermocline. The proposed methylation and demethylation rates vary between 0.3-6.3 % day⁻¹ and 6.5 -25 % day⁻¹, respectively. Based on these values the estimated production in the euphotic zone is between 500 and 1000 kMoles/yr, and degradation was estimated to 750-100 kmoles/yr. It has been shown that the total mercury exchanges at the straits are not unbalanced, while mercury entering the western Mediterranean is mainly in inorganic Hg forms and is exported to the Atlantic partially as methylated species.

RA10-3

Mercury exposure in relation to selenium and glutathione S-transferase gene deletion variants in pregnant women from Mediterranean

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It is well known that an antagonistic effect exists between selenium (Se) and mercury (Hg), and that Se can play a protective role against Hg toxicity in organisms. In order to find an evidence for such connection, total Hg and Se were determined in 1654 umbilical cord blood samples, 1081 maternal blood samples, and 1051 breast milk samples in pregnant women from Slovenia, Croatia, Italy and Greece. Methyl mercury (MeHg) was determined in approx. 30% of the samples. In addition, to observe gene-environment interactions, GSTT1 (Glutathione S-transferase theta 1) and GSTM1 (Glutathione S-transferase Mu 1) gene deletion variants were studied in a subset of 212 participating women. The study was implemented within the EU 6th framework programme PHIME.

Positive and significant linear correlation between Hg and Se was found in cord blood, maternal blood and breast milk. The strongest correlation was observed between inorganic Hg and Se in breast milk of the overall population (r=0.801). Hg and Se were found to be associated positively and significantly also in blood of Slovenian women aged 50-59 and children aged 6-11 from mercury mine area, but not in children from other areas in Slovenia, confirming the association of Se with inorganic Hg, which is the predominant species people are exposed to in the contaminated site. Se in maternal and cord blood, but not in milk, was significantly correlated with the intake of many food items in pregnancy. The strongest direct associations regarded cheese and some vegetables (artichokes and fennels). In addition, both Hg and Se were significantly associated with fish consumption, possibly explaining correlations between these two elements found in selected biomarkers. Moreover, pregnant women with homozygous deletion of GSTT1 gene showed significantly higher MeHg (but not total Hg) in cord blood compared to women with the presence of GSTT1 gene (p=0.028). When adjusted to Se levels in blood, positive and significant associations were observed also for MeHg in maternal blood. No significant differences of MeHg, T-Hg and Se concentrations between GSTM1 gene deletion variants subgroups were obtained.

RA10-4

Mercury and methyl mercury in the trophic chain of the Lagoon of Venice, Italy

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The Lagoon of Venice is one of the numerous coastal zones contaminated by mercury emitted from chemical industry (mainly alkali-chlor). Because of a long residence time of mercury, perpetually recycled between water and sediments, the transfer of mercury to biota in lagoons persists a long time after the reduction or elimination of Hg point sources. Previous studies in the Lagoon of Venice have demonstrated an elevated methylation potential, tide-driven MMHg transfer from sediments to water column and accumulation in some organisms. Here we report for the first time the initial results on Hg and MMHg bioaccumulation in the food web.

Biota and sediment samples were collected in summer 2011 in the moderately polluted northern part of the Lagoon of Venice. Biota samples included pelagic and benthic

organisms from all trophic levels from primary producers to fish. Carbon and nitrogen isotopes were determined with a isotope ratio mass spectrometer (IRMS). Total Hg was analyzed using thermal combustion method (AMA 254) The Hg species were analyzed by species-specific isotope dilution and capillary gas chromatography hyphenated to inductively coupled plasma mass spectrometer.

Based on $\delta^{15}\text{N}$ results the sampled organisms covered 3 trophic levels.

Total mercury concentrations in biota varied by nearly three orders of magnitude from $0.030 \mu\text{g g}^{-1}$ d.w. (dry weight) in seagrass (*Zostera marina*) to $2.3 \mu\text{g g}^{-1}$ d.w. in tissue of shrimp (*Palaemon elegans*). There was a tendency of increasing Hg concentration with increasing trophic level, but the relation was not significant if all species were considered. MMHg concentrations varied between 5 (seagrass, phytoplankton) and about 2000 ng g^{-1} d.w (shrimps, fish) and the proportion of MMHg in total Hg was increasing with the trophic position of organisms. For the full set of data, the relation between MMHg and $\delta^{15}\text{N}$ values was best expressed by an exponential function ($R^2=0.59$).

On average, the accumulation of total Hg and MMHg in the organisms in a moderately polluted area of the Lagoon of Venice increased by one order of magnitude for each of the three trophic levels. However, the deviations from this rule can be considerable for benthic filter feeders (bivalve mollusks) and some fish apparently migrating from less contaminated areas. Results of this and follow up studies should provide a suitable model for risk assessment in more contaminated areas.

RA10-5

Dietary selenium at environmental concentrations reduces methyl mercury retention in some aquatic organisms at the lower trophic levels

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Methyl mercury biomagnifies along aquatic food chains and top predators - including humans with a high intake of fish or aquatic mammals - risk neurological symptoms because of the high dietary exposure to methyl mercury. Methyl mercury is taken up into organisms quite efficiently, whereas - once assimilated - methyl mercury is eliminated slowly. Thereby, the long retention times (of course together with the efficient uptake) are the main contributing factors in the biomagnification process. Selenium interacts with methyl mercury and mercury in multiple, complex and not fully understood ways. Methyl mercury contents in fish tend to be low in selenium rich (natural, manipulated or polluted) ecosystems and dietary selenium increases the elimination of methyl mercury from freshwater fish in laboratory experiments. Lower retention times in organisms at the various trophic levels will inevitably result in reduced biomagnification of methyl mercury along aquatic food chains but the role of dietary selenium on methyl mercury biokinetics in aquatic invertebrates is poorly known. The present experiments were carried out to obtain a better understanding of selenium's role for the retention of methyl mercury in aquatic invertebrates.

Dietary selenium (as selenite, seleno-cystin and seleno-methionine but not selenate) reduced the retention on methyl mercury in the brown shrimp *Crangon crangon*.

Environmentally relevant concentrations ($< 1 \mu\text{g Se/g}$) of selenium in the food correlated negatively with half lives for methyl mercury. Selenium in the food also reduced the retention times for methyl mercury in the marine copepod *Acartia tonsa*, but in a less pronounced way than in the brown shrimp. In shore crabs *Carcinus maenas* no effect of selenium was found.

The finding that there is a negative correlation between low, environmentally realistic selenium concentrations in the food and the half life for methyl mercury in brown shrimps indicates that selenium may play an active role for the biokinetics of methyl mercury in the environment. This is corroborated by similar findings in zebrafish *Danio rerio* [1] where small increases in the selenium concentration in the food also lead to a dose-dependent decrease in the retention of methyl mercury. The potential significance of the somewhat more limited effect of selenium at the lowest trophic levels (the copepods) and lack of effect in the shore crab needs more detailed elucidation.

RA10-6

Mercury pollution in china: releases, uses and impacts

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China currently has the world's largest intentional consumption as well as unintentional environmental release of mercury (Hg). Atmospheric emissions has been estimated to about 700 tons annually, accounting for one third of the global anthropogenic emission. There are also large (but not quantified) releases to local soil and water environments. The intentional use of Hg in industrial processes and consumer products has been estimated at 1000 tons annually, roughly half of the global total.

Mercury is released to the environment by a wide range of sectors, including key industries such as mining, power generation, non-ferrous metal production, and the cement and chemical industries. The industrial use of mercury in China has caused severe pollution incidents in the past. Today, as a result of past practices, high mercury levels are found in water, soil and rice near abandoned mercury mining and smelting areas.

The presentation gives an overview of the major issues regarding China's Hg pollution issues, including releases, intentional use, environmental concentrations as well as human exposure.

RA11 - Guidance documents and guidelines for environmental risk assessment (ERA): needs, developments and progress

RA11-1

Ecological risk assessment of pesticides: linking non-target Arthropod testing with protection goals (ESCORT 3)

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The ESCORT 3 workshop (ESCORT: European Standard Characteristics Of beneficials Regulatory Testing) dealt with questions of the protection of "Non-Target Arthropods" in the context of the use of plant protection products in agriculture. It was the third ESCORT workshop that addressed this question. The Organising Committee put together a programme of discussion topics that were addressed at the workshop in plenary sessions alternating with work in sub-groups. This allowed for in-depth discussions on each of the four areas identified by the Organising Committee: a) Level of protection and testing scheme; b) Off-crop environment; c) Recovery; d) Field studies.

Approximately 60 participants registered for the workshop coming from authorities, the private sector, and academia. The participants of the workshop were assigned to one of the four sub-groups based on their knowledge and expertise, and regular plenary sessions gave participants the opportunity to comment on all areas under discussion. An opening plenary session provided background information with presentations from invited speakers. The ESCORT 3 meeting was held as a review and update of the previous meeting outputs based on current science. It also considered new issues and open points that had arisen in the interim period. The proceedings of this workshop will be finalized in the 2012 and this presentation aims at offering the audience an outline of these proceedings.

RA11-2

Development of OECD guidance on the conduct and evaluation of toxicity tests for endocrine disrupting chemicals

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The science of endocrine disrupting chemicals (EDCs) is maturing, and two jurisdictions (European Union and United States of America) have now enacted regulations for the control of these substances. It was foreseen over a decade ago that such regulations would need to be underpinned by new toxicity assays relevant to human health and wildlife species, because the more traditional suite of internationally-standardised assays was insufficiently sensitive to known EDCs. In response to this perception, the Organisation for Economic Cooperation and Development (OECD) set up its Endocrine Disrupter Testing and Assessment (EDTA) programme to develop and validate new screens with some diagnostic capability for potential EDCs, as well as tests with apical endpoints sensitive to EDCs, several of which have now been published as OECD Testing Guidelines (TG). These include 2 in vitro procedures for detecting substances with potential to affect steroidogenesis (TG 456) or interact with the estrogen receptor (TG 455); 4 in vivo mammalian assays (TG 443, 441, 407 and 440) with diagnostic capability for, and/or sensitivity to, one or more of estrogens, androgens, thyroid disrupters and steroidogenesis disrupters (so-called EATS modalities); 4 in vivo fish assays (TG 229, 230 and 234; GD 148), also with EATS sensitivity; and one in vivo amphibian assay for thyroid-active substances (TG 231). In addition, OECD has developed, or is developing, a range of lifecycle or partial lifecycle assays with birds, fish,

amphibians and invertebrates which are able to measure the apical effects of EDCs without generally being able to diagnose causality. As well as briefly describing these assays, this paper will present a new OECD Guidance Document (GD) which helps users of the assays interpret their results, assists in reaching conclusions about whether test substances possess potential or actual endocrine disrupting properties, and provides advice about a possible further testing step should this be indicated. The basis of this GD is the need to evaluate all relevant information in a weight-of-evidence approach when interpreting assays, including existing data on similar chemicals, physico-chemical properties, results of in vitro tests, and in vivo toxicity in the same and other species. It will rarely be possible to conclude that a substance is an EDC solely on the basis of a single assay, so the GD will provide much-needed assistance to chemical companies and regulators alike.

RA11-3

ECETOC Ecotoxicological assessment of endocrine disrupting chemicals

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European regulations on plant protection products, biocides and chemicals only support the marketing and use of chemical products on the basis that they do not induce endocrine disruption in humans or wildlife species. An ECETOC task force was formed to provide scientific criteria that may be used to identify and evaluate endocrine activity and disruption. This presentation focuses on wildlife species.

For ecotoxicological assessments the key considerations include specificity, potency, population relevance and negligible exposure.

Assessment of specificity is conducted within a study or species, to determine whether the endocrine mediated effect occurs at concentrations lower than those that cause other significant toxicity. Then the evaluation proceeds to consideration of specificity in relation to endpoints obtained in other taxonomic groups, which may drive the overall risk assessment. If the adverse effects are considered not specific the risk assessment is based on the non-endocrine endpoint.

If specificity is confirmed, a risk assessment considers the endocrine endpoint with an assessment factor based on potency. The endocrine-mediated NOEC/NO(A)EL needs to be compared with other endpoints, e.g. by assessing the magnitude of the ACR, comparing the potency of the substance to a reference compound, duration of exposure that induces an adverse effect and the number of species in which the adverse effect is demonstrated.

The protection goal of environmental risk assessments is the population and examples for relevant effects are: age at first reproduction, size of a reproductive event, frequency of reproductive events, duration of reproductive period, viability of young and sex ratio. Some effects are known to be responsive (and even sensitive) to, but not diagnostic of, endocrine modulation (e.g. fecundity, which can be affected by general toxicity). In such cases supporting information will be required to link the population relevant effect to an endocrine mechanism.

There are currently no specified criteria for "negligible exposure" of wildlife species to plant protection products. Based on the wording in the regulation it is evident that negligible exposure must fall somewhere between "no exposure" (i.e. nominal concentrations of 0, or less than the limit of detection/limit of quantification) and a concentration representing an acceptable or low risk.

RA11-4

Environmental Quality Criteria (EQC): a comparison of methods under different regulatory regimes

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Environmental quality criteria (EQC) form the basis for legally binding environmental quality standards (EQS). The EQC's are derived according to different European and national legal frameworks accompanied by technical guideline documents (TGD). Within European chemical and water regimes the TGDs are currently streamlined at European level. This harmonisation of methods is likely to influence both, the other sectors of environmental policy on the European level as well as the existing national risk assessment schemes.

The Federal Environment Agency induced a project comparing the methods under the European REACH-Regulation with national established guidelines for water, soil and the UNECE concept for terrestrial ecosystems. The comparison included the legal frameworks and procedural mechanism for quality assurance. The methodological analysis contained literature work, case studies and interviews with officials to document the experience of authorities and to evaluate the transparency of EQC derivation. The REACH Guidance Documents (GD) served as reference method to identify concordances and differences. Two main procedural "stations" have to be distinguished:

1. Retrieval and Evaluation of all available information [with several "steps" under REACH]

2. Derivation/Identification of the EQS [REACH: PNEC]

As far as the main procedural elements of the first station are concerned a high level of concordances has been identified. However in some respects methodological differences may lead to diverging EQCs:

- Water: The highest level of concordance has been identified between REACH and the European water legislation.

- Soil: The derivation of the EQS differs to some extent since the German approach takes characteristics of the medium soil into account.

- Air: In the field of air quality legislation a case by case approach is dominant basing to a high degree on expert judgement.

Main conceptual differences occur between the REACH-GD and the established concepts in the field of terrestrial ecosystem. In cases where those differences are of minor importance the EQS may be used to fill the gaps in other sectoral legislation, such as provisions for industrial installations (IED 2010/75/EU), water or air quality.

RA11-5

Representativeness of *Eisenia fetida* for the environmental risk assessment of pesticides to soil organisms

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The sole routine testing of the standard earthworm *Eisenia fetida* for the terrestrial risk assessment of pesticides has been under much debate since other soil invertebrates may be more sensitive than this standard test species. Low availability of laboratory toxicity data for taxa other than *E. fetida* has greatly hampered previous sensitivity comparisons. In the present study, the relative tolerance (Trel) approach was applied to enable comparing toxicity thresholds for main terrestrial taxonomic groups and pesticidal types of action (insecticides, fungicides, herbicides, and other) separately. Analyses confirmed previously reported lower and higher sensitivity of collembolans to fungicides and insecticides, respectively. However, various other discrepancies in susceptibility relative to *E. fetida* could be identified using species sensitivity distributions and/or calculated 95% confidence intervals of Trel values. For example, arachnids and isopods were found to be more sensitive to insecticides, and nematodes to fungicides, as compared to *E. fetida*. Implications of study findings for the (first-tier) terrestrial risk assessment of pesticides are discussed.

RA12 - Health and environmental risk assessment of pesticides and biocidal products

RA12-1

Information requirements under the Biocidal Products Regulation and their implications for environmental risk assessment, authorities and applicants

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As of 2013, the current regulatory framework, the Biocidal Product Directive (BPD), is going to be replaced by the Biocidal Products Regulation (BPR). The upcoming regulation will encompass a wide range of regulatory changes. Union-wide authorisation of products, the obligation to substitute active substances with less-hazardous equivalent alternatives, as well as an exposure-based data waiving represent major changes that will affect both authorities and applicants. A further major change concerns the amount of quantitative and qualitative information to be submitted by applicants for both active substances approval and biocidal products authorisation. The BPR requires more compulsory information and exhibits a more extensive list of case-dependent additional information requirements when compared to the BPD. This could represent a major challenge for the applicant, but has to be weighed against the aim to improve hazard and risk assessments in a resource-efficient manner. Current (BPD) and future (BPR) information requirements are compared and discussed and the significance for environmental risk assessments is estimated. In addition, economic implications for applicants are discussed.

RA12-2

Proposal for a harmonized assessment of the mixture ecotoxicity of biocidal products

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Biocidal products are typically mixtures of one or more active substances as well as further ingredients. With the inclusion of the first substances in Annex I of the Biocidal Products Directive (BPD) 98/8/EC the authorisation of the corresponding biocidal products at national level is underway and European Member States (MS) are facing mutual recognitions.

For the authorisation of biocidal products an extensive environmental risk assessment (ERA) is required in accordance with the BPD, i.e. not only every active substance in the product has to be subject of an environmental risk assessment, but also substances of concern, i.e., substances leading to classification of the product or having PBT, endocrine or CMR properties, have to be evaluated separately. In addition estimation of mixture toxicity of the ingredients is required.

As it is well accepted that mixtures of substances usually elicit a different toxicity than the isolated substances itself and additive effects up to synergistic effects are possible, it was agreed between the MS at the Biocides Technical Meeting I / 2011, that the mixture toxicity of the components of biocidal products has to be considered during the ERA and that the concept of Concentration addition is a suitable method for that. However, until now no harmonized concept for the assessment of the mixture toxicity of biocidal products was developed. This means that every MS has its own concept for considering the mixture toxicity during ERA. But this is problematic due to the mutual recognition of authorisations among the Member States.

Therefore the German Federal Environment Agency (Umweltbundesamt, UBA) proposes a tiered approach for the assessment of biocidal products based on the available data for the single product components which also considers the existing approaches of the other MS as well as possible synergistic effects between the product components.

The aim of the approach presented is to harmonize the assessment of the mixture toxicity of complex biocidal products among the MS as well as to assess the mixture ecotoxicity of products and, where relevant, of ecologically relevant mixtures, and at the same time relieve the data requirements for the applicants as well as additional animal experiments.

RA12-3

Prioritisation of biocidal substances for environmental monitoring

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The European Biocidal Product Directive (BPD, 98/8/EC) causes a change of the use of biocidal active substances in EU member states. The placing on the market of a number of substances has already stopped or they will be withdrawn soon as consequence of non-inclusion decisions. Additionally, the use of certain biocides will be restricted by risk mitigation schemes. The expected result of both measures should be a decrease of discharges into the environment of affected biocides. This hypothesis may be proven by an environmental monitoring which might also provide information on the necessity of regulatory improvements (e.g. risk mitigation measures). Therefore, a project was initiated by the Federal Environment Agency of Germany to develop a concept for the selection of biocides for such a monitoring. First, the status of biocide monitoring in Germany was investigated. A questionnaire was sent to authorities involved in routine monitoring as well as to research groups which run projects in this field. Evaluation of about 25 answers revealed that the current monitoring mainly covers surface waters. Often biocidal compounds are monitored due to legal requirements (Water Framework Directive or German Surface Water Ordinance priority substances). However, most of the biocides currently considered are those which are also used as plant protection products. As result of the survey and an additional literature research a database with monitoring data was compiled. Then, a concept was suggested to prioritise biocidal substances for an environmental monitoring. In a first step compounds are evaluated for emission characteristics (mainly based on intended use in BPD product types). The second step covers potential effects. The scores from both steps are combined and used to prioritize compounds. In a third step it is evaluated in which environmental compartment a compound should be investigated (e.g. water, sediment, biota, soil). This evaluation is based on use patterns (product type specific emissions) and substance specific properties relevant for the compartment regarded (e.g. partition between compartments, persistence or BCF). The procedure was tested with a set of 80 biocides which are either already authorised biocides (BPA Annex I) or candidates (biocidal substances currently in the BPA review programme). The plausibility of the prioritisation is discussed with regard to the compiled monitoring data as well as to prioritisation results from other studies.

RA12-4

Pyrethroids: new contaminants in human breast milk

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Pyrethroids are synthesized derivatives of pyrethrins, which are natural insecticides that are produced by certain species of chrysanthemum. In the last decades, they have increasingly replaced organochlorine pesticides due to their relatively lower mammalian toxicity, selective insecticide activity and lower environmental persistence. Thus, they are applied in urban area primarily for structural pest control, in agricultural areas on crops, and in the home in pet sprays and shampoos.

Recently some new works point out that its massive use could make pyrethroids considered as pseudopersistent organic pollutant. This means that they may be constantly present in environment not because of its stability, but given that its continuous dumping. Moreover, some studies show that they are toxic, at specific doses on diverse organisms, rats included. As a result of that and as long as World Health Organization marked them as potentially carcinogenic substances, interest for this family compound is increased and some works are come out, mostly about its presence on different kinds of food and natural environment. Nevertheless, studies in breast milk are rarely.

The objective of this work is to study the presence of 12 different pyrethroids in human breast milk samples collected from different areas, including European and South-American countries, with urban and rural areas where different pyrethroids are used. Analytical methodology includes a liquid extraction from freeze-dry samples with an organic solvent and sonication assistant, followed by a SPE cleanup and finally analyzed by GC coupled with MS-MS.

Results indicate the presence of different pyrethroids, with the higher contribution for cypermethrin (with concentration levels up to 16 ng/g lw), and cyhalothrin (up to 8 ng/g dw). Moreover, different pyrethroid distribution between samples was observed comparing different countries. A relationship between total pyrethroids concentration and number of children has been also founded. Pyrethroid molecules have different stereoisomers, and that because the isomerspecific enrichment in human breast milk was also studied and checked with environmental data. Finally, and based on the calculated pyrethroid concentrations in human breast milk, the daily ingestion rate (EDI) of each pyrethroid was estimated. The maximum nursing infant dietary intake was 4.60 µg (kg of body weight)⁻¹ day⁻¹ for cypermethrin. This value is lower than ADI (Acceptable Daily Intake) WHO recommends.

RA12-5

New physiology-based pharmacokinetic model for predicting mammalian tissue distribution of pesticides

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Physiology-based pharmacokinetic (PBPK) models are a type of mass-balance model used to predict the concentration profile of chemicals in the blood and tissues of a living animal. PBPK models describe four main processes; absorption, distribution, metabolism and excretion (ADME). Three of the ADME processes are strongly dependent on chemical partitioning properties; absorption, distribution and excretion. A common approximation to describe the partitioning properties involved in these processes is to use a surrogate for storage lipids to estimate the lipophilicity and to assume that the sorption capacity of membrane lipids and proteins is related to the fraction of those phases that has a storage lipid-like sorption capacity. For highly lipophilic, non-polar chemicals the predictions are quite accurate. However, polar chemicals and chemicals with hydrogen bond donors and acceptors can deviate significantly. A more robust method with regard to neutral polar chemicals are poly-parameter linear free energy relationships (PPLFER). In PPLFER equations explicit consideration is given to the different molecular level interactions that may exist between a solute and the solvent molecules of the phase in which it is sorbed; specifically dipole interactions, hydrogen bonding, and van der Waals forces.

An improved PBPK model for predicting total mammalian body burden and tissue distribution of neutral organic chemicals is developed. Partitioning to blood and tissues is built up from their basic as described by appropriate PPLFER equations. The model was calibrated and validated on biomonitoring, occupational exposure and epidemiological studies available in the literature. The model was subsequently applied to a dataset of pesticides with experimentally determined values for the solute descriptors required for application of PPLFER equations. Three different exposure scenarios were considered, corresponding to the three different absorption routes: oral, dermal and inhalation. Overall mammalian body burdens of pesticides resulting from exposure through the three routes of exposure were comparable. However, pesticides with certain combinations of properties have notably different tissue distributions when modelled by the PBPK model based on PPLFER equations.

RA12-6

Evaluation of pesticides in food - A dynamic multicrop model

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Human intake of pesticide residues via food ingestion plays an important role for evaluating current agricultural practice. However, human health impacts of pesticides are still poorly represented in existing assessments, since almost exclusively effects from diffuse emissions are considered, thereby disregarding exposure from residues in field crops after pesticide application. Pesticide uptake and translocation mechanisms vary considerably between crop species and require a dynamic assessment.

A new modeling approach was designed to account for pesticide residues in various food crops as source for human pesticide intake. Modeled residues are compared with measured concentrations of 12 different pesticide-crop combinations and correspond well with total crop-specific residual errors ranging between a factor of 1.5 for lettuce and a factor 19 for rice. Intake fractions calculated per unit mass of applied pesticide for 121 substances applied to all crops but potato are usually in the range of 10^{-2} and 10^{-8} kg intake per kg applied for typical times between application and harvest. Intake fractions obtained after direct application were 1 to 5 orders of magnitude higher than intake fractions estimated for indirect emissions, i.e. fractions lost to air and freshwater during application. Main factors influencing the fate behavior of pesticides are the degradation half-life in plants and on plant surfaces, the residence time in soil as well as the time between pesticide application and harvest. A simplified model based on the most influential input variables enables the prediction of residues within a factor of 10 of those calculated with the complex model. Highest impacts are expected via consumption of herbaceous crops and fruit trees with usually high intake fractions and consumption, while roots and tubers only contribute little due to very low intake fractions. Substitution scenarios enable us to reduce health impacts by choosing alternative pesticides with similar ability to control unwanted pests, but with lower toxicity. However, substitution must be discussed separately within each pesticide target class.

RA14 - Landscape ecotoxicology and spatially explicit risk assessment

RA14-1

Effects of scale on pesticide loss patterns and ecosystem services

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RA14-2

Exposure and ecological risk assessment of herbicides in Japan: validation and factor analysis for predicted concentrations of herbicides by the geo-referenced multimedia environmental model G-CIEMS

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The predicted environmental concentration (PEC) is calculated based on fate models or survey results. Pesticides are characteristically used at limited time points and places, hence for environmental risk assessment it is important to predict temporal-spatial variations of the exposure concentrations. The multimedia environmental fate model G-CIEMS (Grid-Catchment Integrated Multimedia Modeling System) based on Japanese GIS data was developed in the past. In addition, emission estimation methods for herbicides used typically in paddy fields were developed, that were applied to calculate temporal-spatial variations of herbicides concentrations in river waters for the total area of Japan. The model simulations were validated using field survey data from seven rivers. In this study, we focused on the analysis of factors which contribute to the accuracy of model predictions in order to improve the reproducibility of model results. The emission estimation method was divided into following three phases. In the first phase, used amounts of each herbicide formulation, which is the commercially available herbicide product, were predicted for each day for each prefecture. In the second phase, we predicted variations of concentrations for each herbicide in paddy fields and the daily emission ratios to a river and air. In the third phase, we allocated emission amounts of herbicides to GIS segments based on land use data. Finally we calculated environmental concentration for all pesticides for the total area of Japan by the G-CIEMS model. We performed field survey from April 2009 to July 2009 for validation. The maximum concentrations were compared between prediction and observation for the 166 pairs from total 175 pairs (= 7 river sites x 25 herbicides). For compound- or site-related average, more than 70% of the plots had an prediction error of less than one order of magnitude. Based on comparison of several physicochemical properties between higher accuracy herbicide group and lower accuracy one, degradation rate was significantly higher in the lower accuracy group with $p < 0.05$. We confirmed our method have high accuracy for predicting herbicide concentration variations in river for several sites and several herbicides. For improving our method, it is important to investigate actual condition of degradation mechanisms of herbicides in the environment.

RA14-3

Linking exposure and effects of pesticides using passive samplers and the SPEARpesticides bioindicator - a case study in Central Germany, 2010

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Pesticides have always been an important stressor for non-target organisms, and today's modern pesticides are no exemption. Our investigations show the still high impact from pesticides on freshwater communities in selected Central German agricultural landscapes, despite continuous attempts to reduce any unintended effects of the pesticides on the environment.

The study took place in the Bode river catchment located in the TERENO Harz/Central German Lowland Observatory, Central Germany, during the peak pesticide application period in 2010.

At 19 sampling sites along six streams, passive samplers of the type Chemcatcher® were deployed for 2 to 3 weeks to detect any pesticides originating from agricultural field run-off.

For each site, the composition of the aquatic macroinvertebrate community was established and the ratio of pesticide sensitive taxa was calculated according to the bio indicator system SPEARpesticides (SPEAR value; SPEAcies At Risk).

The deployed passive samplers detected 16 pesticides in very low concentrations ranging from 0.12 ng to 48.49 ng, proving themselves a reliable tool for the detection of chemicals in surface waters.

Toxic units (TU's) were initially calculated for the obtained time-weighted average pesticide concentrations (TWA) and later re-calculated for realistically estimated pulse exposure time frames of 24 hours and 6 hours, respectively, based on the individual soil organic carbon-water partitioning coefficients (Koc) of all detected substances. The range of TU's shifted from previously -7.7 to -3.4 to now -6.5 to -2.0.

443 Macroinvertebrates belonging to 69 taxa were collected.

The SPEAR values, i. e. the site specific percentages of SPEAR taxa present, ranged from 3.16 to 67.49 (bad < 11 ≤ poor < 22 ≤ moderate < 33 ≤ good < 44 ≤ high).

A significant correlation of the SPEAR values with the respective TU's indicates effects on stream macroinvertebrate communities. Furthermore, the different levels of toxicity towards invertebrates of the different pesticide classes were reflected by their contribution to the site specific TU; herbicides had almost no impact on the macroinvertebrate community whereas fungicides were partially and insecticides mainly responsible for the adverse effects on the macroinvertebrate community.

The re-calculation approach is considered to allow for a more realistic assessment of the dose response relationships (exposure and effects) of pesticides in the fields.

RA14-4

Spatially-explicit exposure and ecological risk modeling tools: SEEM and FISHRAND

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We report on two wildlife exposure models that incorporate the impact of chemical distribution in the environment, habitat suitability relative to areas of contamination, and foraging behaviors of the modeled species. The Spatially Explicit Exposure Model (SEEM) is being developed for the US Army to improve the realism of terrestrial wildlife exposure modeling. SEEM tracks exposures for all individuals in a user-defined population rather than evaluating a single representative individual. Foraging for each individual is guided by habitat suitability preferences. As a result, individuals are less likely to forage in areas where the habitat suitability is low. Also, users may select two different foraging strategies, a free-ranging strategy and a static home range strategy. The model generates population-effects curves. The aquatic model, FISHRAND (FR), is a mechanistic, time-varying bioaccumulation model. The second-order probabilistic model incorporates both sediment and water sources to predict the uptake of organic chemicals based on prey consumption and food web dynamics. The Monte Carlo probabilistic approach allows users to distinguish between sources of uncertainty and variability in predicted fish body burdens. The model allows for more realistic exposure estimates by incorporating data on fish home range, attraction factor (e.g., habitat suitability), and relative abundance. Users can specify preferred habitat areas relative to the contamination profile at the site. Both models offer assessors the tools necessary for advanced exploration of risk drivers and portions of a site in which habitat overlaps with chemical concentrations (and conversely areas where chemicals are present, but habitat is limited).

RA14-5

An agent-based woodpigeon population model used to estimate pesticide exposure in realistic landscapes

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Population modelling is a recommended method to refine risk assessment after the pesticide has failed the first-tier step. We constructed a spatially-explicit agent-based framework (SkyPig) with models of two species: skylark (*Alauda arvensis*) and woodpigeon (*Columba palumbus*) that can be used for population-level assessment of exposure

and effects of pesticides. In our model the birds are characterised by state variables changing dynamically as an effect of their interaction with other agents, environmental resources and time. The common algorithms (mortality check, managing the energy budget, foraging and conducting seasonal activities) operating on these variables result in representation of diverse patterns observed in real ecosystems: population dynamics over the years and foraging patterns including choice of feeding grounds and flocking. These emergent patterns reliably reflect the patterns observed in data describing 40 years long population study in Carlton, Cambridgeshire and radiotracking studies. The distinctive property of our model is the landscape representation: natural structures (like fields or hedgerows) read from GIS maps are implemented as single landscape units without the need for an artificially created grid of hexagonal or square cells.

In this study we present the exposure assessment for a realistic landscape scenario for two fictitious pesticides: pesticide A used as a cereal seed treatment and pesticide B used as a spray applied to oilseed rape. Woodpigeons are known to forage extensively on both cereal grain and oilseed rape leaves and therefore the use of these pesticides potentially exposes them to risk of poisoning. The model predictions on woodpigeon exposure in terms of toxicity-exposure ratios (TER) values obtained by individual birds are compared with TER values calculated for pesticides A and B as used in first-tier exposure model and refined exposure model used in higher tier risk assessment assuming PT and PD values for woodpigeons foraging on sown cereals and oilseed rape leaves.

RA14-6

Accumulation of trace metals in a complex world, validation of a spatially explicit model: BERISP

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Recently, research on accumulation of contaminants to higher organisms has focussed on several environmental factors that may affect food web accumulation. Abiotic factors, species specific traits and habitat have been described to affect accumulation patterns. This implies that spatial variation in environmental factors may play an important role in modulating accumulation. In the current presentation we will present a spatially explicit accumulation model (www.berisp.org), with specific focus on validation of results with field data. The model includes two types of food webs, an omnivorous and an herbivorous one. At the top of the omnivorous food web are two birds species as final receptors: the little owl (*Athene noctua*) and the blackbird (*Turdus merula*). Bovine grazer is the consumer in the herbivorous food web. For this study, three field cases were available to validate the models results. For small mammals, data from two case studies are available: the Metaleurop Nord area, near a former Pb and Zn smelter in the North of France, and the Afferdensche en Deetsche Waarden (ADW), a metal-contaminated floodplain in the Netherlands. For the bovine grazers, results from a case study from Hageven, Northern Belgium are used. In that study, two herds of cows were followed in a natural area, and their feeding behaviour was monitored. Preliminary results on small mammals show that in the Metaleurop Nord case the general pattern of the measured concentrations is reflected in the modelled concentrations: shrews > bank vole ~ wood mouse > common vole. The differences between measured and modelled concentrations are not significant for the bank vole. For the wood mouse and shrews, however, the modelled concentrations are higher. For the common vole, this is the other way around. Difference between modelled and measured concentrations are not related to systematic model assumptions, but may be explained by differences in the assumed diet. In the ADW case, modelled concentrations in bank voles and wood mice are similar to the measured concentrations, as is also the case for the beetles. In the presentation, more detailed information on other receptors will be provided, including the effect of inclusion of spatially explicit foraging.

RA15 - Linking exposure to effects in environmental risk assessment

RA15-1

Development of a toxicokinetic/toxicodynamic model for the sublethal endpoint growth of a synchronized *Scenedesmus vacuolatus* cell population

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Most common ecotoxicological tests assess acute toxic effects by exposing the organism with high dose and short exposure time under laboratory conditions. Process-based toxicokinetic/toxicodynamic (TK/TD) models may be used for extrapolation to chronic toxic effects under field conditions such as fluctuating and pulsed exposures. A TK/TD model simulates the temporal effect course by linking the toxicant exposure concentration to the observable adverse effect via an internal concentration and effect propagation in the organism. Various TK/TD models describe the time course of the endpoint survival of an organism. However, only a few TK/TD models exist which simulate the toxicity time course of sublethal endpoints such as development, growth or reproduction of an organism. The objective of this study was the development of a process-based TK/TD model that describes the sublethal effect on growth of unicellular algae cell populations exposed to toxicants.

We performed a 24 hr algae bioassay with synchronized *Scenedesmus vacuolatus* cell suspensions which were exposed to six concentrations of Norflurazon, Triclosan and N-Phenyl-2-Naphtylamin, respectively. The endpoints cell volume and cell number were measured in a time resolution of two hours within a one-generation algae cell-cycle.

A model that describes cancer cell growth and the effect of anticancer treatments on cancer cell growth was adopted and modified to the observed growth of *Scenedesmus vacuolatus* cell population. A cell-cycle kinetic model of the untreated algae cell population was linked to a one-compartment toxicokinetic model. The toxicodynamic model expresses the time-dependent dynamics of the affected algae cell growth. Different toxicodynamic compartments represent the effect propagation observable through a cumulative damage with continuous exposure duration. A transition time indicates the propagation duration between two effect levels.

The TK/TD model successfully fitted the averaged algae cell volume exposed to six concentrations of Norflurazon, Triclosan, and N-Phenyl-2-Naphtylamin, respectively. This based on our mechanistic interpretation of algae life-cycle process and toxic effects on algae populations. Moreover, the TK/TD model has the potential to link several effect cascades, such as algae cell growth and algae cell reproduction, within a damage progress. Consequently, chronic toxic effects under field conditions may be simulated.

RA15-2

Putting toxicokinetic-toxicodynamic theory into practice

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Toxicokinetic-toxicodynamic (TKTD) modelling provides applications both for chemical risk assessment and ecotoxicological research. TKTD models quantify the time-course of internal concentration, which is defined by uptake, elimination and biotransformation (TK), and the processes which lead to the toxic effects (TD). We present three different applications of TKTD modelling: 1) modelling survival in multiple pulse exposures, where we tested the calibration data requirements and model assumptions, 2) studying the mode of toxic action of pesticides and 3) explaining differences in sensitivity between species. We calibrated TKTD models for *Gammarus pulex* and *Gammarus fossarum*, exposed to pesticides diazinon, imidacloprid and propiconazole. To calibrate the models, we conducted toxicokinetic experiments (internal and external concentrations measured), acute toxicity experiments (4-d standard LC50 tests) and pulsed toxicity experiments (1-d exposure pulses). 1) We simulated survival of *G. pulex* in pulsed exposure to propiconazole using a TKTD model calibrated with acute toxicity data and compared the prediction with experimental results. The comparison showed that the model overestimated mortality in pulsed scenarios, which suggests that acute toxicity data could be used to calibrate TKTD models to achieve protective predictions in pulsed exposure profiles. To assess the need of TK in survival models, we included or excluded simulated internal concentrations based on pre-calibrated TK. The differences between goodness of fits of the models including or excluding TK were small. 2) To investigate whether propiconazole is acting specifically or as a baseline toxicant in *G. pulex*, we compared internal propiconazole concentrations in *G. pulex* during toxicity tests with internal lethal concentrations (ILC50) of known baseline toxicants in *Daphnia magna*. The internal concentrations of *G. pulex* reached the ILC50 range of baseline toxicants in *D. magna*, which indicates that propiconazole acts as a baseline toxicant during 10-day exposure for the endpoint survival. 3) We observed that *G. pulex* is more sensitive to diazinon than *G. fossarum*. Diazinon is an acetylcholinesterase (AChE) inhibitor and it requires metabolic transformation to diazoxon to inhibit AChE efficiently. When we compare the TKTD parameter estimates of *G. pulex* and *G. fossarum*, we see that the elimination of diazoxon from *G. pulex* is much slower, which might explain its higher sensitivity to diazinon.

RA15-3

Chronic effects on pairing behaviour and reproduction of *Hyaella azteca* following pulse exposure to permethrin

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Exposure of non-target aquatic organisms to pesticides often occurs in short pulses following periods of drain flow, surface run-off or spray drift. Both acute and chronic laboratory studies use maintained exposures whereas in the field time varying exposures are likely to occur, with the potential to cause both acute and sub-lethal effects. There is therefore a mismatch between laboratory and field exposure patterns.

The aim of the present study was to examine short-term and long-term effects of 1 hour pulse exposure of different concentrations of the pyrethroid pesticide, permethrin, on pre-reproductive pairs of *Hyaella azteca* and to determine the effect on pairing behaviour and subsequent reproduction over an 8 week period after exposure.

Before molting, pairs of *H. azteca* swim together in amplexus where the adult male grasps the female with its gnathopods. Swimming in amplexus plays a key role in the reproductive cycle and is therefore essential for successful reproduction in *H. azteca*. Very little is known concerning the effect of pesticides on pairing behaviour and subsequent reproduction of *H. azteca*.

Pairs of *H. azteca* were pulse exposed to a single pulse of 1 hour to different nominal concentrations of permethrin; 0, 0.3, 0.9 and 2.7 µg/L. After exposure the pairs were transferred to clean water. During an 8 week test period the treatments were monitored for pairing behaviour and every second week the offspring were counted. Pulse exposure of pairs to permethrin affected the time to reform pairs. Pairs exposed to 0.9 and 2.7 µg/L took longer to re-form compared to controls and pairs exposed to 0.3 µg/L. Furthermore the average reproductive output of *H. azteca* was lower for pairs exposed to 0.9 and 2.7 µg/L during the 8 week test period compared to the control groups.

RA15-4

The relevance of toxicokinetic-toxicodynamic processes for the population recovery of *Gammarus pulex* after exposure to pesticides

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Exposure to pesticides can lead to adverse effects on their survival, the magnitude of which depends on pesticide fate in the environment and the intrinsic sensitivity of exposed organisms. Standard ecotoxicological practices calculate the magnitude of these effects based on maintained exposure concentrations using dose-response models. However, in the field exposure patterns are often more complex. In order to take account of such exposure patterns, an approach that includes toxicokinetic (uptake and elimination) and toxicodynamic (internal damage and recovery), TK-TD, can be used to describe processes within exposed organisms. Recovery of populations from adverse effects is used as a proxy for acceptability of some adverse effects on non-target populations. The potential for population recovery depends toxicant, species specific factors and habitat structure and timing of pesticide applications.

Here we explore the joint impact of intrinsic sensitivity and pesticide specific factors on the population recovery. We developed an individual-based model (IBM) to simulate the response and the subsequent recovery of amphipod populations exposed to four pesticides in different exposure scenarios. Pesticide induced mortality was implemented with two different models. Toxicokinetic-toxicodynamic processes were accounted for by implementing the threshold damage model for survival in half of the treated populations. For the other half, we used a logistic dose-response model. Simulated populations were exposed to 24h, 96h and 16 day LC50 of diazinon, chlorpyrifos, carbaryl and pentachlorophenol on May 1st in the simulated year. Population recovery was compared in populations where we accounted for TK-TD processes, hereafter termed TDM-populations, and those where we used a dose-response model for survival, termed DR-populations.

Calculations of the mortality rate in populations exposed to chlorpyrifos, resulted in much more severe mortality in TDM-populations, when compared to DR-populations. These differences resulted in substantial differences in population recovery times. Recovery after 16 day exposure to LC50 of diazinon took the longest, followed by recoveries after chlorpyrifos, carbaryl and pentachlorophenol.

In conclusion, accounting for toxicokinetic-toxicodynamic processes results in differences, based on different pesticide characteristics, in magnitude of mortality and subsequent recovery times and yielded, on average, longer recovery periods.

RA15-5

Linking pesticide exposure to spatial dynamics: an individual-based model of wood mouse populations

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In pesticide risk assessment, risk is defined as a result of hazard and exposure. The less time animals spend in treated fields the less they will be exposed to pesticides and the risk is thus lower. Therefore, the spatial and temporal distribution and behaviour of non-target species are important in risk assessment. We studied the relationship between the spatial and temporal dynamics and the level of exposure by constructing a spatially explicit individual-based population model, using wood mice (*Apodemus sylvaticus*) on arable fields typical of the UK as an example. The model was constructed based on literature data on wood mouse life-history traits and behaviour. To capture the presence or absence of wood mice in certain fields on certain days, nesting and foraging behaviour and the corresponding movements were represented phenomenologically. Additionally, crop rotation and farming practices affecting the wood mice's behaviour were included. The model is designed to link pesticide exposure and farming practices to the spatial movements and location of individual mice to study the effects of exposure at the population level. We briefly describe the model and then use two example scenarios to demonstrate the potential use of the model for ecological risk assessment of pesticides, addressing the question: how is the (i) spatial and (ii) temporal distribution of the mice, depending on crop type and season, related to pesticide exposure?

RA16 - Monitoring data and post-registration studies: generation, compilation and use in the environmental risk assessment and management

RA16-1

Pre and post-authorisation monitoring for pesticides focussing on birds and mammals - recent developments from the SETAC environmental monitoring action group (EMAG)

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Bird and mammal risk assessments for individual pesticides carried out under EU Regulation 1107/2009 routinely use the relevant guidance document issued by EFSA (EFSA, 2009)¹. This guidance outlines procedures of toxicity and exposure assessments for lower tiers. If concern is raised regarding lower tier assessments it may be possible to refine the risk assessment and although a range of options are available, detailed guidance is lacking as regards how to proceed. One possible refinement mentioned in EFSA (2009) is the use of field studies where 'field studies' refers both to studies of effects following experimental pesticide applications (i.e. applications made as part of a regulatory study) and also to 'active monitoring' of effects following applications of authorised products in agricultural practice. EFSA (2009) also highlights the potential usefulness of 'passive' wildlife incident monitoring or surveillance, involving investigation of suspected incidents reported by farmers and members of the public. The Environmental Monitoring Action Group for Pesticides (EMAG-PEST) of SETAC has been investigating the range of both pre and post-authorisation studies that have been conducted as well as the experience gained, with a view to consolidating 'best practice'.

RA16-2

Post-authorisation monitoring for mammals, birds and insects - wildlife incident investigation scheme - England

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The Wildlife Incident Investigation Scheme (WIIS) is a reactive surveillance scheme that monitors the suspected pesticide poisoning of wild animals, companion animals (pets) and beneficial insects. The suspected pesticides include plant protection products and biocides used to control vertebrate pests and similar schemes operate in England, Wales, Scotland and Northern Ireland. WIIS is partly funded by a levy on pesticide sales in the UK and it is co-ordinated by the Chemicals Regulation Directorate of the Health and Safety Executive (HSE), which is the lead authority on pesticide legislation. It has operated within a legal framework since the Control of Pesticides Regulations (COPR) 1986 and given this longevity and funding arrangements, it is probably unique in the world. The results from WIIS form part of the pesticide regulatory process and are reported quarterly on the internet. The majority of poisonings involve the intentional, illegal use of pesticides and where there is an infringement of legislation on pesticides or the environment, appropriate enforcement action may be taken by the Police, HSE or the local authority. How WIIS operates and the results of investigations into suspected poisonings in England will be discussed. This will focus on investigations where pesticides are used for their intended purpose. For example, honeybee poisonings from spray applications or feral bee treatments and slug pellet applications and rodent control for incidents with mammals and birds. The extent and possible reasons for the intentional, illegal use of pesticides will also be highlighted. Due to improved analytical methods that were introduced in 2010 (unpublished), WIIS has reported an increase in the number of bee incidents where multiple pesticides and/or very small pesticide residues have been confirmed. It is unlikely that these were the cause of death of the bees, but pesticides found include thiacloprid, dieldrin, chlorpyrifos, fipronil and propiconazole. The agricultural use of pesticides may not be involved in some of these incidents and hive wood treatments, amateur garden uses, or pet animal treatments are suspected. An effective, comprehensive and consistent approach to a monitoring scheme is an expensive commitment and a collaborative project among the major disease and contaminant monitoring schemes in the United Kingdom is now facilitated by the Wildlife Disease & Contaminant Monitoring and Surveillance WILDCOMS Network.

RA16-3

Bee health in Europe - facts & figures, A OPERA document

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Declines of managed honey bee colonies and also of some wild bee species have been reported by many countries, leading to intensive work and actions in the areas of research and regulations. Declines in pollinating insect numbers can have significant adverse effects ecologically on the diversity of plant species and economically in the productivity of crops. However, up until now, the status and relative importance of the stress factors that may affect bee populations have been relatively unclear and, in many instances, widely disputed.

In this context, OPERA, has undertaken to produce an updated review on the issue of honey bees and pollinators in Europe, with some highlights to other continents, which would cover ecological and economical aspects related to these species in relation to agriculture.

The expert invited have gathered the latest information available on the factors influencing the health of both managed honeybees and populations of native wild bees, including solitary bees and bumble bees. The main conclusions indicate that the honey bee can cohabitate with modern agricultural practices provided necessary precautions are taken to maintain viable food resources for bees and avoiding practices that may cause adverse effects. These precautions include the design of agricultural landscapes and the implementation of practices that account for the presence of pollinators. Essential developments also concern the availability of effective and regulated veterinary compounds to help beekeepers eradicate the most important pests from apiaries. An analysis of beekeeping activity in its economical context is also provided. Finally, modern agriculture and beekeeping demands better technical knowledge and a critical lack of training and communication to better accompany the updates in science and technology to the farm and the field is identified.

The case of wild bees may be considered to be very similar to that of the domesticated honey bee albeit far less well documented.

Recommendations are emitted towards all those involved in agriculture, bee keeping regulatory authorities and research, which should be communicated to all as the effectiveness of the actions will rely on their common effort to implement them.

RA16-4

Contamination of flowering crops by insecticidal dust drift - effects on honey bees (*Apis mellifera* L.)

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In 2008 a large-scale honey bee poisoning in parts of southern Germany occurred during sowing of maize. This incident was caused by contamination of flowering bee forage plants with drift of dust from the insecticidal seed-dressing containing the active substance Clothianidin.

Since early 2009, practical and large-scale drift experiments during sowing insecticide treated seeds were realized to investigate the link between abrasion potential of treated maize and oilseed rape seeds (determined by Heubach values), dust drift and resulting residues in adjacent flowering crops as well as the effects on honey bees. In 'worst case' scenario experiments bee colonies were set up in semi-field and field trials along the edges of the drilled area (treatment) to study the impacts of dust drift on mortality, foraging activity and brood development in bee colonies (exposure to dust during sowing and contaminated pollen and nectar) using drift-reduced pneumatic sowing techniques. Drilling was conducted during bee flight activity, so foragers were continuously exposed to dust. Two control variants were set up with hives in about 50 m (control) and more than 500 m (remote) distance to the exposed forage plants.

No adverse effect on bees and bee colonies were detected after rape sowing in 2009 and 2011. However, during maize sowing in 2010 and 2011 bee mortality was clearly increased.

Semi-field experiments with manual application of insecticide-loaded maize dust in *Phacelia* (*Phacelia tanacetifolia* Benth.) were carried out to analyse the impact of different rates of the active ingredient (0.5 g a.i. / ha and 2.0 g a.i. / ha Clothianidin) and different particle sizes ($x \leq 160 \mu\text{m}$, $250 < x \leq 450 \mu\text{m}$, $x > 500 \mu\text{m}$) of dust on mortality, foraging activity and brood development in bee colonies.

In contrast to the other dust fractions, at the same rate of Clothianidin for fine dust particles ' $x \leq 160 \mu\text{m}$ ' significant mortality was detected. Studies on different rates showed effects on mortality only for the higher rate, whereas no differences were detected between the low rate and the untreated control.

Despite improvements in seed dressing quality and development of drift-reducing sowing technique since 2008, there is a continuous need for further improvements to exclude adverse effects on bees, especially for maize seeds.

Acknowledgement: The work was financed through the Diabrotica research program funded by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV).

RA16-5

Exposure of soil organisms to plant protection products - Monitoring the vertical niche differentiation of soil microarthropods in an arable field in the course of a year

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According to the new pesticide regulation 1107/2009 the necessity to conduct soil macro-organism studies is triggered by the mode of application and the impact of substances on NTA rather than by the DT90. As a consequence the need to test springtails and mites in lab and field studies is increasing. Additionally more attention is drawn on the ecology of soil microarthropods. A crucial point under discussion is the exposure of soil fauna. Recently published "EFSA opinions" pointing at the fate of pesticides in the soil compartment, the relevance of the soil litter layer and the soil ecoregion concept take this feature particularly into account. As a result it is assumed, that the "worst case soil depth profile for short term risk assessment would be litter (if present) or 0-1 cm depth instead of the currently used 0-5 cm depth" (EFSA 2010). With our recent results we complete the dataset presented at the last SETAC Europe meeting in Milano 2010 (Theißen et al. 2010) to increase knowledge on the spatio-temporal dynamics of microarthropod-assemblages in the topsoil of arable fields. Within one year soil cores (5 cm in diameter; 10 cm in depth) were taken monthly, divided into four layers (0-1 cm, 1-3 cm and 3-5 cm and 5-10 cm respectively) and extracted for Collembola, Oribatida and Mesostigmata by means of heat extraction in a Macfadyen apparatus. The results contribute to a sound development and evaluation of exposure scenarios compared to effect thresholds obtained in standardised ETX-tests. EFSA Panel on Plant Protection Products and their Residues (PPR) (2010): Scientific Opinion on the development of a soil ecoregions concept using distribution data on invertebrates. EFSA Journal; 8(10):1820. [77 pp.] Theißen, B., Larnaudie Lopez, M.L., Michala, M., Leicher, T.: Spatial and temporal distribution of soil microarthropods in in-crop habitats. Poster SETAC Europe 21st Annual meeting 15.- 19. May 2011, Milano, Italy.

RA16-6

Use of groundwater monitoring data for existing agrochemicals to support re-registration

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In order to fulfil regulatory requirements for agrochemicals in the EU, the predicted environmental concentrations in groundwater (PEC_{GW}) are estimated using FOCUS models. Depending upon the uses to be registered, up to nine groundwater scenarios are modelled. The resulting concentrations are compared to a non-health-based cut-off criterion of 0.1 $\mu\text{g/litre}$. This modelling is designed to be conservative and thus tends to overestimate the concentrations in even the shallowest aquifer. Recent revisions to the criteria governing the selection of modelling endpoints, such as the FOCUS Kinetics guidance, have tended to increase the conservative nature of the modelled PEC_{GW} . As increasing numbers of agrochemicals will not pass this arbitrary assessment step in the future, it is necessary to develop acceptable higher tier methodologies to refine the modelled output using real-life retrospective groundwater monitoring data. Data from a number of such studies with the maize herbicide, terbuthylazine, will be described and the potential acceptability of such data sets for the regulatory process will be discussed with special reference to key criteria that must be met for the data to be considered sufficiently robust to be used. Implications of the application of groundwater monitoring study data as a higher tier of assessment will be discussed.

RA17 - Multiple stressors in a changing world

RA17A-1

A close look at the temperature-dependent chemical toxicity to aquatic organisms and its implication on derivation of water quality guidelines for protecting aquatic life

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The anticipated anthropogenically-driven climate change not only can increase the average air and water temperatures and prolong the hottest period, but also result in

increased incidents of temperature extremes that will have profound implications on the toxicity of chemical contaminants and hence their ecological risks to aquatic organisms. In this talk, I will comprehensively examine and discuss the mechanisms of temperature-dependent chemical toxicities to aquatic ectotherms based on both literature review and empirical laboratory studies. Examples will be drawn from an array of marine organisms including amphipod, copepod, diatom, and fish after exposure to a chemical (e.g., chlorothalonil, copper, copper pyrrhione, DDT or nano-zinc oxide (nZnO)). In general, we observed that chemical toxicity often increases with increasing temperature over the thermal tolerance range (TTR) of a species, and is further exacerbated at extreme temperatures (i.e., lower or higher than the TTR). Most of the aquatic organisms showed the highest tolerance to chemicals at their optimal temperature(s) where they displayed the highest value of median lethal or effect concentration. For aquatic animals, such universal phenomena can be well explained by the oxygen limited thermal tolerance theory established by Hans Pörtner. Moreover, the overall temperature-dependent toxicity profiles vary considerably amongst different chemicals. Such differences may be partially attributable to the differences in temperature-mediated modifications of their physicochemical properties, toxicokinetics and bioavailability. For instance, we observed that ion dissolution of nZnO in seawater significantly increases with decreasing water temperatures. The diatoms exposed to nZnO had a significantly reduced growth rate at the lowest experimental temperature in contrast to the control; this response was probably due to the increased availability of toxic Zn ions at low temperatures. Recently, we have been conducting a novel meta-analysis to address whether an assessment factor of 10 (AF10) applied to fresh water quality guidelines (WQGs) would be sufficient to account for variation in chemical toxicity brought by thermal extremes. Our preliminary results suggest that AF10 seems adequately protective. Implications of our results will be discussed in relation to ecological risk assessments of chemical contaminants and derivation of WQGs for protecting aquatic life.

RA17A-2

The calculation of risks due to mercury and other stressors to multiple endpoints at a regional scale for the South River and Upper Shenandoah River, Virginia USA.

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A cumulative integrated risk assessment has been performed for the South River from the area upstream of Waynesboro VA to the uppermost part of the Shenandoah River. The area is a site of historic mercury contamination from synthetic fiber production in Waynesboro. Six risk regions have been delineated. Other sources of other stressors include urban and agricultural run-of, channelization, erosion, and contaminated sediments and biota. The current iteration of the relative risk model (RRM) incorporating the hierarchical patch dynamics paradigm was used to construct a conceptual model, designate risk regions, create a ranking system and calculating risks to the stakeholder derived endpoints and impacts. In concert with this effort a Bayesian network was constructed using the same risk regions, sources, stressors, habitats and impacts. The use of the Bayesian network allows a straightforward calculation of management requirements to reduce risk in each of the six risk regions. Atypically, the current source of the Hg contamination is the environment itself. The warm water fish species have been found to have consistently high tissue concentrations downstream of the original source. Temperature may also be a risk factor affecting fish reproduction. Nutrients from upstream of Waynesboro also may be contributing to risk to a variety of endpoints. Although intensively studied for a number of years, the lack of data from upstream and from within the watershed are contributing to the uncertainties in the risk estimates. Our results demonstrate that the risk is unevenly distributed along the course of the river. Mercury is the stressor contributing the highest risk but other stressors are large contributors. The endpoints at highest risk are those directly associated with ecological services. The Bayesian networks excelled at examining risks to specific endpoints while the RRM demonstrated relationships between endpoints. This research program identifies risks and demonstrates the benefits of an integrated risk, research and management program in the restoration of watersheds at a regional scale.

RA17A-3

Application of toxicological and ecological concepts to analyse multiple stress in aquatic communities

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As a result of the increasing human impact on aquatic ecosystems, freshwater organisms are often exposed to multiple stressors simultaneously, which can result in combined effects, leading to serious consequences for ecosystems. There is a clear need to develop a better understanding of interactive effects on ecosystems. Therefore, already existing concepts and tools in ecotoxicology and ecology have to be proofed for their validity and applicability in complex community responses to multiple stress.

A microcosm study was conducted under controlled conditions and periphyton was used as test community. The effects of a resource stressor (elevated conductivity) and a toxic stressor (prometryn) were analysed independently and both in combination. Effects were compared to untreated controls. Structural (biomass, algal class and diatom composition, and functional parameters (tolerance development) were determined over a growth period of 6 weeks.

Ionic stress clearly influenced structural properties of communities, whereas prometryn as single and combined toxic stressor led to functional and structural changes of communities. Diatom compositions clearly respond to different treatments, representing *Navicula halophila* as salt tolerant species and *Fragilaria capucina* as tolerant to toxic stress. Both stressors in combination lead to a completely new community structure with high abundances of omnipresent diatom species. Functional changes of the community were quantified by PICT (pollution-induced community tolerance). The exposure to prometryn increased the tolerance of all exposed communities, independently from the ionic load of the treatments. According to the EC₅₀ of P5000, high ionic loads (5000 µS cm⁻¹) and prometryn showed a synergistic interaction because their combined effect was clearly larger than the predicted EC₅₀ (calculated by the model of independent action, based on the individual single effects).

RA17A-4

Responses of mean species traits in communities to multiple environmental stressors and the ecological risk assessment based on ecosystem function

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A trait-based analysis was conducted on a long-term (25 years) monitoring data of zooplankton community of Lake Kasumigaura, to elucidate relative importance of environmental stressor (temperature changes, nutrient loadings, and chemical pollution) for the temporal changes in zooplankton community with respect to their contribution to an ecosystem function. I used the trophic transfer efficiency across three trophic levels as a measure of ecosystem function. Based on this measure, I identified species traits that were important for this function with a simple ecosystem model (the conversion efficiency of zooplankton was one of the most important traits). For the trait-based analysis, I made a database of traits (e.g. conversion efficiency, optimal temperature, range of suitable temperature, saprobic index, tolerance to chemical [carbaryl acute toxicity], median food size, and body mass) for major zooplankton species in Lake Kasumigaura. The long-term temporal changes of biomass-weighted mean traits in two seasons (summer: May-September, and winter: October-April) were separately analyzed in conjunction with the environmental factors. The water temperature has been increasing at approximately 0.027 °C per year (climatic warming). There were long-term trends in traits: the mean body size and the mean tolerance to chemical had peaks on early 1990s but are decreasing in summer. In winter, however, they are increasing in recent two decades. The conversion efficiency is also increasing in summer but decreasing in winter. These observations suggest that the ecosystem function is severely deteriorated in winter. Similarities of temporal patterns between the functional traits and the environmental factors were examined with the Wavelet analysis. The results indicated that the zooplankton community synchronized clearly with water temperature in both seasons. The effect of inflated temperature was positive for the conversion efficiency in summer although this effect would be conditional of reduced chemical pollution because the tolerance to chemical was highly correlated with the temperature preference of zooplankton.

RA17A-5

Discriminating the effects of pesticide stress and habitat degradation on stream benthic invertebrates

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Achieving a good ecological status for waterbodies requires the identification of the relevant stressors to implement adequate measures. While the reduction of organic load resulted in acceptable low levels, restoration of the natural river morphology is now believed to be the most urgent task for water managers. Yet, several of these measures failed to rehabilitate the expected near-natural communities. At the same time, pesticides are frequently detected in surface waters that may limit the recolonisation of sensitive invertebrate species. The aim of the present study was therefore to discriminate between the effects of morphological degradation and pesticide stress on invertebrate communities. For this purpose, the SPEAR[%]_{habitat} index was introduced to identify the effects of habitat loss due to morphological degradation, which was not intercorrelated to the other metrics. To quantify habitat loss, relevant in-stream variables available from field surveys were identified by PCA and combined to an overall habitat degradation score (HDS) for multiple regression modelling. To quantify pesticide input, the established run-off potential (RP) model was applied. Many of the classical metrics, such as the EPT Index or the German Fauna Index were highly interrelated and identified the predominant alkalinity gradient as major stressor. This was also the case for the SPEAR[%] index, which was expected to solely indicate pesticide stress. However, also the RP and the number of recolonization stretches (RS) were included in the best fit model of this metric, which had the overall best correlation of biological indices and environmental variables. The SPEAR[%]_{habitat} was not intercorrelated to the other metrics and identified the MDS as most important variable, although the correlation was not as strong as others. This correlation was even stronger for sites with low potential pesticide pollution, as indicated by high SPEAR[%] values and a low percentage of agriculture in the catchment. Our results suggest that pesticide stress has a similar or even higher impact on the ecological status than morphological degradation and that current-use degradation metrics are also influenced by pesticide stress. The latter may have important implications on future assessments of ecological status. Finally, the presence of recolonization stretches upstream alleviated the effects of pesticides in terms of higher SPEAR values, which could be used as potential management option.

RA17A-6

Ranking the impacts of multiple environmental stressors on freshwater invertebrate and fish communities

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Community composition and structure are largely determined by environmental stressors which have selected species able to survive, grow and reproduce under given conditions. Human activities like habitat degradation or toxicant release represent additional stressors potentially modifying the selection processes and affecting biological communities. Understanding those filtering mechanisms and disentangling the impacts of multiple stressors is vital to underpin management measures to avoid or reduce biological impairment. The goal of this study was to disentangle the individual influences of 20 natural and non-natural stressors pertaining to four categories (geography, physical habitat quality, water chemistry and toxic pressure) on eight endpoints summarizing freshwater fish and invertebrate communities. Toxic pressure was expressed for five groups of chemicals as the multi-substances Potentially Affected Fraction (msPAF) of species, derived from species sensitivity distributions (SSDs). We used a relatively new, non-parametric modelling technique, Boosted Regression Trees (BRT), to separate and assess individual stressors' impacts and visualize data-driven response curves. The models behaved well in terms of predictive power, with an explained variance above 60% for five out of eight endpoints. Overall ranking was relatively similar among the different endpoints, with recurrent predictors including geography (especially drainage area and latitude/longitude), physical habitat quality and water chemistry variables (particularly the phosphorus concentration, pH and total Kjeldahl nitrogen). The five toxicant groups together explained between 5.2 and 17.4% of the total variance in the biotic endpoints, and specific patterns of response were observed for endpoints related to sensitive taxa or physiological impairment. Partial dependency plots, free from distributional assumptions, provided insight into the direction, shape and thresholds of single predictor-response relationships. Boosted Regression Trees proved an appropriate approach to investigate the individual impacts of combined stressors on species assemblages in the field.

RA17B-1

The importance of seasonal resolution to modelling the interaction of bioaccumulation and climate change

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From 16-21 July, 2011 a SETAC Pellston workshop was convened to assess the influence of global climate change (GCC) on the scientific foundation and practice of environmental toxicology and chemistry. Workgroup 1 focused on influences on chemical occurrence, fate and bioavailability. Here, I present our results for GCC impacts on bioaccumulation. These impacts occur through three primary mechanisms: influences on environmental exposure, on dietary exposure and on within-organism uptake and loss rates driven by bioenergetic processes. This work focuses on the bioenergetic-mediated impacts of GCC, building on previous modeling of species in the Laurentian Great Lakes.

A bioenergetics model for round goby (*Apollonia melanostomus*) was parameterized to reflect growth rates observed in Lake Erie, Laurentian Great Lakes. The impacts of climate change on bioenergetics was assessed based on a shift towards warmer annual lake surface temperatures. Simulations were run for the baseline case and for a 1, 2, and 3-degree Celsius increased annual average temperature. The effects of this warming on chemical bioaccumulation were then estimated by coupling the bioenergetics model to a mechanistic bioaccumulation model for a broad range of hypothetical chemicals with log octanol-water partition coefficients (KOW) ranging from 0 to 8 and biotransformation half-lives ranging from 0.1 to 1000 days. The impact of GCC on round goby growth illustrated the non-linear interaction between growth rate and fish thermal range. Two scenarios were chosen to showcase the impacts of GCC on bioaccumulation: the highest growth scenario, and the warmest scenario. When only annual average concentrations were considered, little difference among scenarios was evident. However, when the seasonal patterns of bioaccumulation were considered, substantial changes, both increasing and decreasing bioaccumulation, emerged. These seasonal impacts, which were more pronounced for metabolizable chemicals than for persistent ones, could be particularly important when they intersect with other time points, such as commercial fishing seasons or critical life stages for toxicological impact. The magnitude of the deviation from the baseline case depends most strongly on the biotransformation half-life, and then on KOW.

RA17B-2

Additive pressures from herbicides and elevated sea surface temperatures on symbiont-bearing foraminifera from the Great Barrier Reef

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Coral reefs are threatened globally by the combined effects of climate change and decreased water quality, especially in the tropics where monsoonal rainfall can deliver vast amounts of terrestrial contaminants to inshore reef systems. It has been hypothesized that reducing pollution could increase resilience of tropical ecosystems to climate change but empirical support for this strategy is extremely limited. Our research focuses on how temperature thresholds of symbiont-bearing species are impacted by different water quality scenarios. Both herbicides and elevated temperatures have been shown to disrupt the relationship of reef building invertebrates with their microalgal symbionts and as both factors potentially interfere with photosynthetic electron flow, additive or interactive effects may occur. In a series of laboratory studies, we examined the potential of stress caused by low concentrations of the photosystem II (PSII) inhibiting herbicide diuron on several types of symbiotic algae (diatom, dinoflagellate or rhodophyte) within the tissues of benthic foraminifera from the Great Barrier Reef. Symbionts within foraminifera were always more sensitive to thermal stress in the presence of diuron ($\geq 1 \mu\text{g L}^{-1}$). Diuron had a direct effect on photosynthetic efficiency (reduced effective PSII yield $\Delta F/F_m$), while elevated temperatures ($> 30^\circ\text{C}$, only 2°C above current average summer temperatures) were observed to impact photosynthesis indirectly by causing reductions in maximum PSII yield (F_v/F_m), interpreted as photodamage. The mixture toxicity model of independent action revealed response additive combined effects of temperature and diuron for inhibition of photosynthesis and the onset of photodamage. Additionally, combinations of diuron and elevated temperatures were shown to cause bleaching through loss of chlorophyll a in foraminifera hosting either diatoms or dinoflagellates. We thus demonstrate that locally improving water quality can enhance resilience of symbiotic phototrophs to global stressors such as projected increases in ocean temperatures.

RA17B-3

How climate change scenarios will affect toxicity: assessing multiple stressors using a *Heliocidaris tuberculata* sperm bioassay

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Marine ecosystems are facing dramatic changes as a result of climate change. Changes in the physicochemical parameters of seawater systems, as a result of climate change, can potentially alter a number of factors that determine toxicity for any given pollutant. For marine systems, it is accepted that the world will contain warmer, more acidic oceans under future climate change conditions. These changes alone (increased temperature and/or decreased pH) have been shown to influence survival, development and reproduction for many marine species. However, such effects have always been in uncontaminated waters. The reality is that many marine species will face the effects of changes in seawater physicochemistry in combination with toxicant stress.

In order to better understand the combined effects of copper, elevated temperature and lowered pH on fertilisation success of an endemic Australian sea urchin, *Heliocidaris tuberculata*, we present a study incorporating multiple stressors (3 temperatures $\times$ 3 pH $\times$ presence/absence of copper). The nominal copper concentration applied in these tests was based upon an EC_{50} calculated from five standardised toxicity bioassays. Potentiometric titration was performed to ensure $p\text{CO}_2$ levels were maintained at conditions expected for eastern Australian coastal waters for the end of 2100 scenarios.

Results show that temperature and copper had a synergistic impact upon fertilisation, significantly ($p < 0.05$) reducing fertilisation success by $> 50\%$. With temperature alone also being a significant factor ($p < 0.001$), we suggest that exceptional future warming of eastern Australian coastline waters may limit the spawning season of *H. tuberculata*, which is already sensitive to high summer temperatures. Acidification, as a sole stressor, showed no significant effect on fertilisation ($p=0.098$). However, in combination with copper, low pH caused a significant decline in *H. tuberculata* fertilisation success ($p < 0.001$). As found in other studies, we conclude that sea urchin fertilisation may be robust to changes in $p\text{CO}_2$ under clean water conditions, but that in combination with other stressors, such as temperature and/or pollution, changes in ocean physicochemistry may pose a risk to continued recruitment. This study particularly highlights the importance of addressing the potential for interactive effects between climate change stressors and common marine pollutants.

RA17B-4

Evaluation of the combined action of natural stressors and chemical pollutants in algae. Assessment of functional, structural and metabolism alteration

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Nowadays, there is a growing number of studies exploring the combined effect of different stressors on ecosystems. However, the interactions between natural stressors and toxicants are still poorly understood. Among the stressors that might influence ecosystems, toxicants can directly or indirectly affect all relevant ecosystem processes such as primary production. On the other hand, fast and intense changes in the water temperature is one of the major threats for freshwater ecosystems.

The objective of this study was to characterize the physiological responses of cultured algae, *Scenedesmus vacuolatus* in response to physical (temperature) and chemical (anthropogenic contaminants) stressors. The pollutants selected for this study were the priority pollutant diuron (herbicide, PSII inhibitor) and the emerging compounds propranolol (β -blocker) and fluoxetine (antidepressant).

The experiment followed a factorial design with 2 factors (water temperature and toxicant concentration) and their interaction. A previous experiment determined that the optimal temperature was 20°C , while 30°C was used for simulating physical stress. Chemical stress was reproduced at the level of EC_{30} for each toxicant (5, 850 and 500 $\mu\text{g/L}$ for diuron, propranolol and fluoxetine, respectively). Changes in photosynthetic efficiency (Y_{eff}), photosynthetic capacity (Y_{max}), photochemical quenching (qP) and non photochemical quenching (NPQ) were studied in control and exposed algal cultures.

The algal toxicity of the three compounds differed considerably. Diuron was the most toxic compound, followed by fluoxetine and propranolol. Effects of diuron occurred

immediately after addition, which indicates a very specific toxic action of this compound to algae (inhibition of photosynthesis). Propanolol and fluoxetine toxicity effects appeared later than those detected with diuron exposure (after 30 minutes). Most of the endpoints were affected after 12 hours for all the compounds tested, except in the case of diuron, where NPQ mechanisms were inhibited after 12 hours of exposure, suggesting damage in the pigments where the NPQ takes place. The increase of temperature reduced the toxic effect of the herbicide diuron and propanolol at the beginning of the exposure as well as at the final time. In the case of fluoxetine, the temperature increase reduced the toxicity at the initial time, but a synergistic effect was observed at final time with an enhanced toxicity.

RA17B-5

Chemical exposure enhances outbreak of infectious disease in fish

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Heavy oil (HO) pollution is one of the most important environmental issues in the world. In our previous study, we evaluated immunotoxic effects of HO on Japanese flounder *Paralichthys olivaceus*, and found that 0.3 g/L of HO led immune suppression at cellular and molecular levels in the fish. Moreover, in the experimental infection with viral hemorrhagic septicemia virus (VHSV) to the fish exposed to HO, we obtained higher mortalities in dual stressor group (virus infection with HO exposure) than those in control groups. In this study, we investigated the mechanisms of disease occurrence in the fish given the dual stressor by microarray experiment and dynamic of viral replication in the host fish. After applying stress to the fish by each single stressor or by the dual stressor, the mortalities of the groups were calculated and the live fish were dissected for sampling of kidney for the microarray experiment and heart for virus titration. As the results, fifty percent of the fish died in the dual group, while no mortality was observed in single stressor groups as well as in control. These results indicated that the dual stressor induced lethal effect in the fish, even when each single stressor had no effect. To investigate if the mortality in the dual group attributed to viral hemorrhagic septicemia (VHS), we measured the virus titre in the heart sampled from each group. The infectivities per g heart tissue were higher in dual group (105 to 108.25 TCID₅₀/g) than those in VHSV group (103 to 106.8 TCID₅₀/g). This suggests that VHSV easily replicates in the fish affected by the dual stressor, and a cause of death is VHS in the group. From the results of microarray experiment, the expressions of antiviral activity related genes such as interferon and apoptosis induction were relatively lower in the dual group compared with those of VHSV infection group. These results supported the high virus titre in the fish given the dual stressor. In conclusion, interferon production in the virus-infected cells and apoptosis induction by NK cells worked in a normal manner in the VHSV-infected fish without HO exposure, but these antiviral activities were suppressed in the fish affected by the dual stressor, which might lead to extensive viral replication in the host cells, resulting in the occurrence of VHS.

RA17B-6

Interactive mixture toxicity effects of a cyanobacterial stressor and insecticides may partly be grouped according to insect mode of action

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We investigated mixture toxicity of a harmful cyanobacteria (i.e., *Microcystis aeruginosa*) and insecticides with different modes of action to *Daphnia pulex*. The widespread occurrence of such toxic cyanobacterial blooms has become an important concern for aquatic ecosystems. In agricultural areas, these toxins may act in combination with plant protection products, including insecticides. In order to study such combined effects, we exposed the waterflea *Daphnia pulex* to binary mixture combinations of *Microcystis* and 8 insecticides for a period of 21 days according to a modified central composite design. Results were statistically analyzed with the concentration addition and independent action reference models to test for additive, synergistic or antagonistic mixture toxicity. Synergistic effects were observed on reproduction for the combination endosulfan and *M. aeruginosa* while fenoxycarb, imidacloprid and spinosad caused synergistic effects on length in combined with *M. aeruginosa*. Linking the results with the mode of action, we observe no interactive effects in combinations with insecticides affecting the acetylcholine pathway by inhibition (carbaryl and chlorpyrifos). However, synergistic effects on daphnid size are observed with those insecticides that affect the acetylcholine pathway by agonistic or allosteric activity (imidacloprid and spinosad). Although endosulfan has a different molecular target compared with imidacloprid and spinosad, both of these receptors belong to the same superfamily of Cys-loop ligand-gated ion channels. This could explain the common observation of synergistic effects in all combinations with these three insecticides. Combination of tebufenpyrad and *M. aeruginosa* resulted in antagonistic effects on both length and reproduction. Interestingly, both can affect the oxidative phosphorylation through completely different mechanisms. Tebufenpyrad specifically inhibits electron transport in mitochondrial complex I while *Microcystis* is known to affect the mitochondria. More research is needed to verify whether the antagonistic effects on reproduction are the results of combined antagonistic effects in the mitochondria. These results suggest the existence of potentially complex interaction patterns between insecticides and harmful cyanobacteria, which may require consideration in future risk assessment of insecticides in the context of climate change.

RA18 - Oil spill effects and risk assessment

RA18-1

Risk of severe oil spills - was deep water horizon an outlier?

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Following the explosion of the drill rig Deep Water Horizon (DWH) on April 20, 2010 that killed eleven workers, an estimated 670000 tons of oil were spilled in the Gulf of Mexico until the well could be capped on July 15th. This makes DWH the largest single unintentional spill in the history of drilling for oil.

Based on global data of oil spills since 1974, we estimate the expected return frequencies of such very severe oil spill events for different spill sources throughout the oil chain to answer the question if the DWH spill can be considered an outlier.

This is particularly important in view of the rapid increase in deep and ultra-deep offshore activities, where both a geographical expansion as well as a trend towards drilling at ever greater depths can be seen over the last decade.

We also compare the risk of oil spills from offshore drilling with the risk of spills that is posed throughout the entire oil chain, separately for different infrastructures such as pipelines, tanker ship transport and storage. Data is extracted from our uniquely comprehensive global Energy Related Severe Accident database (ENSAD) that contains accident data from a large number of different sources. The severity is modeled with a generalized Pareto distribution, to measure specifically the risk of very severe accidents. The results give a mean return frequency based on historical data since 1974 of spills from offshore platforms and rigs exceeding the 670000 tons of the Deep Water Horizon oil spill of around 19 years with 5% and 95% quantiles of 7 years and 49 years respectively. Based on this result this accident cannot be considered an outlier.

RA18-2

Where has all the oil gone? Identification and toxicity of oil degradation products

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Oil is a highly complex mixture containing hundreds of thousands of compounds. Recent analyses using two-dimensional gas-chromatography - mass spectrometry (GCxGC-MS) of the acid-extractable fraction of oil sands process-affected waters (OSPW) from Alberta Canada has shown that even hydrocarbons thought to be extremely resistant to biodegradation will eventually be converted to acid products. Some of these so-called 'naphthenic acids' (NA) also have also been identified in extracts derived from crude oils and therefore likely also occur in the environment as a result of degradation of oil following accidental spillage. Previously we have reported quantitative structure-activity relationships for several structural groups of individual NA using a standard microbial toxicity assay. Now we present the toxicity of individual NA when combined as mixtures. For oil-industry produced waters, marine species may be exposed to these acids. We have now tested the effects of acute exposures to three commercial preparations of NA (CNA) on marine mussels. Many NA are not available in sufficient quantities for toxicity testing. Consequentially, we have modelled the toxicity of over 50 acids for a large number of environmental endpoints (e.g. fathead minnow lethality) and an array of human health measures such as endocrine disruption.

Structural class mixtures of individual NA plus a 35-component mixture was tested using the Microtox[TRADEMARK] assay. Mussels, *Mytilus galloprovincialis*, were exposed for 72h to three CNA and their clearance rates measured. The toxicity of 54 NA, reported to be modelled for a range of both environmental and human endpoints. The results of microbial toxicity tests of NA mixtures were consistent with a narcosis mode of action and concentration addition. This suggests that most of the compounds present in OSPW and CNA were contributing equally to their overall narcotic toxicity. The toxicity of CNA to *V. fischeri* has previously been shown to be greater than that of OSPW. Our tests now show that mussels are more sensitive to CNA than *V. fischeri*. The models predicted some NA to be several orders of magnitude more toxic to fathead minnows than other structures. In addition, some steroidal-type structures were predicted to be endocrine disruptors. When oil degrades in the environment it does not simply disappear. Instead, many hydrocarbons are converted into complex mixtures of acids that are water soluble and toxic to a range of species.

RA18-3

Use of ecosystem service valuation in quantifying ecological impacts and compensatory restoration associated with oil spills in the environment

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Since the Exxon Valdez spill, approaches to quantifying the potential impacts of an oil spill on the environment have evolved significantly. These approaches were derived out of the need to make the public whole for impacts to the environment. The approaches evolved from a strict monetary compensation framework to an ecosystem service based compensation process. That is, given that there was an impact on the environment, impacts to ecosystem services (ecological and human use services) were to be quantified and then, based upon the lost ecosystem services, the public was to be compensated by the provision of an equivalent amount of ecosystem services through the

implementation of site restoration projects. As such, the compensation (restoration) was scaled to the level of injury. This overall approach is termed the service-to-service or restoration-based compensation approach.

The author's experience, through the response and/or damage assessment phases on over 20 releases, provides insight into the methods and approaches used to quantify the impacts to the environment as well as the benefits associated with site restoration. Key ecological service quantification methodologies include the habitat equivalency analysis (HEA) and resource equivalency analysis (REA) approaches. HEA is an economic method of assessing natural resource injuries and compensatory restoration and is increasingly being used in damage assessment cases. The HEA model attempts to (1) quantify the change in service flows over time from ecological resources that have been injured, and (2) determine how much of a similar asset must be preserved, created or enhanced to provide an equivalent flow of services to those that were injured. Although the author will focus on the application of the HEA approach to evaluate ecological service losses, they will also touch on human use impact analyses that are typically conducted by a variety of standard economic approaches. The author will discuss both the debit and credit calculation phases as they relate to the evaluation of impacts associated with an oil release and the scaling of an appropriate level of restoration (compensation). This presentation will provide an overview and case application as to how these methodologies have been used to quantify impacts and scale restoration as the result of oil spills. Case examples will be provided.

RA18-4

Effects of chemically and mechanically dispersed oil on fitness-related and molecular endpoints in the North Atlantic copepod *Calanus finmarchicus*

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In recent years there has been an increasing emphasis on the significance of invertebrates for assessing environmental impact of oil spills. In the event of accidental oil spills in sub-Arctic and Arctic marine environments planktonic organisms like copepods are realistic targets for oil toxicity based on their significant role in the food web, their abundance and their large lipid content. For many years the SINTEF/NTNU culture of *Calanus finmarchicus* has functioned as relevant model for standard ecotoxicity testing of North Atlantic crude oils, and a large database of ecotoxicity data exists for this species. Parameterized experimental data on the effects of oil (single oil components, water soluble fraction of oil, and dispersed oil) on copepod survival and reproduction are used as input for development of numerical models for environmental risk and damage assessment. The accessibility of a continuous copepod culture provides homogeneous specimen in terms of developmental stage and lipid content. This, along with sophisticated experimental systems, is a major foundation for investigating effects of stressors on molecular systems. A 15K oligonucleotide microarray has been developed based on 260,000 ESTs sequenced using FLX454 technologies. Several methods, like 1H-nuclear magnetic resonance and mass spectrometry, have also been developed and applied in order to investigate metabolic profiles and alterations. Together these complementary methods give valuable supporting information to well known fitness-related endpoints, as they contribute with modes of toxic action of stressors, and output data may also be used to determine effect limits. The presentation will include results from an experiment aimed at comparing the effects of chemically and mechanically dispersed oil, their modes of toxic action, and proposed effect limits of toxicity based on fitness-related endpoints as well as molecular profiling. These data will provide input to environmental models for risk and damage assessment following acute oil spills.

RA18-5

Temperate and polar marine species sensitivity to oil components in relation to accumulation kinetics

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Risk assessments for polar marine ecosystems are mostly based on toxicity data obtained for temperate species. Yet, it is unclear whether toxicity data of temperate organisms can be used to derive reference values for protection of polar species, as differences in physiological characteristics, like metabolism and lipid composition, may yield differences in sensitivity to toxicants. The first aim of our study was therefore to compare polar and temperate marine species on their sensitivity to oil. To that end, species sensitivity distributions (SSDs) were constructed for crude oil, 2-methyl-naphthalene, and naphthalene based on acute toxicity data from scientific literature and databases. To allow for an estimation of the overall risk of oil contamination in the field, toxicity thresholds of oil components are needed. Therefore, our second aim was to model oil toxicity based on accumulation and transformation of oil fractions in marine organisms and to validate the outcome by comparison with lab and field measurements. LC_{50} values (lethal concentration for 50 % of the organisms) were calculated by using a critical body residue for compounds acting by narcosis and a weighted average of log octanol-water partition ratios (K_{ow}) of the individual components constituting oil.

The HC_{50} value (hazardous concentration for 50% of the organisms) and estimated LC_{50} value for naphthalene, $5.0[GREEKX]10^3 \mu g/l$ and $5.7[GREEKX]10^3 \mu g/l$ respectively, showed little difference. Therefore, the average toxicity of naphthalene to temperate marine species can be predicted based on accumulation kinetics. A K_{ow} of 10^4 and molecular mass of 200 yielded an LC_{50} of $2[GREEKX]10^3 \mu g/l$ for oil in general. A more refined calculation taking into account biotransformation and specific modes of action underpinned this value.

The sensitivity of polar and temperate marine species to oil and oil components differed on average less than a factor of 3. In addition, most of the differences were not statistically significant and there was no taxonomic group that was consistently more sensitive than the other groups. Apparently, physiological mechanisms suggested to cause differences between polar and temperate species have little overall effect on sensitivity to oil. As a consequence, toxicity data obtained for temperate organisms may serve to obtain a first indication of the risks in polar regions. Yet, exceptions may result from biotransformation and specific modes of action.

RA18-6

Integrating marine physics, biological and eco-toxicological models into a unified simulation system for oil spill risk and impact assessment

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A 3-year project called SYMBIOSES has been initiated to develop a holistic, integrated modeling framework for ecosystem-based risk and impact assessments. The strategy is to couple existing numerical models, each of which has been previously tested, validated, and proven useful. This approach avoids the cost of basic code development and testing, but introduces different challenges in that the various components are based on quite different numerical solution methods. The system consists of physics, biological, oil/chemical fates and eco-toxicological components. The physics models are Eulerian, as are some of the biological models, whereas the pollutant fates and some of the biological components are Lagrangian. The related process equations of the component models are solved in general on different time-steps, and at different spatial resolutions. The design of the system requires the possibility of two-way communication between model components (e.g. zooplankton feeding on phytoplankton, zooplankton consuming oil droplets of appropriate size, chemical concentrations affecting growth of phytoplankton, mortality, growth and reproduction of zooplankton, and growth and mortality of ichthyoplankton). It is also desirable that alternate components can be inserted into the system, to facilitate geographic transportability as well as uncertainty analyses. We therefore seek a relatively flexible and transparent set of methods to achieve integration of the separate models into the system. We assume that the basic physics models are always Eulerian: regardless of numerical solution, the computations are carried out on a regular or irregular grid that is spatially fixed for the duration of a given simulation. The biological and oil/chemical transport and fates models may be either grid-based or particle-based (pseudo-Lagrangian), and will in general produce data on spatial scales different from that of the physical basis models. This paper will describe the proposed design plan to meet these challenges.

RA19 - Plants and chemicals in the environment: risk assessment, pest management and phytoremediation

RA19-1

Risk assessment of herbicides for the common buttercup *Ranunculus acris* in field margins - an experimental field study

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Field margins comprise the majority of semi-natural habitats in the intensively farmed agricultural landscape and thus they can benefit the conservation of biodiversity in agroecosystems. However, field margins can be affected by pesticide by direct overspray and spray drift from field applications. The risk assessments of herbicides are often performed with single and annual plants species in early development stages in greenhouse experiments. But the vegetation of field margins usually consists of annual and perennial plant species in different development stages. Also an insufficient knowledge about the sublethal and long term effects of herbicides on plants is available. The present perennial field study (start 2010), was undertaken to investigate the effects of repeated herbicide applications in successive growing seasons on *Ranunculus acris* in field margins. The test design followed a randomized block design (7 treatments, 1 control, 8 replicates). The applications of the treatments and their application sequences mimic the field management of winter wheat fields with their recommended agrochemical products and application rates. The applied fertiliser and pesticide concentrations

are consistent with their inputs (drift+overspray) in the first meter of a field margin directly adjacent to a field under Good Agricultural Practices. To detect the effects of the applications vegetation assessments and a photo-documentation of the flower intensity of *R. acris* was performed in May 2010 and 2011. Additionally the experiment was accompanied by monitoring of *R. acris* in field margins in the study area in May 2011. *R. acris* revealed sublethal effects after the herbicide applications. Flower intensity was significantly reduced in the herbicide treated plots, whereas plant presence was not affected. So far these sublethal effects are not accounted for in risk assessment procedures. However in the long run these effects will cause the decline of specific plants and set in community shifts in agricultural field margins. This was confirmed by the monitoring where *R. acris* could hardly be observed in field margins adjacent to cereals, whereas in field margins located next to pastures or orchards (no herbicide use) the species was recorded more frequently. Besides the implications for the plants the described sublethal effects can also cause secondary effects for flower visiting arthropods and thereby negatively affect the biodiversity of the agricultural landscape.

RA19-2

Enhancing risk assessment by using a toxicokinetic and toxicodynamic (TK/TD) growth model of *Myriophyllum spicatum*

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Toxicological studies used for risk assessment can only cover a limited range of exposure scenarios due to their work intensity. Ecological models considering the toxicokinetic and toxicodynamic of chemicals are capable to close the lack of exposure scenarios in toxicological studies and, thus, enhance risk assessments by predicting the effects of variable exposure scenarios.

In this work a toxicokinetic and toxicodynamic growth model of *Myriophyllum spicatum* is presented giving the ability to predict the effects of chemical substances on the growth of *M. spicatum*. Therefore, a growth model considering the most important factors influencing the growth was developed. A toxicokinetic part calculating the uptake and elimination of chemicals depending on their physicochemical properties and the physiology of *M. spicatum* was included in the growth model. Moreover, a toxicodynamic part using the internal concentration in *M. spicatum* and experimentally established dose-response relationships to modulate the growth rate of *M. spicatum* was added to the model. The model is able to predict reversible growth inhibition under different environmental and exposure conditions based on standard test results.

The model was verified with experimental data on growth inhibition of *M. spicatum* after fourteen days due to different concentrations of 3,5-dichlorophenol (DCP). The model was able to predict the effect of 3,5-DCP exposure on *M. spicatum* very well but cannot fully explain the total inter experiment variability of growth, particularly under untreated conditions. The model can be used to predict growth inhibition for 3,5-DCP. Further validation and verification of the model is needed to ensure that the model works properly and to show that the model can be used for a wide range of substances with different physico-chemical properties.

RA19-3

Herbicide impacts on macrophytes: can we predict community-wide effects from single-species toxicity tests?

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RA19-4

Linking aquatic plant toxicity data for pesticides to risk assessment endpoints and environmental protection goals

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This presentation discusses protection goals for aquatic plants, assessment endpoints that address those protection goals, and the relationship of standard toxicity data to the assessment endpoints and protection goals. Protection goals for aquatic plants generally focus on the plant community as a whole, rather than particular species or individuals as is the case for aquatic animals. Consistent with these protection goals, it is not necessary to completely protect every sensitive plant species; protecting some fraction of plant species (represented by a certain percentile of the Species Sensitivity Distribution, SSD) is sufficient to protect overall community function and structure. Assessment endpoints used to characterize effects of chemicals on aquatic plants should represent impacts of similar severity to assessment endpoints used for animals, in relation to their respective protection goals. Toxic endpoints for aquatic plants are based on non-lethal responses, unlike acute endpoints for animals which are based on mortality. Furthermore, aquatic plant communities usually recover more quickly than animal populations (especially fish) from effects of stressors. These considerations suggest that assessment endpoints for aquatic plants should be based on a higher SSD percentile than the 5% typically used for animals. When data are unavailable for a sufficient number of species for SSD analysis, a small number of surrogate test species (*Lemna gibba*, *Myriophyllum spicatum*, and certain algae) can indicate the toxicity of herbicides and fungicides to the most sensitive aquatic plants.

RA19-5

Uptake and intra-cellular accumulation of hydrophobic chemicals in charophytes and implications for ecosystem exposure control and remediation

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Uptake to primary producers (constituting an important pool of organic carbon in lakes) is a key step for addressing hydrophobic chemicals to upper trophic levels. It has been hypothesized that permeation and diffusion through biota surface structures such as epitelia, cell walls or membranes may be inhibited for highly hydrophobic substances by steric hindrance of membrane passage. *Chara rudis* is a macroscopic, perennial, benthic taxon having a protective layer of cortex cells. We used ¹⁴C-12 radiolabelled Hexachlorobenzene (HCB) to i) investigate whether or not HCB can reach the internode cell, ii) to assess the distribution of HCB in different plant parts, and iii) to discuss the implications of the accumulation on benthic macroalgae for fresh water ecosystems. We found that i) about 65% of the total amount of HCB added to the microcosm was associated to the algae tissues, ii) the HCB in the cortex cells represented about 90 to 95% of the total found in the plant, and iii) HCB was detected at measurable levels in 57% of the sampled internode cell cytoplasm, demonstrating the occurrence of intracellular transport and storage of chemicals. In some water bodies charophytes grow in very dense meadows up to 2m tall and can cover a large fraction of the water body bottom. The high efficiency of taking up hydrophobic chemicals directly from the water phase suggests that they can also represent a key control for the mass balance of POPs in the water ecosystem wherever they are abundant.

RA19-6

'Halophyte filters': the potential of using halophyte species for phytoremediation purposes in saline aquaculture

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There is a growing consumer demand for seafood as protein source, and land-based (marine) aquaculture is seen as a sustainable way to meet this growing demand. A major problem with aquaculture is the nutrient waste, since most of the nutrients added through feed are released into the environment, predominantly in dissolved form. Filtration and sedimentation techniques to reduce nutrient concentrations are not adequate for the large volumes of waste water produced in aquaculture. The use of constructed wetlands has been shown to be successful in freshwater aquaculture practices. Freshwater constructed wetlands are worldwide used to treat various types of wastes. However, there is only limited experience in saline systems. The main aim of this study is to analyse the potential of constructed saline wetlands in land-based marine aquaculture. The main advantage of using wetlands is that these are relative simple systems that need little control after construction. The location of the wetland can be located close to the source, limiting transport of waste water through pipelines. Further, wetlands can simultaneously reduce several contaminants (BOD, suspended solids, nutrients, pesticides, pathogens). The primary function of a constructed wetland is the purification of water, but there are also several ancillary benefits that can be incorporated in wetland treatment designs. For example a high vegetation biodiversity, offering a habitat for fauna, and giving aesthetic, recreational, commercial, and educational human uses. Different approaches exist to utilize plants (halophytes, macro-algae, micro-algae) in the treatment of marine aquaculture effluent; these are described in a conceptual framework. In essence, all the approaches attempt to maximize the ecosystem service of water purification. The approaches differ in the balance between economic benefits and nature benefits. The potential use of this framework is demonstrated with a Dutch case study.

RA20 - Risk assessment of chemical mixtures: where do we stand? what are the next steps?

RA20-1

Predictive regulatory risk assessment of chemical mixtures in the aquatic environment: a conceptual framework

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Risks of chemicals in the aquatic environment are still often assessed substance-by-substance, neglecting mixture effects. This may result in risk underestimations, as the typical exposure is towards multi-component chemical "cocktails". We used the two well established mixture toxicity concepts of concentration addition (CA) and independent action (IA) for developing a tiered scheme for environmental hazard and risk assessments of mixtures, focusing on general industrial chemicals regulated in the EU under REACH and assuming that the so-called "base set" of toxicity data is available, i.e. EC50 values for algae, crustaceans, and fish.

As mixture toxicities higher than predicted by CA are rare findings, we suggest applying CA as a precautionous first tier - irrespective of the modes of action of the mixture

components. In particular, we show that summing up PEC/PNEC ratios might serve as a justifiable CA-approximation, in order to extrapolate from the “base set” data to the aquatic ecosystem in a regulatory first tier assessment. This approach makes optimum use of already existing single substance assessments. More in-depth mixture investigations may be requested only if the first tier estimates give an indication of a potential environmental risk. Finally we suggest to call for mode-of-action driven analyses only if error estimations indicate the possibility for substantial differences between CA- and IA-based assessments.

RA20-2

Bearing down the borders? - Considerations on an environmental risk assessment of substance combinations across different regulations

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Across Europe, the prospective environmental risk assessment (ERA) of chemical substances is conducted under separate regulatory frameworks, i.e. for plant protection products, biocides, industrial chemicals as well as human and veterinary pharmaceuticals.

The aim of this prospective assessment is to avoid adverse effects of these chemicals a priori to an exposure of the environment. Under all regulations, the current assessment methods focus on single substances only. As it is well accepted that multiple substances typically reach environmental compartments together and act jointly on organisms, strategies and methods for an assessment and regulation of chemical mixtures are currently under development for all these regulatory frameworks.

The presentation focuses on the activities at the “Chemical and Biological Safety” Division of the German Federal Environment Agency (Umweltbundesamt, UBA) with a focus on the development of common methods and strategies for the consideration of substance combinations under the different legislations. Furthermore the limitations and needs for an assessment of mixtures that reach the environment from multiple sources, i.e. substances that traditionally fall under different legislations, are pointed out. Existing approaches for overarching concepts beyond substance-oriented regulations are discussed from a regulatory perception. Common agreements on definitions, assessment methods and strategies across the substance-oriented regulations are important. The same applies for an exchange of knowledge and data between regulations, which would enable an assessment of substances from a variety of sources. Overarching concepts might be helpful for certain situations and substances, but it needs to be clarified for which cases and who might conduct such an assessment.

RA20-3

The threshold of (eco)toxicological concern: a suitable tool for mixture risk assessment and ranking?

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The threshold of toxicological concern is defined as a safe level: a concentration at or below which there is no appreciable risk for humans (TTC) or the environment (ecoTTC). Recently, the TTC concept has also been put forward as a tool for the risk assessment and ranking of chemical mixtures, for example in the recent opinion on mixture toxicity assessment by the scientific committees of the European Union. In particular it has been argued that compounds that are present at or below their individual (eco)TTC do not contribute to the (eco)toxicity of a mixture. That is, a mixture would pose no risk for human health or the environment, as long as all components are present only in concentrations at or below their individual (eco)TTC.

The TTC approach has a range of appealing features. It would be extremely helpful for simplifying the (eco)toxicological assessment of complex mixtures by allowing to ignore compounds below their individual (eco)TTC. It would also simplify the problem of mixture toxicity assessment to a single substance assessment, as we can safeguard against any mixture effect, as long as we can ensure that the concentrations of all compounds never exceed their individual (eco)TTC. Furthermore, the (eco)TTC seems to provide a good basis for the exposure-based waiving of risk assessment in the context of e.g. REACH. A first ecoTTC-like approach is already implemented in the form of the so-called action limit in the European guideline for the environmental risk assessment of pharmaceuticals.

I will briefly review the conceptual basis of the (eco)TTC, the suggested applications in the context of mixture toxicity assessment and will then discuss the implications of its application in view of the classical mixture toxicity concepts (Concentration Addition and Independent Action), which are the cornerstones of our current understanding of mixture (eco)toxicology. The main conclusions are that (i) the (eco)TTC concept requires constant update of the underlying (eco)toxicological data, as the chemicals in use change over time, (ii) the (eco)TTC concept needs to be adjusted for mixtures that contain at least partly compounds with a similar mode of action, which might make it primarily useful for risk management, and (iii) that in particular the ecoTTC requires further development and refinement if it is to be used in the context of ecological protection goals.

RA20-4

Long-term trends in potential toxicity of unknown organic micro-pollutants in rivers

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During these years, the effects of toxic substances on the ecosystem in Dutch inland waters were measured with a complementary method, i.e., by means of so-called bioassays. This approach provides information on the effects of unknown chemicals in water which are overlooked by traditional analytical techniques. The latter cover only a small portion of the large number of chemicals that are present in surface water. Moreover, classical chemical techniques do not give insight into the auxiliary effect that several toxic substances may have.

The results from the bioassays confirm that damage to the aquatic ecosystem during the last decade due to the presence of toxic substances has decreased. Toxic pressure in the river Rhine in the year 2000 was already very low but has decreased further. Toxic pressure in the water of the rivers Meuse and Scheldt was significantly higher than that in the river Rhine, but has decreased considerably since the year 2000. The results also indicate that the toxic pressure is higher upstream and decreases downstream.

The reaction of five organisms to toxic chemicals in a water sample was measured with bioassays. Applying the species sensitivity distribution method to this small set of data results in a snapshot of the toxic stress. This approach is however flawed by large uncertainty margins which make a series of snapshots unsuitable for discerning a trend in water quality. A trend became apparent when all bioassay results collected over the whole period were combined by means of an advanced statistical technique. As a result, information on toxic trends becomes more accurate because the multitude of data has reduced the spread in toxic stress in river water sampled at a given river bank location. Responses to the bioassays provided insight into the nature of the toxic compounds. The cocktail of toxic substances in the river Rhine was found to consist of non-polar chemicals, i.e., substances without a specific mode of action which affect all aquatic organisms. In the other rivers, pesticides are probably responsible for the observed effects. In the summer of 2002, the river Meuse must have been polluted by both known (albeit banned for more than ten years at that time) and unknown herbicides, as revealed by a comparison of chemical measurement and bioassay results.

RA20-5

Toxicity of metal mixtures to *Daphnia magna*: Implications for a multi-metal, multi-site biotic ligand model

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In real-world aquatic systems, organisms are usually exposed to metal mixtures instead of individual metals. To predict the toxicity of metal mixtures, we have been developing a mechanistic model based on tissue residues of metals and on the concept that the toxicity of a mixture of metals can be either dose-additive or response-additive, depending on the mechanisms of action. To calculate tissue residues and thereby predict toxicity across wide ranges of water quality, we have been developing a multi-metal, multi-site biotic ligand model (MMMS BLM) that concurrently accounts for metal-metal competition for binding on dissolved ligands in the water and at sites of toxicity on organisms. We have exposed *Daphnia magna* to mixtures of Cu and Zn, Cd and Cu, or Cd and Zn in moderately hard reconstituted water containing dissolved organic matter (added as Suwannee River fulvic acid) at 3 mg DOC/L, and compared observed mortality to the null assumption of additive mortality predicted from results of concurrent Cd-only, Cu-only, and/or Zn-only toxicity tests. This research has revealed several apparent metal-metal interactions that otherwise might lead to conclusions that metals interact in non-additive ways, yet simple geochemical speciation in the BLM can explain these interactions and reconcile the apparent non-additive toxicity. For example, the toxicity of Cu-Zn mixtures always appeared to be more-than-additive or additive when based on dissolved metal concentrations, whether Cu was varied while Zn was held constant, or vice versa; whereas in the same tests, the toxicity of the Cu-Zn mixtures always appeared to be less-than-additive or additive when based on free-metal-ion concentrations. In contrast to the results for Cu-Zn mixtures, the toxicity of Cd-Cu and Cd-Zn mixtures appeared to be less-than-additive or additive when based on dissolved metal concentrations. These results demonstrate that different metal mixtures can appear to interact differently based on dissolved-metal or free-ion concentrations, but a MMMS BLM could help reconcile those apparent inconsistencies and could be an effective tool to help water quality agencies implement appropriate methods to regulate metal mixtures.

RA20-6

Low dose mixture effects - detectable using toxicogenomic approaches ?

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The advent of toxicogenomic techniques have raised large expectations that some of the central questions of mixture toxicology such as for mechanisms of low dose interactions can be provided with novel perspectives if not definite answers. After the first decade of experimental studies this review summarizes mixtures toxicity studies that

address diagnostic, mechanistic or extrapolation questions. Since 2002 almost 40 studies were published with their major focus on mixture toxicity assessment by means of toxicogenomic techniques, mainly through microarray or qPCR techniques, though metabolomic and proteomic analysis of joint exposures have also been undertaken. It is now standard to explicitly state criteria for selected concentrations and provide insight into employed data transformation, and statistical treatment with respect to minimising sources of undue variation. Bioinformatic analysis of toxicogenomic data, by contrast, is still a field with diverse and rapidly evolving tools. The combined effect assessments achieved are discussed in the light of established toxicological dose-response and mixture toxicity models. Often transcriptomic responses are discussed based on the presence or absence of signals. As there are yet no consented ways of how to interpret these effects, there are ambiguous interpretations. Furthermore, mixture studies in their majority designed their experiments and compared their recorded outcomes against individual treatments i.e. focus was to retrieve signals of individual components under mixture exposure. This stands in stark contrast to our existing understanding of biological activity at the levels of chemical target interactions and apparent apical combined effects. Here models are employed to calculate expected combined effects based on information of the mixture components' individual dose-response relationships. By joining these mixture effect models with toxicokinetic and -dynamic thinking we suggest a theoretical framework that may help to overcome the current limitation of providing mainly anecdotal evidence on mixture effects and progress into more hypothesis driven mixture studies. As ways forward we suggest to study and establish quantitative relationships between dose dependency and time dependency of responses.

RA21 - Standard vs non-standard methods for hazard and risk assessment

RA21-1

Is there a need to better standardise test organisms in ecotoxicology? A case study based on sexual development in inbred and outbred zebrafish (*Danio rerio*)

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The use of outbred laboratory animal strains representing wild populations is often advocated but rarely demonstrated in ecotoxicology. Due to practical constraints limiting effective population sizes, laboratory animal strains are generally more inbred and less genetically diverse than their wild counterparts. Furthermore, breeding history may vary considerably between strains, driving differences in genetic variation and ultimately phenotypes used for assessing effects of chemical exposure. Developmental phenotypes are particularly susceptible to variation due to phenotypic plasticity caused by genotype[environment] interactions. Sexual development in the zebrafish is a potential case in point. Sex determination in this species has been shown to be highly plastic and therefore influenced greatly by environmental factors (including chemical exposure), as well as genetic factors.

We compared a range of phenotypic endpoints in zebrafish from four different "breeding treatments" comprising a WIK zebrafish strain and a WIK/Wild strain with three levels of inbreeding (FIT=n, n+0.25, n+0.375) in the new OECD234: Fish Sexual Development Test (FSDT), where one of the core endpoints is sex ratio. There were no differences between treatments in terms of egg viability, hatch success or fry survival. However, compared with WIKs, WIK/Wild hybrids were significantly larger in size with more advanced gonadal (germ cell) development at the end of the test. Increasing the levels of inbreeding in the related WIK/Wild lines did not affect body size, but led to a male bias (72%) in the most inbred line (FIT=n+0.375). Conversely, in the reference WIK strain there was a significant female-bias in the population (80% females). Overall, our results support the use of outbred zebrafish strains in the FSDT. Despite increased variance in some endpoints, WIK/Wild outbreds (FIT=n) met all acceptance criteria for controls, whereas WIKs failed to comply with tolerance limits for sex ratio (30-70% females). Sexual development was also more advanced in WIK/Wild outbreds (c.f. WIKs), providing greater scope for detecting developmental toxicity following chemical exposure.

Whilst considerable attention is paid to the standardisation of environmental conditions in test guidelines, pedigree and/or genetic information confirming the use of outbred strains in ecotoxicology is generally lacking. This additional information should aid further standardisation of regulatory studies.

RA21-2

Improved protocols for sediment toxicity testing: a review

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At present, tests to assess the toxicity of sediment-bound chemicals to sediment-dwelling organisms are relatively well established for a few test species. However, there is a lack of cost-effective and widely accepted methods to assess potential effects on microorganisms, macrophytes and animals across taxonomic groups, as well as methods to translate results of such tests between freshwater and marine ecosystems and to the population and community levels. Such methods, however, are crucial within the current context of criteria setting and prospective risk assessment (PRA). Here, we critically review the state of the art on prospective sediment toxicity testing with individual organic compounds in order to identify knowledge gaps and to provide guidance for optimum sediment toxicity test designs for microorganisms, macrophytes and benthic invertebrates. Focus is on freshwater, estuarine and marine systems in the temperate zone, however also with relevance for other climate zones. We synthesise an overview of recommendations from the literature on sediment preparation and spiking, microorganism, macrophyte and invertebrate tests, microcosm and mesocosm tests in the regulatory context and framework of PRA.

Standard test protocols are only available for benthic invertebrates, whereas a protocol for the freshwater macrophyte *Myriophyllum* sp. is currently under development. Tests for macrophytes, invertebrates and microorganisms to a lesser extent are described in the literature but prospective micro- and mesocosm tests are rare and highly diverse. Compared to freshwater sediment tests, marine and estuarine tests have received much less attention. In order to perform a proper sediment toxicity test, it is recommended to use spiked artificial sediment and artificial water, assess exposure by passive sampling and use selected test species. Standard species together with optimized standard test protocols form the basis of an improved first tier of sediment PRA.

RA21-3

Use of public literature and dossier data in WFD EQS derivation compared to risk limit derivation in other regulatory frameworks

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In the Netherlands, RIVM has a broad experience on both the derivation of Environmental Quality Standards (EQSs) under the Water Framework Directive (WFD) and the evaluation of dossier data within various regulatory frameworks. An overview will be given of the use of public literature in EQS derivation, which will then be compared to the use of public literature in the derivation of risk limits, e.g. PNECs, in REACH and the framework of plant protection products.

For EQS derivation according to the draft guideline of the WFD, all relevant available information should be considered. This means that dossier data submitted under the relevant frameworks should be used, but also data from open literature should be included, provided that the scientific reliability is sufficient and endpoints are relevant. For reliability, the scores of Klimisch [2] are used, with a division into four categories: 1 = reliable; 2 = reliable with restrictions; 3 = unreliable; and 4=unassignable. Only studies with a validity score of 1 or 2 can be used for EQS derivation. However, not all valid studies are also relevant for EQS derivation. Only endpoints which are relevant to the population should be taken into account. Thus, endpoints like histopathology, blood parameters, general activity, swimming speed, organ weight and results from *in vitro* tests cannot be used for EQS derivation, even if this would result in more critical values.

The use of public literature differs among the various frameworks, partly because of the specific guidelines but also because public literature cannot always be assessed for its validity. The use of public literature in regulatory framework risk assessments could seriously improve if all relevant methodological details would be described and results are reported in a clear way. Then, even if a study is not performed according to OECD guidelines or GLP, it could still be used for EQS derivation and other regulatory work. The datasets used may differ among the frameworks, not only because of different ways of literature searching, but also because of diverging choices made in assessing reliability and relevance. This means that EQSs and PNECs may be focussed on the same protection goal, but they can numerically still differ among frameworks. The challenge is to harmonise the data sources used and ensure enough transparency so that data can be used across all frameworks, irrespective if industrial or governmental parties derived the risk limit.

RA21-4

Standard and non-standard ecotoxicity tests in regulatory risk assessments of chemicals

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Guidance documents recommend the use of standard ecotoxicity tests in regulatory risk assessments [1-6]. These recommendations have decreased the use of non-standard test data for several groups of chemicals. On the positive side, this has increased the reliability of the data since the strict reporting requirement for standard tests enhance reliability. On the negative side, it could have implications for the relevance of the risk assessment. Standard tests may not always be sensitive enough to measure the specific effects expected for instance from endocrine disrupting chemicals, nanoparticles and pharmaceuticals. Bisphenol A is an illustrative example since three standard tests are preferred by several risk assessors over 200 non-standard studies with lower effect values [7].

Previous studies indicate that ecotoxicity studies published in the open scientific literature are surprisingly often insufficiently reported [8]. This may be a major reason why they are often seen as less reliable in a risk assessment context. Choice of reliability evaluation method could also affect the outcome of the evaluation [8]. Several guidance

documents recommend that the method described by Klimisch et al. [9] should be used for evaluating the reliability of ecotoxicity and toxicity data but the Klimisch criteria give a strong preference for standard tests.

We present a novel and more comprehensive method for evaluating and reporting non-standard ecotoxicity data [10]. Its aim is to enable an increased use of non-standard data in risk assessments. As part of this work we clarify the definitions of “reliability” and “relevance” in order to promote the consistent use and application of these concepts in risk assessment procedures. Lastly we present examples from environmental risk assessments of pharmaceuticals showing how non-standard data can complement standard data to arrive at robust and transparent risk assessments.

RA21-5

Toxicity and detoxification of chemicals in detergents, softeners and shampoos

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Previously environmental concern was mainly focused on the intentional release of chemicals from use of pesticides and discharges from industries. Presently concern has also been raised on non-intentional release of chemicals from articles and products like paints, textiles, tires, plastics, pharmaceuticals, and personal care and cleaning products. For all chemicals their hazard and risk is based on toxicity (including genotoxicity and sensitization), degradation and bioconcentration, either from real testing or chemometric modelling. However, for products and articles, which may contain many chemicals at confidential concentrations, assessments of biodegradation, bioconcentration and combined toxicity based on ingredient toxicity are not possible to make. Therefore, an approach similar to that used for industrial wastewaters using toxicity testing and, when necessary, TIE (Toxicity Identification Evaluation) might be used. Since many chemicals will reach the environment via sewage treatment works, their biodegradation and detoxification are important for their environmental ecotoxicity. Research on detergents, softeners and shampoos has shown that surfactants are key chemicals for the toxicity of these products, and that there is a wide range in product toxicity, as well as in their detoxification. Recent studies, using the standard ISO test with *Daphnia* (ISO 6341) and inoculation by activated sludge, have shown that toxicity (24-h EC50) have ranged from 4 to 1615 mg L⁻¹ for 26 detergents, from 25 to 225 mg L⁻¹ for 5 softeners, and from 0.54 - 163 mg L⁻¹ for 9 shampoos. This variation suggests that there is a considerable potential for toxicity reduction among these products either through replacement of entire products or by chemical substitution. The former needs more transparency of environmental labelling and a more sophisticated labelling into more than one category. The latter implies a better communication and co-operation between academia, industry and regulators. Recent research on detoxification has shown a wide range also in detoxification and differences between products in abiotic (without activated sludge) and biotic (with activated sludge) degradation. Even if the reasons for all variation is not known it is obvious that this aspect should be considered together with toxicity in the hazard and risk assessment.

RA21-6

Aquatic toxicity of halogen-free flame retardants

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Several halogenated flame retardants have been banned due to their persistence, bioaccumulation potential, toxicity (PBT) and probable environmental risk. Hence, alternative flame retardants are required and a range of new flame retardants have been proposed. The PBT properties of most alternative flame retardants are, however, poorly characterized. Therefore, the aim of the present study was to make an inventory of the available data on aquatic toxicity of a selection of halogen-free flame retardants (HFFRs) that are already commercially available. The data were classified according to the REACH system, meaning that we assigned the categories “high”, “moderate” and “low” (< 1 mg/L, 1-10 mg/L & > 10 mg/L respectively). Since data gaps exist, the acute aquatic toxicity to *Daphnia magna* was determined for the selected compounds. To determine the EC50 values, the compounds were subjected to 48 h immobility tests with *Daphnia magna*, based on OECD guideline 202. For each compound, first the concentration that equals the maximal water solubility (max. Sw) was tested. Second, if an effect was observed at max. Sw, a dilution range was prepared (4 concentrations per compound) and tested to determine the EC₅₀. The alternative flame retardants studied included six organic phosphates (Aluminium Diethyl Phosphinate (ALPI), Bisphenol A bis(diphenyl phosphate) (BDP), Dihydro Oxa Phosphaphenanthrene (DOPO), Melamine Polyphosphate (MPP), Resorcinol bis(diphenyl phosphate) (RDP), Triphenyl Phosphate (TPP) and six inorganic compounds (Aluminium Trihydroxide (ATH), Ammonium Polyphosphate (APP), Antimony Trioxide (ATO), Magnesium Hydroxide (Mg(OH)₂), Zinc Hydroxy Stannate (ZHS) and Zinc Stannate (ZS)). Tetra bromobisphenol A (TBBPA) was tested as well, as a reference compound for halogenated flame retardants. TPP and TBBPA had a high toxicity to *D. magna*. In contrast, the literature review revealed that ATO, APP and ZHS did not show high aquatic toxicity. Also, these compounds and Mg(OH)₂ and ZS showed low acute toxicity to *Daphnia magna*. Therefore, these compounds were considered here to be the most promising HFFRs. However, the review showed as well that for these compounds still large data gaps exist. To assess whether the presently studied HFFRs are actual viable alternatives, these gaps should be filled and each compound should be examined individually by comparing its toxicity values with those of the relevant halogenated flame retardant.

RA22 - The use of rodenticides, a nagging issue on effectiveness and risks

RA22-1

Anticoagulant rodenticide uptake in resistant rat populations

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Anticoagulant rodenticide (AR) resistance in rat populations has been a problem for fifty years. However, its impact on non-target species, particularly predatory and scavenging animals has received little attention. Field trials were conducted by RRAC on farms in Germany and by the University of Reading on farms in England, where resistance to anticoagulant rodenticides had been confirmed. Resistance is conferred by different mutations of the VKORC1 gene in each of these regions: tyrosine139cysteine in Germany and leucine120glutamine in England. A modelling approach was used to study the transference of the ARs into the environment during AR treatments for Norway rat control. Baiting with brodifacoum resulted in lower levels of AR entering the food chain via the rats and lower numbers of live rats carrying residues of AR during and after the trials due to its effectiveness against resistant rats. Bromadiolone and difenacoum resulted in markedly higher levels of AR uptake into the rat population and more live rats carrying residues during the trials and for long periods after the baiting period. Neither of these compounds provided full control on any of the trial farms. Secondary non-target predators were predicted to take up more AR when residues in rats were higher and the more rats remained alive. In resistant areas where ineffective compounds are used there is the potential for higher levels of AR exposure to non-target animals, particularly predators of rats and scavengers of rat carcasses. Thus, resistance influences the total amount of AR available to non-targets and should be considered when dealing with rat infestations, as resistance-breakers may present a lower risk to wildlife.

RA22-2

What do anticoagulant rodenticide residues in predatory birds and mammals tell us about non-target exposure and risk?

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Anticoagulant rodenticides (ARs) are non-specific in their toxicity and so liver residues have been measured in non-target species to gain information on scale of exposure and to assess the likelihood of effects. The second-generation anticoagulant rodenticides (SGARs) have been a focus for monitoring because their widespread use, high acute toxicity and persistence enhance potential for secondary exposure and poisoning. We review what monitoring has told us about the key factors that mediate exposure of non-target species and the likelihood of adverse effects.

Exposure of predatory birds and mammals to SGARs is widespread but species vary in their accumulation of liver residues. *Diet* is assumed [but rarely shown] to be a major factor yet significant differences in residue accumulation occur between species with similar diets. However, a recent study strongly suggests that diet can indeed be a key factor. When prey guild is restricted, foxes (*Vulpes vulpes*) feed relatively extensively on commensal rodents and other species likely to take AR bait. As a result, they accumulate greater SGAR residues than animals from areas where prey choice is wider and includes species unlikely to encounter bait. *Usage* is also likely to influence exposure. At large spatial scales, the overall pattern of wildlife exposure reflects SGAR usage but the relationship between use and exposure appears more complex at a local scale. The way SGARs are used, rather than the total amount used, is likely to be of prime importance. *Resistance* to ARs in commensal species may also affect exposure in predators. Resistant rats survive (and are available to predators) for longer than non-resistant individuals and may also accumulate higher AR body burdens. The importance of resistance in mediating exposure and risk remains a key question. It can be addressed through analysis of wildlife residues but first requires that resistance areas are mapped.

The relationship between magnitude of liver residue and likelihood of mortality in wildlife is poorly defined. A “potentially lethal range” has been proposed for barn owls (*Tyto alba*) but is poorly defined, not diagnostic and may vary between ARs and species. New probabilistic analyses to assess the likelihood of mortality associated with any given AR residue offers an exciting new approach that may allow, for the first time, estimation of likely toxicity at a population level and identify inherently sensitive species.

RA22-3

Reflections on the risk of first generation Anticoagulant Rodenticides to Raptors

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In 2008, the United States Environmental Protection Agency placed new regulatory restrictions on the use of second generation anticoagulant rodenticides. These changes are expected to result in expanded use of older first generation indandione compounds (FGARs; e.g., diphacinone, chlorophacinone). Recent acute oral and 7-day dietary diphacinone toxicity studies conducted in American kestrels (*Falco sparverius*) and Eastern screech-owls (*Megascops asio*) suggest that raptors are considerably more sensitive to FGARs than predicted by data developed with traditional avian wildlife test species (Northern bobwhite, *Colinus virginianus* and mallards, *Anas platyrhynchos*). Many studies have demonstrated that the toxicity of FGARs to target rodent pest species is more pronounced in a multi-day exposure scenario. Likewise, some data indicate that the toxicity of FGARs to non-target wildlife is greatly enhanced in a repetitive dietary exposure scenario. Regulatory agencies continue to require and use acute oral toxicity data (i.e., LD50 derived from single or multiple doses administered in a 24-hour period) as a significant component of their ecological risk assessments. While a valuable measure of toxicity, the LD50 can underestimate the toxicity of FGARs that can be more toxic when consumed over several days. Additional information examining the effects of varying the frequency and duration of FGAR exposure would improve risk assessments. Furthermore, sublethal FGAR responses (e.g., bleeding, coagulopathy, histopathological lesions) that may constitute biologically significant adverse effects are considered, but are given less weight in the overall risk assessment. Some of these adverse effects could affect survival of free-ranging birds in ways that would not be apparent in a controlled laboratory setting. Choice of test species, laboratory exposure regimens and toxicity endpoints, as well as better characterization of the probability of exposure in field situations, deserve further attention to more accurately assess the risk that FGARs pose to non-target wildlife.

RA22-4

Investigating the potential risk of secondary rodenticide poisoning to urban owls inhabiting and foraging in urban landscapes of the Lower Mainland, British Columbia, Canada

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Anticoagulant rodenticides are widely used to control pest rodents, but poisoning of non-target wildlife has been linked to these practices, including secondary poisoning of birds of prey, particularly owls. In this study, we investigate whether Barred owls (*Strix varia*), Great-horned owls (*Bubo virginianus*) and/or Barn owls (*Tyto alba*) inhabiting and foraging in predominantly urban landscapes of the Lower Mainland, British Columbia are at risk of consuming rodenticide-laden prey, such as rats and house mice. By conducting a pellet study, we found that urban Barred owls had the largest proportion of rats in their diet, with some individuals' diet consisting primarily of rats. Urban Great-horned owl pellets were also comprised mainly of rats, but there was a clear shift towards alternative prey base when urbanization within home ranges decreased. Field voles (*Microtus townsendi*) were the main prey item for Barn owls, regardless of the amount of urbanization within their home range. For all three species, consumption of rats and house mice appears to coincide with increased urbanization within home ranges. The shift in the diet of owls living in urbanized areas may potentially lead to an increased risk of secondary rodenticide poisoning. Radio telemetry was deployed to further investigate which landscape features urban Barn owls select as foraging habitat and whether they forage in proximity to buildings where rodenticide is applied. Urban Barn owls were found to predominantly forage in grass strips along highway interchanges and verges, and untended grass patches within the city. The majority of foraging was done within 100 m of commercial buildings where rodenticide had been applied. These findings will be discussed in conjunction with previous research done on rodenticide residues found in the livers of deceased owls and the current and historic sales of rodenticides in the Lower Mainland, British Columbia.

RA22-5

How the uses of anticoagulant rodenticides influence the distribution of their residues in rodent community?

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In Europe, the uses of anticoagulant rodenticides (AVKs) are regulated as biocides (Directive 98/8/EC) or as plant protection product (Directive 91/414/EE referred as pesticide uses hereafter). According to these uses, the active ingredients (a.i.) found in the commercial products and the quantities that are applied in the environment may be very different. Biocide uses of AVKs correspond to a large diversity of a.i. (8 molecules are homologated) which are applied around villages and habitations at relatively small quantities locally. Plant protection is authorized with 3 a.i. only and is commonly realized in eastern France with large amount of bromadiolone poisoned baits (up to 20 kg / ha) over vast areas (e.g., 12,000 ha in 2006) to control Water vole outbreaks. If large mortality events of rodent predators are generally associated to pesticide uses, secondary exposure of wildlife to biocide AVKs is now widely reported. Here, we aim to document the distribution of 8 AVKs in the rodent community according to the uses in eastern France. For each context of uses, 2 areas were selected and the localization and intensity of AVKs treatments were characterized as precisely as possible. Then, both target and non-target rodents were trapped in autumn up to 1 km from the place where AVKs treatments have been identified. The residues of the 8 AVKs authorized in Europe were measured in the liver and the whole body of 100 specimens selected in each area. Anticoagulant rodenticides were largely distributed in the rodent community of the treated areas whatever the type of uses. In the pesticide area, a high proportion of individuals in both target and non-target species were exposed to bromadiolone (45%) and the high levels of residues in some individuals (> 50,000 ppb in the liver for some Water voles and mice) may explain lethal poisoning of rodent predators. In biocide areas, median liver concentrations of all AVKs were 3.5 and 1.3 ppb in target and non-target species respectively, 25% of the specimens trapped exhibiting detectable residues of AVKs in the liver. This suggests that predators are frequently exposed to low doses of different AVKs but the impact of sublethal exposure of wildlife to rodenticides remains under question.

RA22-6

Risk mitigation measures for anticoagulants used as rodenticides in Sweden

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Anticoagulants used as rodenticides are non-selective, highly toxic substances. Second generation anticoagulants (SGARs) (bromadiolone, difenacoum, brodifacoum, flocoumafen and difethialone) are also persistent with extremely slow elimination from the body, and prone to accumulate in non-target species that consume poisoned rodents (secondary poisoning). They have characteristics which meet the criteria to be classified as potential PBT and potential vPvB substances. Furthermore, monitoring studies in several countries have shown high levels of second generation anticoagulants in predatory mammals, raptors and owls. Nevertheless, SGARs have been included in Annex I to Directive 98/8/EC because of their identified benefits for public health and the lack of established alternatives which are at the same time equally effective and less damaging to the environment.

If SGARs are to be used, extensive risk mitigation measures need to be applied in order to reduce the risks for primary and secondary exposure for humans, non-target animals and the environment. The Swedish Chemicals Agency finds it appropriate to restrict authorisations of rodenticides containing SGARs to user category "class 1 - professional use with a specific permit". In combination with other risk mitigation measures, this is considered an appropriate way to reduce the risks yet allowing for effective rodent control.

RA23 - Wastewater effluent discharges: chemical characterisation and understanding potential risks in receiving waters

RA23A-1

The UKWIR Chemicals Investigation Programme

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Recent EU legislation in the field of water and the environment, in particular the Water Framework Directive (WFD), has important implications for the scope and nature of pollution control measures required to protect surface waters. Environmental Quality Standards (EQSs) have been set for substances that hitherto have not been subject to detailed monitoring or control. These standards have, in turn, generated a need for compliance assessment and, where necessary, the development of appropriate control measures. The UK Water Industry has responded to this challenge by collaborating with national environmental regulators to undertake a £25M programme of investigations of contaminants in wastewater, their fate and behaviour in wastewater treatment and their sources within urban sewer catchments. Effort is focussed on a range of over sixty contaminants, including priority substances regulated at European level, specific pollutants regulated at national level and a range of substances, including pharmaceuticals, of emerging importance. The project is intended to identify and prioritise substances that are likely to require future action under the Water Framework Directive. It also seeks to establish the sources of different substances and to determine the most effective forms of control, including measures not related to end of pipe treatment. This paper provides an overview of the chemicals investigation programme, describes its key outputs and provides examples of how the results of the programme have been used in order to prioritise proposed future measures to be implemented as part of the WFD. The relative performance of different wastewater treatment processes will be discussed in relation to the achievement of good chemical status for surface waters.

RA23A-2

Using target and non-target LC-MS/MS screening to characterise the presence of known and unknown micropollutants in wastewater

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Characterising the presence of organic micropollutants in wastewater effluents is fraught with challenges. The full assessment of the effectiveness of treatment options is often difficult as many organic micropollutants transform during the treatment process. Thus, a pure target screening approach may indicate that a compound is no longer present following treatment, while the compound is still present but in a transformed state. We show here how this can be addressed using a combination of target and non-target screening approach on wastewater effluents. The program *enviMass*, developed in-house, was used to perform target and non-target screening of waste water effluents from around Switzerland. Between 97 and 111 of 376 target compounds were found and quantified using *enviMass*, with concentrations from low ng/L (lowest detection limit 5 ng/L) to µg/L levels. The number of transformation products detected as targets (over 10 per sample of a total of 30 TPs) indicates the significance of accounting for transformation processes in wastewater effluents and thus considering also “unknown” peaks when assessing samples. The number of non-target peaks varied greatly between the different plants, as did the presence of isotope peaks (providing some idea of peak identity) for positive and negative ionisations. Over 40 % of negative non-target peaks had sulfur present, attributed mainly to sulfonic acids. In contrast, many homologous series were detected in positive ionisation samples, generally resulting from peptides. A comparison of peak intensities for target and non-target peaks revealed that of the top 20 peak intensities in negative mode, only 4 were target compounds. These were three artificial sweeteners (acesulfam, saccharin and cyclamate) and a pharmaceutical (diclofenac). The remaining non-target peaks are clearly a significant part of the sample, despite the comprehensive target list of environmentally relevant compounds selected for relevance to Swiss conditions and based on experience. Despite not knowing the identity of the non-target peaks, we show here that we can follow target compounds, transformation products and even non-target masses in wastewater effluents using an in-house software which is available for public use. The information extracted in the *enviMass* workflow can be used to quantify target and TPs as well as prioritise non-target compounds for identification and track these peaks in future monitoring programs.

RA23A-3

Occurrence of Pharmaceuticals on a sewage impacted section of a Mediterranean River (Llobregat, NE Spain) and their behaviour under different hydrological climate conditions

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Mediterranean rivers are characterized by high flow variability, strongly influenced by seasonal rainfall. In addition, the river receives effluent discharges of more than 55 WWTPs, and at some points especially at drought periods, the effluents may represent almost 100 % of the total flow of the river. When water scarcity periods occur, water flow and dilution capacity of the river is reduced. On the other hand, floods contribute to remobilization of pollutants from sediments. Consequently, the potential environmental risk of pollutants is expected to increase. Besides, due to continuing human pressure from extensive urban, industrial and agricultural activities, contamination levels in Mediterranean rivers are frequently higher than in other European river basins.

3,000 different pharmaceuticals (PhACs) are used in human medicine in the European Union. The main route of PhACs into the aquatic environment is excretion by humans and the direct disposal through domestic wastewater. Despite its previous treatment in WWTPs, depending on the efficiency and chemical properties of the compound are able to reach surface and ground waters. PhACs are widespread pollutants. Around 150 PhACs have been detected in the aquatic environment at levels in the ng/L range. However, little attention has been paid to behaviour of PhACs in surface waters.

In this context, this work aimed to trace presence of PhACs in sewage impacted surface waters and to determine relationships of levels of PhACs and flow under different hydrological conditions in order to gain information about effects of floods and droughts on these relationships. Sampling period (October 2009-July 2010) covered storm-flow events and river base-flow periods. Sampling sites were located downstream a dominant WWTP. A multiresidue analytical method based on LC-MS/MS after solid-phase extraction was used for sample analysis. 90 % of compounds targeted were present in at least one of the samples analysed. The most concentrated were at levels higher than 500 ng/L. Analgesics and antiinflammatories were the most ubiquitous and concentrated therapeutic group, determined in the range of 700-1700 ng/L. Target compounds were detected following an increasing gradient together with number of WWTPs distributed along the river section studied. Positive and negative correlations were observed pointing out the relevance of different hydrological phenomena like dilution effects or sediment re-suspension.

RA23A-4

PACs during wastewater treatment and in receiving waters - emerging issues

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Pharmacologically active compounds (PACs) are emerging contaminants found in surface waters at ppt levels. Thousands of PACs are approved for human/veterinary use, although only a very small percentage of these compounds have been studied in the environment. Some of the most commonly used PACs are sold in hundreds of tonnes/year in the UK alone. PACs enter the environment mainly through insufficiently treated sewage, waste effluents from manufacturing processes, runoff and sludge (if used as fertiliser or transported to landfill). They are ubiquitous and persistent (due to their continuous introduction into the environment) with synergistic properties. PACs have also been detected in drinking water, which poses a direct risk to humans and raises the issue of contaminated water sources. The necessity of research into PACs is widely acknowledged and the need for action to further improve our understanding of risks posed by PACs is often highlighted.

The aim of this presentation is to discuss efficiency of different wastewater treatment processes in the removal of PACs and to raise awareness of two important but underreported issues relating to the presence and fate of PACs in wastewater and their ecotoxicity. These are: (i) the possibility of under-reporting of the measured concentrations of certain PACs due to lack of routine measurement of these compounds in solids and (ii) possibility of a significant under or overestimation of toxicity of chiral PACs and incorrect environmental risk assessment due to lack of data on enantiomer-specific fate of these compounds during wastewater treatment and in the environment.

RA23A-5

Assessment of WWTPs efficiency to limit surface water pollution by illicit drugs

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Water contamination resulting from consumption of illicit drugs is a new concern for water management that must be considered, not only because of the social and public health aspects, but also in an environmental context, because of the contamination of surface waters by partially treatment efficiency of WasteWater Treatment Plants (WWTPs) discharging contaminated effluents.

It is now established that WWTPs influents contain illicit compounds at concentration included between some ng.L⁻¹ to several µg.L⁻¹ and leading to surface water pollution by WWTPs effluents incompletely treated. However, data are missing concerning illicit drug metabolites. In this way, we decided to study 18 drugs and metabolites and to compare WWTPs removal efficiency as a function of volume capacity and technologies of the treatment trains.

RA23A-6

Are the concentrations of micropollutants responsible for the reduction in wastewater toxicity for gammarids following the application of ozone?

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Micropollutants, which comprise among others pharmaceuticals and personal care products, enter the aquatic environment via secondary treated wastewater and are thus frequently detected in surface waters. To counteract the continuous release of these micropollutants, advanced treatment methods are currently under discussion. In this context the application of ozone is considered as an effective tool. However, its ecotoxicological implications are largely unknown. Therefore, the aquatic toxicity of secondary (=non-ozone) or ozone treated wastewater were assessed using the feeding rate of the leaf-shredding invertebrate *Gammarus fossarum*, which is known as a key-species in the ecosystem function of leaf litter breakdown. Two repetitive experiments resulted in significantly higher feeding rates for gammarids exposed to ozone compared to non-ozone treated wastewater sampled from a treatment plant equipped with a full-scale ozonation. While ozone was applied at the lab-scale, a further experiment confirmed the detoxification of wastewater from the same treatment plant following its treatment with ozone. Moreover, the deviations in the dissolved organic carbon profiles of ozone and non-ozone treated wastewater could be excluded as a driving factor, which required further experiments addressing this gap of knowledge. The two additional experiments displayed on the one hand a statically significant higher feeding rate of *G. fossarum* if exposed to ten-fold enriched eluates from solid phase extraction cartridges loaded with ozone compared to non-ozone treated wastewater. On the other hand, the mean feeding rate of gammarids exposed to non-ozone treated wastewater, which contained hardly any micropollutants (i.e. pharmaceuticals), was at the same level as wastewater from the same source but additionally treated with ozone. These results suggest the load (concentration) of micropollutants to trigger the effects displayed by the bioassay applied, while alterations in the organic matrix seem to be of minor importance. Hence, the feeding rate of *G. fossarum* appears to be a well-suited bioassay to indicate alterations in ecotoxicological properties of wastewater due to the application of advanced oxidation processes like ozonation.

RA23B-1

Do we need expensive advanced treatment of waste waters to prevent feminisation of wild fish?

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Oestrogenic contaminants are thought to be the major cause of feminisation (intersex) in wild fish populations. The major oestrogenic contaminants; oestrone (E1), 17 β -oestradiol (E2) and the contraceptive pill hormone 17 α -ethinylestradiol (EE2), enter the aquatic environment via wastewater treatment works following human excretion. Widely used waste water treatment technologies are not completely effective at removing these steroids down to concentrations thought to be required to protect aquatic wildlife. A number of advanced tertiary treatment processes have therefore been proposed to reduce the concentrations of oestrogens entering the environment. It is imperative that we assess their efficacy: a) in terms of meeting proposed no-effect concentrations (PNEC) for steroid oestrogens and b) in mitigating the negative impacts of these chemicals on native fish populations.

This study assessed both steroid removal and intersex induction in a native UK fish species; the roach (*Rutilus rutilus*), maintained in a wastewater treatment works effluent following a variety of secondary, tertiary and advanced processes. Both adult and early life stage roach were exposed directly to either secondary activated sludge plant (ASP) effluent or to ASP effluent plus sand filtration (SF), chlorine dioxide (ClO₂), or granular activated charcoal (GAC) for a six month period. Analytical chemistry indicated that whilst ClO₂ and GAC treatments removed E1, E2 and EE2 most effectively, their concentrations still exceeded the suggested environmental quality standards (both individually and when combined) at multiple times during the course of the study. Notwithstanding the failure to remove steroid oestrogens down to proposed PNEC, both GAC and the substantially cheaper ASP plus SF were effective in reducing or preventing the occurrence of intersex in fish exposed to these effluents. With the exception of the control and GAC, intersex was induced in all other treatments, including the ClO₂ treatment, where it was seen at a comparable level to the ASP effluent, despite the fact that this effluent exhibited the lowest concentrations of steroid oestrogens. Our results highlight the possible danger of relying on chemical analyses alone to assess sewage treatment options for removing hazardous substances and indicate that nitrifying ASP treatment followed by SF may provide the most cost effective strategy for mitigating feminisation in wild fish caused by endocrine disrupting chemicals.

RA23B-2

Fate of oestrogens in a tertiary nitrifying sand filter

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The fate of hazardous compounds was assessed in a full-scale nitrifying sand filter as part of the chemical investigations programme, a UK national scheme instigated by the Water Industry and the Environment Agency to examine the fate of target compounds over a wide range of process units with the ultimate goal of developing adequate measures to meet the requirements of the Water Framework Directive.

Natural and synthetic estrogens are endocrine disrupting chemicals that can cause adverse effects on the sexual and reproductive systems in wildlife, fish and humans [1].

The nitrifying sand filter examined was a tertiary treatment process designed to remove wastewater solids and perform nitrification by specialized bacteria growing in a biofilm on the sand particles. The sand was continuously fluidized to control biomass growth and the accumulated solids removed by an airlift system [2].

From the 49 substances investigated a detailed overview on the fate of selected oestrogens: estrone, 17 β estradiol and 17 α -ethinylestradiol is provided. The wastewater quality, the sand filter performance and operation were also taken into consideration as well as the chemical-physical characteristics of the target compounds.

Several studies have reported a possible connection between nitrification and oestrogens removal [3], others have underlined the relevance of solids retention time [4] and the removal through sorption onto wastewater sludge [5]. The implications of using tertiary treatment processes for hazardous pollutants removal on energy and investment are also taken into account.

Final effluent concentrations were found to be in agreement with the values reported on literature [3]. Removals of E2, E1 and EE2 from the main stream were 89%, 78% and 21%, respectively. For proposed environmental quality standards E2 was always below the reference value of 1 ng/L; average concentration of E1 was below the reference 3 ng/L; but EE2 was always above 0.1 ng/L.

Nitrifying sand filters remain a good candidate for an end of the line process for removal of oestrogens from wastewater although improvements are needed to meet environmental quality standards for 17 α ethinylestradiol.

RA23B-3

The response of wild fish to Municipal Wastewater Effluent Exposures at sites in Canada

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The extent and implications of Municipal wastewater effluent (MWE) of fish in the Canadian environment is currently poorly understood. The objective of this research was to examine the impact of MWEs on the status of sentinel fish species at various levels of biological organization (cellular to community). Field studies conducted in the fall (2007) and spring (2009) in the Grand River watershed, Ontario, Canada, investigated the cumulative impact of two sewage discharges in an urban environment at reference sites upstream of the municipality of Waterloo, and at an intermediate site located between the two sewage treatment plant sites for Waterloo and Kitchener. Responses of wild fish [Rainbow Darter (*Etheostoma caeruleum*) and Greenside Darters (*E. blennioides*)] were assessed in terms of energy storage (condition factor), and energy utilization (reproduction: in vitro sex steroid production, gonadosomatic indices, and histology). Both sentinel species collected downstream of both discharges demonstrated greater growth (longer and heavier), however fish were not assimilating additional resources into energy storage (increased condition, liver somatic index). Exposed male darters had impaired capacity to produce androgens in vitro, lower GSI and altered sperm cell staging. Exposed female fish also had impaired capacity to produce estrogens in vitro. Intersex in male darters collected during the fall downstream of both sewage discharges studied while upstream agricultural and urban reference sites demonstrated low levels of intersex. Pre-spawning darters demonstrated dramatically higher incidence of intersex at both the near-field and far-field exposure sites and coincide with reductions in gonadosomatic indices and steroids. The fish communities downstream of these outfalls demonstrated differences in abundance, diversity, and species composition when compared to the fish community upstream or further downstream of the points of effluent discharge. Changes in the species composition downstream of the outfalls occurred with larger more mobile, tolerant fish species such as suckers and sunfish becoming more common. The potential exists for a cumulative impact of multiple outfalls of treated wastewater effluents as changes were more pronounced downstream of the second sewage discharge. This research demonstrates the potential cumulative effects of MWE discharges in Canadian rivers on fish responses at various levels of biological organization.

RA23B-4

Reducing and monitoring toxicity in an industrial effluent, using a regulatory approach

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Between 1997 and 2000 the UK government started to try to regulate complex effluents using Direct Toxicity Assessment (DTA). Sites were selected, effluents screened and then ranked for further investigation. This was followed by characterisation, risk assessment, toxicity reduction efforts and monitoring.

In this case study we explore a data set spanning 8 years where we applied these processes for an industrial chemical company, resulting in a collaboration that significantly reduced the final effluent acute toxicity.

Effluents were tested using *Tisbe battaglia* and *Skeletonema costatum* bioassays. High toxicity was identified and it was decided that the effluent needed moderating before it could be allowed to be disposed of to river. The most toxic samples were analysed and Phenol and formaldehyde were identified as key contaminants. Their contribution to the overall toxicity was calculated using a toxic units approach. Assays were performed according to the UK EAs Standard Committee of Analysts methods and were carried out every month from 2003 until the present. The most toxic samples taken through the industrial process were analysed for phenol and formaldehyde and found to have high concentrations in the 1000's mg/l range. A new piece of equipment was introduced to scrub these specific chemicals from the effluent before it was discharged to the environment.

Following the introduction of the new plant in 2006, detectable toxicity in the two bioassays dropped significantly and has remained low, apart from one unexplained peak in 2008.

This shows the success of the DTA approach and the potential it has to lead to solutions that work for industry and the regulators in reducing toxic inputs to the environment.

RA23B-5

Evaluating advanced treatment of hospital wastewater

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This study aims to evaluate the importance of hospitals as point sources of pharmaceuticals into the environment and to propose possible measures to minimize the pharmaceutical input. For that purpose, a pilot-scale wastewater treatment membrane bioreactor (MBR) was operated at a Swiss cantonal hospital over one year. Part of the

MBR permeate was further treated either by powdered activated carbon (PAC), ozone, or photocatalysis (UV/TiO₂). Employing robust sampling strategy and quantifying over fifty highly consumed pharmaceuticals by online SPE-LC-MS/MS provides robust and reliable results. Measurements of species-specific resistance determinants as well as multi-resistant *Pseudomonas aeruginosa* have shown that hospitals release high amounts of antibiotic resistant and multi-resistant bacteria to the environment. Hospitals are also major contributors to the load of contrast media, some anti-infectives and anesthetics in the environment. Decentralized hospital wastewater treatment is technically possible and ozonation as well as PAC treatment can be recommended as techniques to reduce pharmaceutical load as well as ecotoxicological effects. For most pharmaceuticals high elimination rates were achieved with both treatments; lowest eliminations were observed for the contrast media diatrizoate and ioxitalamic acid. Elimination efficiencies observed in hospital wastewater treatment were similar to the ones in municipal wastewater treatment. Results will be used to support the legal decision whether or not decentralized hospital wastewater treatment would be an option for Switzerland.

RA23B-6

An examination of the toxic properties of water extracts in the vicinity of an oil sand extraction site

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The industrial extraction of oil sands (OS) in northern Alberta, Canada, has raised concern about the water quality of the nearby Athabasca River. The purpose of this work was to examine the toxic properties of various water extracts on *Oncorhynchus mykiss* trout hepatocytes. The water samples were fractionated on a reverse-phase C18 cartridge and the levels of light-, medium- and heavy-weight polycyclic aromatic hydrocarbons (PAHs) were determined by fluorescence spectroscopy. Primary cultures of trout hepatocytes were exposed for 48 h at 15°C to increasing concentrations of the C18 fraction corresponding to 0.02, 0.1, 0.5 and 2.5X concentrations from upstream/downstream sites in the Athabasca River, groundwater samples, OS tailings and interceptor well-water samples. Changes in cell membrane permeability, activity of phase I and phase II biotransformation enzymes (cytochrome P4501A and glutathione S-transferase activities), oxidative damage (lipid peroxidation LPO) and genotoxicity (single and double DNA strand breaks) were monitored in post-exposure cells. The water samples produced minor changes in membrane permeability but did increase all the above endpoints at thresholds of between 0.02 and 0.1X the water concentration. The most responsive biomarker was DNA damage but it also offered the least discrimination among sites. LPO was stronger at sites downstream of the industrial operations compared to upstream sites. A decision tree analysis was performed to formulate a set of rules by which to identify the distinctive properties of each type of water sample. The analysis revealed that OS tailings and interceptor waters were characterized by an increased concentration in light PAHs (> 42 µg/L) and this fraction represented more than 85% of the total PAHs. These samples also inhibited GST activity, which could compromise the elimination of genotoxic PAHs present in the system. An analysis of groundwater samples revealed a contamination pattern similar to that for OS tailings. There is a need for more research into specific biomarkers of toxicity from OS tailings compounds such as naphthenic acids, light PAHs among others, which are a characteristic fingerprint of OS extraction activities.

MO 001

Calibration and field deployment of five integrative samplers for the monitoring of indicator and dioxin-like PCB (ECLIPSE project)

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Various integrative samplers, at different stages of development, are now available to estimate time-weighted average (TWA) concentrations of freely dissolved hydrophobic contaminants in aquatic environments. The ECLIPSE project (2009-2011, coord. C Miege, Irstea) involves 5 laboratories and aims to calibrate and compare 5 different integrative samplers for the monitoring of indicator and dioxin-like PCB in water: semi-permeable membrane device (SPMD, studied by Irstea), low-density polyethylene strip (LDPE, Ifremer), silicone rubber (SRPS, Deltares), Chemcatcher® (BRGM) and continuous-flow integrative sampler (CFIS, LABAQUA).

The first step of the project was the simultaneous calibration of these samplers under laboratory conditions. During summer 2009, samplers were exposed under constant agitation and temperature in a stainless steel tank filled with 200 L of PCB contaminated water. A constant PCB concentration of about 1 ng/L was achieved by immersing a large amount of silicone rubber sheets [Rusina 2010] spiked with indicator and dioxin-like PCB. Prior to deployment, samplers were spiked with Performance Reference Compounds (PRC) and exposure durations ranged from 1 day to 3 months. After exposure, processing and analysis, samplers were compared in terms of sampling rate, linear uptake phase duration, repeatability and accuracy of calculated TWA concentration.

The second step of the ECLIPSE project was the deployment and comparison of these samplers in real aquatic environments. In summer and autumn 2010, and in spring 2011, three field campaigns were carried out along the Rhône River and near the lake Le Bourget (France), known to be contaminated by PCB. Using appropriate cages, canisters and holders, studied samplers were exposed simultaneously at an approximate depth of 1 m during 1, 2, 3 and 4 weeks with duplicates and/or triplicates. These field campaigns allowed to compare in-situ ease of use and robustness of the five integrative samplers in addition to repeatability of sampling and TWA concentration estimate.

MO 002

An international collaborative study on the use of passive samplers in monitoring of emerging pollutants

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It is now being recognised that passive samplers can play a valuable role in monitoring water quality within a legislative framework such as the European Union's Water Framework Directive (WFD). The time-integrated data from these devices can be used to complement chemical monitoring of priority and emerging contaminants which are difficult to analyse by conventional spot or bottle sampling methods, and to improve risk assessment of chemical pollution. In order to increase the acceptance of passive sampling technology amongst end users and to gain further information about the robustness of the calibration and analytical steps, several inter-laboratory field studies have recently been performed in Europe. Such trials are essential to further validate this sampling principle and to increase the confidence of the technological approach for end users. An inter-laboratory study on the use of passive samplers for the monitoring of emerging pollutants was organised in 2011 by the NORMAN association (Network of reference laboratories for monitoring emerging environmental pollutants; www.norman-network.net) together with the European DG Joint Research Centre to support the Common Implementation Strategy of the WFD. Thirty academic, commercial and regulatory laboratories participated in the passive sampler comparison exercise and each was allowed to select their own sampler design. All the different devices were exposed at a single sampling site to treated waste water from a large municipal treatment plant. In addition, for each target analyte class the organisers deployed in parallel, multiple samplers of a single type which were subsequently distributed to the participants for analysis. This allowed an evaluation of the contribution of the different analytical laboratory procedures to the data variability. The results obtained allow an evaluation of the potential of different passive sampling methods for monitoring selected emerging organic pollutants (pharmaceuticals, polar pesticides, steroid hormones, fluorinated surfactants, triclosan, bisphenol A and brominated flame retardants). The exercise was a great learning experience for organizers and participants. The results will be used to inform EU Member States about the potential application of passive sampling methods for monitoring these compounds within the framework of the WFD.

MO 003

Method for passive sampling of TBT in surface waters using silicone rubber - field application

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Maximum permissible concentrations of tributyltin (TBT) in the aqueous phase are lower than the limits of detection for common analytical methods. Passive sampling can accumulate contaminants from large volumes of water and was suggested as a method for the determination of TBT in surface waters. Analytical aspects of passive sampling using silicone rubber and the determination of parameters to convert sampler uptake into aqueous phase concentrations, i.e. sampler-water partition coefficients as well as diffusion coefficients in sampler material were investigated. Sampler water partition coefficients were higher than expected from octanol water partition coefficients and suffered from the presence of particulate matter. Diffusion coefficients for mono and di-substituted organotin compounds were low, such that diffusion in the sampler is partly controlling the uptake. This requires that for calculation of aqueous phase concentrations both water boundary layer transport and diffusion in the sampler needs to be taken into account. Several field trials were executed. Samplers were deployed for 6-8 weeks, in harbor areas in the Netherlands and at 6 sites in the UK, on a transect from the tidal limit of the Thames down to the Outer Estuary. This poster describes the method developed as well as results of the field and evaluates the applicability of silicon rubber as a suitable passive sampler for butyltin compounds.

MO 004

Comparison of uptake rates of pharmaceuticals and organophosphate flame retardants in a range of passive samplers

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Passive samplers have been widely used to a range of contaminants. In this study, a range of passive samplers (Chemcatcher (polar), Chemcatcher (non-polar), POCIS, SPMD and PDMS (silicon rubber)) were assessed in a laboratory study. The samplers were deployed in a tank with a continuous flow of water from a header tank, which was set at a rate to allow complete replacement of the water in 30 minutes. Into the header tank, a known concentration of chemical was dosed. Compounds investigated were pharmaceuticals and Organophosphate flame retardants.

The purpose of this tank test is to determine suitability of each of the four samplers in the analysis of these compounds, and to establish sampling rates for the compounds of interest.

The water replenishment meant that we could be confident that any removal of the contaminant by samplers was not sufficient to significantly reduce the concentration of that contaminant in the water phase (meaning that the concentration could be assumed to be constant). Samplers were removed from the tank weekly and the study was terminated after four weeks exposure. Samplers were then extracted and the extracts analysed for the spiked compounds. Relative uptake rates of various compounds to different samplers were established.

MO 006

Simultaneous quantitative analysis of pesticides including Phenoxy-Acids in surface water using UPLC-MS/MS with direct injection

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LC/MS/MS is widely used, in combination with solid phase extraction techniques, to achieve the very low detection levels (low ppt range) needed for the monitoring of pesticides and other pollutants like pharmaceutical drugs in surface and drinking water. Various approaches are possible, depending on the performances on the MS instrument, on the needed sensitivity and on the nature of the sample (drinking or surface water). SPE is usually done off-line, starting from a large volume of water (up to 500 ml to 1 l), with associated drawbacks (long protocols, transportation of the samples, cost). Preconcentration can also be done automatically on-line, with different drawbacks (recoveries for compounds from different classes, robustness of the method for surface water). The evolution of HPLC to Ultra Performance LC (UPLC) and new generation of tandem-quadrupole mass spectrometer bring additional possibilities. The analysis of most pesticides is now possible at low ppt by direct injection.

The LC-MS/MS method that we present has been developed for the quantitative analysis of pesticides including phenoxy-acids. The chromatographic separation is done using a Waters ACQUITY UPLC system. The MS detector is a Waters Xevo TQ-S tandem quadrupole, operated in ESI mode, with fast polarity switching. For each analyte, the most intense MRM transition was quantified and reported. This method allows the analysis of more than 4 samples per hour. Surface water samples were filtrated, spiked with the internal standard (deuterated compounds) before injection (250µl).

The analysis of more than 25 basic pesticides residues and of phenoxy-acids was done simultaneously in less than 15 minutes, injection to injection. This method revealed an excellent linearity across the studied range (5-500 ppt) and an excellent repeatability with RSDs typically lower than 2% over 10 injections at mid concentration. Limits of detection range from 5 ppt for the less sensitive compound (MCPB), to below 0,02 ppt for the most sensitive compound (Carbendazim). Despite the very minimum sample

preparation, the UPLC column lifetime was several thousand injections. This method brings a significant gain in time (acidic and basic compounds in a single injection), and in cost (sample preparation is limited to filtration). Possible developments on this method is the inclusion of other molecules, including PPCP (Pharmaceutical and Personal Care Products).

MO 007

Monitoring trials for detection of ionic herbicide residues in water using passive samplers

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Passive samplers for routine monitoring of pesticide residues in irrigation channels and rivers have already been developed for a number of hydrophobic compounds. However, some polar, hydrophilic ionic herbicides such as glyphosate and amitrole are either not retained in such samplers or are difficult to dislodge from membranes specifically designed to capture polar compounds. Development of new passive sampling devices for such herbicides required first the development of a suitable analytical method for detection of their residual amounts in environmental samples. An electro-chemical detector fitted to a HPLC system detected with accuracy and sensitivity these and other common herbicides found in agricultural waters (1). Secondly, SDB-RPS Empore® disk membranes were found to perform best for accumulating amitrole, and were chosen for calibrating a passive sampling device containing these membranes under flow-through laboratory conditions. Whilst SDB-XC Empore® disk membranes could be used to retain and extract glyphosate from spiked water samples filtered through them, this membrane was unable to accumulate this herbicide in a flow-through system. Instead, glyphosate accumulated in a passive sampler device consisting of Amberlite® IRA67 resin pre-washed with 1M NaCl, dried and then packed in nylon mesh bags (240 micron) that were subsequently tested under the same flow-through laboratory conditions. Both herbicides could be extracted from their respective samplers by the same extraction method using 10 mM NaOH, which facilitated the processing and analysis of the herbicides taken up by the passive samplers. A field trial to test the performance of both types of sampling devices when deployed in agricultural irrigation channels was carried out. Results from the field trials are still under way, and will be presented to show whether the mean time-integrated pesticide concentrations taken up by the passive samplers compare with the cumulative mean water concentrations calculated from daily extractions using the Empore disk membranes.

(1) Sánchez-Bayo, F., Hyne, R.V. and Desseille, K.L. (2010) An amperometric method for the detection of amitrole, glyphosate and its aminomethyl-phosphonic acid metabolite in environmental waters using passive samplers. *Anal. Chim. Acta* 675(2): 125-131.

MO 008

Ionic compounds and SPE materials - What do you have to bear in mind?

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A wide variety of environmental compounds of concern, e.g. pharmaceuticals or illicit drugs, are acids or bases that may be predominantly present as charged species in drinking water sources. These charged micro pollutants happen to exist in the environment in large numbers due to anthropogenic activities. Many of these compounds have a high affinity towards water and sampling is often difficult due to the fact that many commonly employed sorption materials are designed to adsorb neutral compounds. Therefore, sorption materials allowing coulomb interaction are often used to enable sampling of these compounds. Detailed knowledge on their sorption behaviour, however, is sparse, especially concerning passive sampling in environmental monitoring. The exact influence of both the charged and the neutral moiety on the sorption behaviour is not yet well-investigated. The influence of various inorganic ions (e.g. Ca²⁺, Cl⁻), that are commonly present in water, on the sorption affinity is just as little understood. Competition for the charged sorption sites can be expected and consequently a decrease in sorption affinity.

For this research sorption coefficients in different water matrices for various charged compounds, such as pharmaceuticals and ionic liquids, were derived from measured retention times on columns filled with ion exchangers and SiC as inert support material. The results show that apolar interaction of charged compounds with the sorption material is an important factor regarding sorption affinity. The more pronounced the apolar moiety is, the higher the sorption coefficients. Equally important is the influence of various inorganic salts in the water phase. This study shows that for e.g. metformin changing from Ca²⁺ to Na⁺ the sorption coefficients on a cation exchanger rise by one order of magnitude.

These results help to evaluate if the employment of passive samplers for a compound is useful under certain conditions. It becomes clear that sampling in e.g. Ca²⁺ rich water is more challenging than in Na⁺ containing water and that small organic ions with a lack of apolar moieties are difficult to concentrate on a passive sampling materials so far.

MO 009

Development of novel monitoring, analytical chemistry and modelling methods for improving the ecological risk assessment of radioactive caesium in the aquatic environment in East Japan

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Pollution by radiocaesium of the area surrounding the Fukushima Daiichi nuclear power plant is expected to be persistent for many decades in surface waters, soil, foodstuffs and in aquatic and terrestrial organisms. Following a nuclear accident, it is crucial to accurately measure, monitor, and model concentrations of radioactive caesium in the environment. Due to widespread dispersion of this radioelement across Japan, low-cost, simple (but accurate) monitoring methods are needed and these should be easy to interface with existing instrumental analytical techniques.

We aim to develop a passive sampling device to measure TWA concentrations of radioactive caesium in aquatic environments in East Japan based on the Chemcatcher®. No passive sampler currently exists for measuring radioactive caesium. Chemcatcher® was developed and patented by the Portsmouth University team (UoP). It is proposed that UoP models be adapted to predict long-term contamination of freshwater ecosystems in Japan using Chemcatcher® sampling data.

We are also developing new analytical procedure of radioactive caesium in aquatic environments including surface water, sediment, aquatic biota, which will take much shorter time to analyze the concentration. This presentation will show novel monitoring methods, analytical procedure and results from field monitoring for radioactive caesium in aquatic environments.

MO 010

Seasonal monitoring of organic UV filters and UV light stabilizers in coral reef water and beach water by passive samplers

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A great deal of attention has been directed to the ecological risk associated with organic UV filters and UV light stabilizers in aquatic environment, especially in beaches and coral reefs. Recreational activities such as swimming may be one of major direct inputs of organic UV filters into beaches and coral reefs. Moreover coral reefs near estuaries could be affected by river water which often contains organic UV filters and UV light stabilizers originated from human activities.

Seasonal monitoring of eight organic UV filters and 10 UV light stabilizers in coral reef water and beach water was conducted in Okinawa from March to September 2011.

Passive sampling method was adopted using TRIMPs in order to reveal seasonal variation of 18 analytes in reef waters. The Chemcatcher® was also deployed for the measurement of preservatives from cosmetics. Grab samples were also collected monthly in beach water.

Passive sampling in coral reefs revealed more than 100 times higher concentration of octyl salicylate, homosalate, benzophenone-3, EHMC and octocrylene in summer than in winter. They were also observed at much higher concentration in beach waters in summer. These results indicated that these compounds released from recreational activities, especially swimming, could have a potential ecological risk to coral reef ecosystems.

MO 011

Calibration data for Polar organic chemical integrative samplers (POCIS)

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Polar Organic Chemical Integrative Samplers (POCIS) have been originally developed for sampling polar compounds from the aquatic phase. POCIS consist of an adsorbent which is enclosed by a polyethersulfone membrane. A crucial step in order to utilize POCIS for the quantitative determination of aqueous concentrations is to determine uptake rates, which is usually done in the laboratory in separate calibration experiments.

Calibration data based on own experiments and uptake rates retrieved from literature have been collected in a database. The data set comprises more than 300 single values of uptake rates. This contribution will compare results from calibration experiments and look for relationships between the compound's properties and its uptake rate, with the final goal to predict uptake rates by properties or structures of the targeted compounds. The influence of varying environmental conditions during the calibration process will be discussed, and uncertainties in uptake rates will be examined.

MO 012

In-situ validation of 3 PRCs and calibration of 14 pharmaceuticals and 20 endocrine disruptors on the polar C18 Chemcatcher

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In response to the growth of organic pollutants identified in surface water many multi-residue analytical methods have been developed to quantify multi-families of emerging pollutants in water. Many strategies include enrichment steps for water samplings due to very low levels of pollution [1]. Recently, sampling systems more realistic of

environmental exposure have been designed to get time weighted average (TWA) concentrations of those xenobiotics. Those passive samplers enable further investigation for toxicological effects on the environment or populations of such mixture and concentrations. The polar C18 Chemcatcher has been evaluated for the monitoring of multi-families of Endocrine Disruptors and Pharmaceuticals. First C18 Chemcatcher with PES diffusion membrane have been rigorously calibrated for 34 emerging organic pollutants. Secondly a PRC approach (Performance Reference Compounds) has been investigated [2]. Samplers have been exposed for 28 days in a 50L flow through microcosm for the adsorption study and spiked samplers with tap water for the desorption experiment. Finally samplers were exposed for 3, 7 and 14 days in surface water in two rivers near Lyon. All the 34 calibrated molecules showed good correlation to first order accumulation with half time of equilibrium above 14 days for most of those molecules. Good linear relationships were obtained (R^2 from 0.90 to 0.99) for 7 to 21 days on the C18 Chemcatcher. Sampling rates were calculated between 0.005 and 0.16 L/d. 3 molecules showed accumulation and desorption consistent with first order kinetics with similar half time of equilibrium and thus verifying all criteria for their use as PRCs. Exposure campaign allowed to validate PRCs comparing TWA concentrations obtained from passive samplers to average concentrations detected in grab sampling analyzed by online-SPE-LC-MS/MS method.

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MO 013

Comparative analysis of pesticide monitoring via traditional surface water sampling and Chemcatcher® passive samplers

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Pesticide monitoring in European surface waters is regulated by the Water Framework Directive (WFD) establishing environmental quality standards (EQS) for the good chemical status of water bodies. EQS monitoring is based on analyses of pesticides in water samples. WFD requires at least monthly sampling for assessment of maximum annual average concentrations. Due to the high temporal variation of pesticide use and leaching and discharge conditions in running waters, such robust monitoring approach may fail to detect pesticides, especially peak concentrations. We compared traditional water sampling results to pesticide concentrations detected with Chemcatcher® passive samplers in a large peatland river Kyrönjoki and its small tributary Lehmäjoki. The Chemcatchers® were deployed in four separate trials on both sites for two weeks time period during 21 Jun - 30 Aug. The traditional water samples were analysed twice a month in Lehmäjoki and monthly in Kyrönjoki.

The number of compounds found in Chemcatchers® was 32, while in water samples 27 pesticides were detected but only 15 of them were quantified. High concentrations of some pesticides not detected in traditional water sampling were found with Chemcatchers®.

The preliminary results clearly indicate that measurement of pesticides from water samples is prone to failures to detect their actual presence in water bodies. Passive sampling with Chemcatcher® should also be compared simultaneously with other methods which have commonly been used earlier in monitoring organic pollutants, like natural biota, sediments and bioaccumulation using mussels.

MO 014

Estimating herbicide contamination in flowing waters using passive sampling (POCIS) coupled with an algal bioassay

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Herbicide contamination of water bodies occurs mainly through run-off and leaching from agricultural fields as well as through spray drift. In-stream concentrations of herbicides may be highly variable with peak concentrations occurring in pulses following rain events. Traditional grab sampling is of limited value because this point of time estimate of herbicide contamination fails to accurately integrate pulses in concentration. Passive sampling techniques have the potential to eliminate this problem by providing time weighted average concentrations. Of the existing passive sampling technologies available, polar organic chemical integrative samplers (POCIS) show the most promise for assessing herbicide contamination and may also be used to measure concentrations of a wide variety of polar organic contaminants. In spring and summer 2010, POCIS were deployed at sites located throughout the South Nation River watershed, an agricultural watershed in Ontario, Canada. Three POCIS per site were deployed for two 28-day periods. One litre grab samples were taken at the beginning and end of each deployment to compare passive sampling with grab sampling. Grab samples were filtered, spiked with 1 µg/L deuterated atrazine and contaminants concentrated on 500 mg Oasis® HLB cartridges. Extracts were analyzed for atrazine via LC/MS/MS. Relative toxicity of the extracts was compared by exposing the green alga *Pseudokircheriella subcapitata* to extracts using an existing bioassay method. One issue with passive sampling in general is that daily sampling rates are influenced by a number of different factors such as temperature, stream velocity and biofouling. A calibration study to estimate how widely sampling rates vary in streams with different chemical and physical properties was conducted in fall 2010 and summer 2011 at six different streams. POCIS were spiked with 5 µg deuterated desisopropyl atrazine (a metabolite with high fugacity). Both the fall and summer experiments were conducted over 28-days, with three POCIS removed from each field site weekly. Comparisons between grab sampling and passive sampling will be discussed, along with the feasibility of using desisopropyl atrazine as a performance reference compound. In addition, the merits of using of passive sampling coupled with an algal bioassay to assess herbicide contamination and direct further monitoring will be discussed.

MO 015

Performance of three passive samplers and sediment for detecting agrochemicals

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Due to intensive agricultural land use, Victorian waterways (South-East Australia) may contain a large number of agrochemicals (pesticides), some of which may prove directly toxic to organisms living therein, while others may elicit more subtle effects. Managing the effects of such contaminants ultimately requires information on effluent toxicity and chemical concentrations. Time integrative sampling of waterways with passive samplers (or passive sampling) is a technique which facilitates cost-effective monitoring of chemicals found at very low concentration in a range of aquatic ecosystems. In our presentation, we present the results of one of the most comprehensive examinations of the input of pesticides in surface waters encompassing the measurement of 84 pesticides in sediments and three different passive sampling devices in 24 streams over a period of 5 months.

Of the three passive samplers used, in short there were 28 individual pesticides observed amongst the 319 detections in the SDB-XC disk extracts. The most commonly observed chemicals ($n > 10$) were simazine, myclobutanol, pirimicarb, pyrimethanil, imidacloprid, hexazinone, metalaxyl, atrazine and carbaryl. There were 27 individual pesticides observed amongst the 319 detections in passive TRIMP sampler solvents, with the most commonly observed chemicals ($n > 10$) being dieldrin, trifloxystrobin, pyrimethanil, and pirimicarb. Only seven agrochemicals were observed in extracts, although these observations included two chemicals not observed in the TRIMP or SDB-XC disk extracts, namely malathion and propyzamide. In natural sediment 34 pesticides were observed amidst a total of 266 detections, with the most commonly observed chemicals ($n > 10$) being p,p'-DDE, simazine, myclobutanol, pyrimethanil, dieldrin and spinosad.

This study confirmed that passive samplers appear most suited to investigation of non-polar compounds, with the Chemcatcher system utilizing an SDB-XC disk most suitable for moderately polar pesticides. Passive samplers with receiving phases of different thickness such as the silicone rubber passive samplers may give valuable insights into uptake kinetics of compounds. Sediments collected a wide range of chemicals. Overall, sediments are probably most suitable to monitor a wide range of hydrophobic compounds, while passive samplers can complement important information on exposure kinetics, water concentrations and hydrophilic compounds.

MO 016

Analysis of Polar organic pollutants in the environment by means of continuous flow integrative sampler (CFIS)

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In order to obtain a good ecological state in body of water it is necessary to implant new continuous sampling system as passive or integrative samplers. In this way, the WFD and UNE-NORMES are introducing and recommending the use of new methodologies.

However, the range of possibilities for the development of new devices is relatively restricted by the theoretical principles governing passive sampling. In most of the actual passive samplers, the variation in the uptake values which result from the variations in turbulence can generate important problems in determining accurately time weighted average concentrations obtained from sampling rates calculated from tests under laboratory conditions. In samplers which are based on sorbents, the problem has been partially resolved by using performance reference compounds (PRCs). Nevertheless, the problems of turbulence vs. sampling rate are still the main sticking point of passive samplers of polar compounds in general, with sorbents governed by adsorption principles. In these cases the Rs must be estimated with a theoretical approximation. Apart of from, the Rs have been influenced by temperature and a control of this parameter is crucial in order to obtain an accurate time weighted average concentrations.

Another important limitation of passive sampling techniques resides in the fact that only the dissolved fraction (bio available fraction) is sampled. The suspended solid and colloid fraction information is not given as required in different legislations.

In this work we present the results obtained with a new device (Continuous Flow Integrative Sample (CFIS) for the time weighted average monitoring of water quality has been present. The results obtained in some different campaigns (waste water, river water and marine water) in different sampling points:

- Waste water. WWTD after secondary treatment.

- Surface water. Ebro river.

- Marine water. Soler Port.

The results obtained with CFIS have been compared with spot samples

MO 017

Impact of biofouling on DGT measurements in water

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The technique of diffusive gradient in thin film (DGT) is commonly used to assess metal contamination in natural waters. In this study, we assess the effect of biofouling on DGT measured labile concentrations in water and investigate whether an additional nucleopore polycarbonate membrane on the surface of DGT devices can limit biofilm growth. Simultaneous field deployments of DGT equipped with and without the additional membrane in a canal receiving wastewater were compared. The effect of the biofilm was also assessed in controlled laboratory experiments, completed by the experimental determination of several metals diffusion coefficients in the hydrogel and membrane systems. Biofilms effect was problematic only from the tenth day of accumulation. Accumulation of some elements is highly biased by the presence of a thick biofilm (Zn, Ni, Cd). It improved the quantification of Cd and Ni, but adversely affects the quantification of Cr and Co. A kinetic model is proposed to explain biofilm role on the DGT measurement. Depending on the metals of interest, it is possible to limit bias due to biofilms by using an additional polycarbonate membrane.

MO 018

Estimation of water concentrations of PAH, PCB and OCP by using semi permeable membrane devices with performance reference compounds

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Among the passive samplers, lipid containing semipermeable membrane devices (SPMDs) have been most commonly utilized in several matrices of the environment such as water, sediment and air. Lipophilic environmental contaminants have been frequently identified in several compartments of the global ecosystems. In this study, the water sampling rates and concentrations of polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB) and OCP organochlorine pesticides were estimated by using SPMDs spiked with performance reference compounds (PRCs) at five sampling sites in the Istanbul Strait and Marmara Sea. Water sampling rates (Rs) for PAHs calculated by using the sampling rates of selected PRCs were negatively correlated to the Kow values of the corresponding PAHs. Generally, the sampling rates for all compounds after 7 days of deployment were similar indicating the similar conditions during the exposure period. The differences in sampling rates were more pronounced after 21 days of exposure. Water concentrations (Cw) of total PAHs vary depending on the Rs values used during the calculations and range from 69-193 pg/L and 47-211 pg/L and for Rs1 and Rs2 respectively for 7 days of exposure. Naphthalene, Fluorene, Phenanthrene, Pyrene and Fluoranthene were the dominant PAHs in seawater. Estimated PCB water concentrations by using Rs1 and Rs2 values were very similar and found between nd - 2.17 pg/L and between 0.28 pg/L -2.4 pg/L respectively. According to the estimations, indicator PCBs were the dominant compounds in sea water and most of the non-ortho and mono-ortho PCBs were absent in the water phase. Among OCPs, HCH and DDT derivatives were dominant at all stations.

MO 019

Vertical fluxes of Polycyclic Aromatic Hydrocarbons in the Pelegrino Field (Southeastern Brazilian Shelf): preliminary results

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Pelegrino Field is a region located at 23°20'S - 41°12'W on Southeastern Brazilian Shelf that recently has being explored for petroleum resources. Therefore, the adjacent marine environment may be impacted by the introduction of material from the drilling activities. Polycyclic aromatic hydrocarbons (PAHs) are an important proxy for the identification of this impact, because they are present in petroleum and its products, indicating introduction of these materials in the marine environment. Sediment traps are a passive sampling tool used to monitor the impact caused by drilling activities. The aim of this study is to evaluate the amounts and fluxes of PAHs on Pelegrino field. Settling suspended matter (SSM) were sampled using two sediment traps (S1 - 23°18'S, 41°13'W; and S3 - 23°18'S, 41°12'W) equipped with 20 cups filled with mercury chloride (HgCl₂) to prevent organic matter bacterial degradation. The sediment trap area was 0.650 m² and sampling interval was three days. PAHs concentrations were determined by gas chromatography coupled to mass spectrometer detector (GC/MS). Overall, PAHs concentrations suggest a low level of contamination, since levels were lower than 250 ng g⁻¹. PAHs amounts show no difference between S1 and S3. Total PAHs (parental and alkylated PAHs) ranged from 12.2 to 687 ng g⁻¹, with similar average for both sediment traps (S1 223 ng g⁻¹; S3 199 ng g⁻¹). There is also no difference between amounts and proportions of low molecular weight (LMW) and high molecular weight (HMW) PAHs. S1 presented on average 48.9% of LMW and 51.1% of HMW, while S3 shown 42.2% of LMW and 57.8% of HMW. It suggests a mixture of PAHs derived from both petrogenic and pyrolytic sources on the SSM, which may results from both drilling and sailing activities in the area. SSM vertical flux shows a great difference between S1 and S3. S1 presented average flux of 600 mg m⁻² d⁻¹, whereas S3 showed much higher values, 2881 mg m⁻² d⁻¹. This was reflected on total PAHs fluxes. S1 ranged from 10.4 to 317 ng m⁻² d⁻¹, while S3 ranged from 43.2 to 2400 ng m⁻² d⁻¹. Although PAHs levels were similar between S1 and S3, vertical fluxes showed that S3 area exports more PAHs through the water column suggesting that S3 area is being more impact by drilling activities than S1.

MO 020

Evaluation of different passive samplers to predict uptake of polycyclic aromatic hydrocarbons by crop plants

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Polycyclic aromatic hydrocarbons (PAHs) belong to a class of hydrophobic organic pollutants, produced during the incomplete combustion or pyrolysis of organic materials from primarily anthropogenic sources such as fossil fuels and agricultural waste. The fate of PAHs in the nature is of great concern to human health as these contaminants are widely distributed in the environment and are known to be toxic, mutagenic and carcinogenic. In the past decades, various passive sampler devices (PSDs) have been developed to concentrate hydrophobic organic compounds (HOCs) from environmental matrices and to mimic bioconcentration. Previous research has indicated that triolein embedded cellulose acetate membrane (TECAM) and petroselinic acid embedded cellulose acetate membrane (PECAM), types of lipid containing membranes as well as samplers made of polydimethylsiloxane (PDMS) have potential to accumulate certain polycyclic aromatic hydrocarbons (PAHs) from water and soil and to estimate the bioavailability of these pollutants to living organisms. The present study compares the ability of this suite of passive samplers to mimic uptake of 15 PAH prioritised by the European Scientific Committee on Food (ESCF) by crop plants grown in a hydroponic nutrient solution.

MO 021

Use of the semipermeable membrane devices (SPMD) as integrative tool for monitoring polycyclic aromatic hydrocarbons in tropical seawater

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To understand the bioavailability of contaminants in any system it is necessary to measure the concentration of these contaminants. It can be done using an organism surrogate, or biomimetic model, such as a passive sampling device as semi-permeable membrane devices (SPMDs). SPMDs are membranes composed of low density polyethylene layflat tubing filled with a neutral lipid and have been shown to effectively mimic the function of lipid membranes in uptake of lipophilic contaminants. These devices have a number of advantages over live organisms including ease of deployment, low maintenance costs, transportability, and applications to a wide variety of environments.

In the two last decades, several works have been developed to measures polycyclic aromatic hydrocarbons (PAHs) in seawater by using this technique. However almost all of these studies were carried out in cold and oligotrophic waters from the North Hemisphere, where biofouling effect is minimized.

The purpose of the present study was to evaluate the use of SPMDs in tropical and eutrophic waters to measure PAHs as part of a bioaccumulation study near produced water discharges. This is particularly important due the necessity of monitoring seawater contaminants in the new offshore oil fields, including the Brazilian Pre-Salt.

Field studies to assess PAHs in the seawater by using SPMDs were carried out in the Potiguar basin, located at Rio Grande do Norte State, Brazil. The SPMDs were deployed in three different months of the year at five sampling points. Three sampling points were located near an ocean outfall which discharges produced water and two at 12 Km distant from the outfall, as a reference area. It was analyzed the EPA's 16 priority pollutant PAHs and the respective homologue alkylated compounds. Near the outfall concentration of PAHs ranged from 25 ng L⁻¹ to 623 ng L⁻¹ with the presence of alkylated compounds from 2 to 4 rings and presenting PAHs distribution with weathering characteristics. At the control area the total PAHs concentration were quite similar to the SPMD blank, ranging from 0.8 ng L⁻¹ to 4.8 ng L⁻¹.

PAHs concentration found in seawater near the outfall was within the concentration expected by the dilution model of the effluent. To sum up, based on this study, the SPMD technique has shown considerable promise in studies in tropical and eutrophic waters.

MO 022

Development and application of a passive sampling technique for determining biomagnification factors and elimination half-lives in fish following dietary exposure

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Recent revisions to the OECD 305 test protocol have led to the introduction of a dietary exposure test in which the bioaccumulation potential of a substance is characterized in terms of a lipid normalized biomagnification factor (BMF). The BMF expresses the lipid-normalized concentration ratio in fish tissue to that in the diet to which the fish is exposed at steady-state. Research was conducted to demonstrate the utility of passive sampling techniques for estimating BMFs and half-lives of several hydrophobic chemical in fish tissue following a dietary exposure in rainbow trout. In this study, five test substances (hexachlorobenzene, musk xylene, o-terphenyl, methoxychlor, benzo[a]pyrene) with a range of susceptibility to metabolic transformation by fish were selected to allow comparison of this approach to results obtained using conventional exhaustive extraction of diet and fish tissues. Method development consisted of determining optimal sampler volumes and equilibration times for chemical analysis of spiked diets, oils and fish tissue samples using disposable polydimethylsiloxane fibers (2 cm length by 100 µm film thickness) which were solvent desorbed and analyzed by GC-MS. Empirically derived lipid to PDMS partition coefficients (K_{lipid-PDMS}) were determined for fish feed, fish tissue, corn oil, and fish oil and found to be in good agreement across the four matrices for all compounds, with the exception of benzo[a]pyrene. These results were comparable to published research investigating K_{lipid-PDMS} in lipids from various trophic levels. Laboratory derived BMFs and elimination half-lives determined using passive sampling techniques will be compared to results obtained using conventional

exhaustive extraction techniques. This approach offers a promising screening alternative test method for determining tissue concentrations, half-lives and biomagnification potential with potentially less fish and at a fraction of the cost than traditional test protocols.

MO 023

Study of partitioning between lipid and passive sampling materials for equilibrium sampling

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Samplers consisting of two phases like semi permeable membrane devices (SPMDs) as well as single phase polymer samplers (e.g. polydimethylsiloxane (PDMS), low density polyethylene (LDPE)) are widely used for monitoring of hydrophobic contaminants in the aqueous environment. Application of such passive samplers can be extended to monitoring of hydrophobic contaminants in fish tissue. Equilibrium concentration in PDMS sheets immersed in fish tissue can be easily converted to a concentration on lipid basis using separately determined lipid-PDMS partition coefficients. Such concentration in lipid showed a good relation with lipid-normalized concentration measured with classical analysis. So far, limited data on partition coefficients between lipid and passive sampler (Klps) are reported in literature. Klps can be calculated as the ratio of contaminant concentration in each phase at equilibrium. In the present work partitioning of major groups of hydrophobic contaminants as PCBs, PAHs, BDEs and various pesticides between several polymer materials (e.g. PDMS, LDPE) and various lipids (e.g. olive, fish, seal oil and a model lipid, triolein) is studied. The influence of temperature on the Klps is investigated. Equilibrium is confirmed by using performance reference compounds (PRCs) with properties equal to the properties of target compounds. Target compounds and PRCs are dosed to different media and an equal distribution of both compounds after equilibration demonstrates that equilibrium is achieved. Additionally diffusion of lipids in passive sampling materials at different temperatures is measured, including characterization by analysing the lipid patterns. The Klps values will contribute to implementation of monitoring in biota through passive sampling. Additionally when combined with existing water-LDPE partition coefficients, the obtained triolein-LDPE partition coefficients also allow to estimate water-SPMD partition coefficients for comparison with values presently applied in water passive sampling.

MO 024

Implementing WFD biota standards - a possible role for passive samplers?

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Environmental Quality Standards (EQS) define tolerable upper limits for concentrations of chemicals in the environment. For some substances, there is now the option of monitoring compliance in the body tissues of aquatic biota, or sediments. This may offer a more reliable and cost-effective measure of potential environmental risk than measurements in the water column for substances that bio-accumulate strongly and have very low aqueous EQSs.

Under the EU EQS Daughter Directive (EQSD), biota standards have been set for:

- mercury;
- hexachlorobenzene (HCB); and
- hexachlorobutadiene (HCBd).

The EQSD also sets out a requirement to monitor temporal trends of the levels of substances in biota and/or sediment.

The use of biota for assessing compliance and trends presents a number of challenges, e.g. choice of appropriate species, availability of species at different sites, and the destruction of large numbers of fish or invertebrates to collect the required data. We are not yet at a stage where we can implement biota standards with confidence.

To address these concerns, we are exploring alternative ways for assessing compliance with biota EQSs. Passive Sampling Devices (PSDs) are an emerging technique that could provide useful data on compounds present in the water environment without the requirement of sacrificing fish. To develop more confidence in a strategy for implementing biota standards and trend monitoring under the WFD, we need to compare the uptake of compounds in sediment, water, PSDs and biota (fish and mussels) sampled at the same sites at the same time.

Our poster describes a study to determine the potential role of PSDs as surrogates for biota. If quantifiable relationships can be discerned between chemical residues in biota, sediment and PSDs, we may be able to use PSDs as a surrogate for biota when assessing compliance with biota EQSs.

PSDs and caged fish (*Leuciscus idus*) or mussels (*Mytilus edulis*) have been deployed at a number of sites along the River Thames (freshwater and estuarine) and water and sediment samples have also been taken from the same locations. Analyses were conducted to determine the concentration of 13 compounds, including, HCB, HCBd, Pentachlorobenzene and TBT in all four matrices. The results from the trial and the potential for using PSDs for assessing compliance with biota standards and for trend analysis under the WFD will be presented.

MO 025

Measuring contaminant partitioning in Norwegian rivers

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Chemical monitoring under legislative structures such as the European Water Framework Directive (WFD) heavily relies on the application of environmental quality standards (EQS). Under the WFD, EQS values for hydrophobic organic priority substances have been set for the "whole water". Conventional sampling-analytical methodologies fail to meet target performance criteria for WFD method selection. Passive sampling devices are able to provide improved limits of detection (when compared with bottle sampling) and time-integrated measurements. However, concentrations measured with passive samplers are for the truly dissolved fraction and do not include those bound to particle or dissolved organic matter (i.e. the "whole water"). Reconciliation between passive sampling measurement and EQS is needed. Derivation of EQS values for the truly dissolved phase is a long-term option. A shorter-term initiative may be to estimate river-specific suspended particle matter-water partitioning coefficients. These may be based on measurements in the dissolved phase with passive samplers combined with techniques such as continuous flow centrifugation and in situ integrative SPM samplers. In this study, we have measured with the help of semipermeable membrane devices, silicone strips and low density polyethylene membranes dissolved phase concentrations of a range of nonpolar priority substances in 6 river of the South East of Norway. These measurements were complemented by measurements in the suspended particulate fraction. Contaminant partitioning coefficients were estimated from these measurements and put in context with literature data. Variability in partition coefficients were estimated with repeated seasonal sampling in 2 of the rivers over a 2 year period. We propose here to derive estimates of whole water concentrations based on passive sampling measurements. Uncertainties associated with for example contaminants sorbed to dissolved organic carbon are discussed.

MO 026

Development of passive surrogate addition devices for remote water sampling

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Most procedures for sampling remote bodies of water require the collection and shipment of the water samples from the site to the laboratory. In addition to the cost and inconvenience of collecting, bottling, preserving and shipping the water, there are possibilities for cross-contamination and errors at each step in the process. In another scenario, analytical instrumentation is available that is designed to be deployed in the field so that the analyses occur at-site. This second scenario for field testing is of course limited to the types of techniques supported by the instrumentation. A third scenario is the extraction of analytes from the water followed by shipment of sample-loaded concentration devices to a remote lab for analytical testing. We are most interested in this third scenario due to the potentially lower cost and complexity and we report on our efforts to improve and perhaps validate data quality for this type of system by developing a passive surrogate addition device.

A surrogate is a compound or analyte that is added to all samples during preparation. The use of a surrogate is required in most sampling procedures that are approved for environmental testing in order to insure that the method is actually delivering data in the 70-130% recovery range. A surrogate generally has similar properties and responses in the analytical process to the target analytes and can be used to prove that the process is both capable and actually did deliver quality data.

In remote sampling, there can be an issue with providing sufficient energy to power instrumentation including sampling pumps, valves etc. This can be easily solved by supplying a dual solar/battery power supply combined with consumption-minimizing components. If the system contains a mechanism for the addition of one or more method surrogates then one option is the use of a metering pump which of course would require power. In this poster we discuss our development of several passive systems for the addition of organic surrogates to sampling streams.

MO 027

Ambient thermal desorption ionization for rapid mass spectrometric analysis of contaminants

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To ensure the safety of consumers following an oil spill, a rapid screening method is required to analyze food for compounds of concern. Of the many compounds found in oil, a subset of major concern is the polyaromatic hydrocarbons (PAHs). These compounds are known to be carcinogenic and the US Environmental Protection Agency has defined these compounds as priority pollutants.

The application of ambient desorption techniques for sample introduction into mass spectrometers is an emerging technology that has applicability in many areas of contaminants analysis. The atmospheric-pressure solids analysis probe (ASAP) is a unique mass spectrometry technique for direct analysis of volatile, semi-volatile, solid and liquid samples. In ASAP, samples are introduced to the mass spectrometer on a glass capillary and vaporized with heated nitrogen desolvation gas. Ionization is achieved using a corona discharge. While conventional analytical tools, such as LC or LC/MS, require time-consuming sample preparation procedures, ASAP can provide mass spectra within seconds of sampling.

The use of ASAP coupled to a tandem quadrupole mass spectrometer for the detection of PAHs in seafood samples was investigated. Spiked samples of fish and shrimp were homogenized and then sampled directly with the glass capillary. PAHs were screened using multiple reaction monitoring (MRM) mode. In addition, PAHs were extracted from

the samples using a simple QuEChERS protocol and the extract sampled directly using the capillary. Results from both approaches will be presented. This work provides an interesting example of the potential applications of ASAP for the analysis of environmental contaminants and will discuss the benefits and challenges of the technique.

MO 028

Development of a communicating portable analytical system of organic micropollutants in water based on UV spectrometry-fluorimetry detection after multiple solid phases selective preconcentration

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Lots of organic contaminants such as regulated molecules (phytosanitary products, polycyclic aromatic hydrocarbon (PAH), [3DOTS]) as well as emerging pollutants (pharmaceutical products, endocrine disruptors, [3DOTS]) are generally present at trace level in environmental waters (in particular natural waters) except in case of accidental or intentional pollution where concentrations are stronger. In general, detection methods include, sampling, solid phase extraction (SPE) and finally gas or liquid chromatography-mass spectrometry (GC-MS or LC-MS) (Hilton *et al.* 2003, Momplaisir *et al.* 2010). These protocols are necessary because standardized, but, they are limited when sudden pollution/contamination occurs and when rapid decision making is required. In this case, there is a growing interest to have available, rapid and on site detection techniques able to give information (qualitative, (semi)quantitative) of the type of pollution and the potential corresponding hazard for the environment and human health.

In this work, we develop a rapid, reliable and low cost solution for the detection of organic micropollutants directly on site, based on multiple solid phases selective preconcentration (MSP2) coupled to UV/Visible spectrometry and/or fluorimetry.

The principle of the development is to combine several solid phase preconcentration cartridges (different chemical sorbents can be used) and various washing/elution solvents for the on site extraction, separation and detection of specific chemical micropollutants or family of substances.

A first approach have considered 13 substances among which pesticides, pharmaceutical products, PAH and endocrine disruptors in more or less complex mixtures. From the physico-chemical (pK_a , K_{ow}) and optical (UV and fluorimetric spectra) proprieties of each target, and by mixing several sorbents and elution solvents, we tried to propose protocols for the detection of each component in the considered mixtures.

Such system can be characterised by its availability (rapid use and result in real time), simplicity (easy to transport and use by everybody), and rapidity (adapted procedure based on simple principle).

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MO 029

Ultra low level determination of bisphenol A, 17- β estradiol and poly aromatic hydrocarbons in river water using fully automated column-switching HPLC

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Automated solid phase extraction (SPE) is one of potential candidates for improving experimental productivity. We have reported novel auto-SPE column-switching HPLC method for the determination of trace amounts of chemical substances in surface water using specific detectors such as mass-spectrometry (MS), along with electrochemical detector. Bisphenol A (BPA), which has been suspected as an endocrine disruptor and 17- β estradiol, (E2) most famous natural estrogenic compound were determined at ng/L levels with this method[1, 2]. Here we have introduced multi-valve column switching HPLC system with a specially designed pretreatment column[3]. This column-switching system solved a recurrent issue of column clogging and the pretreatment column provided remarkable performance for removal of humic compounds from environmental water samples. Use of this combination controlled ion suppression and increased peak response while using single stage MS as a detection system.

On the other hand, there is still a demand to do trace analysis with simple and inexpensive way. We found it possible to detect ng/L levels of BPA and poly aromatic hydrocarbons (PAHs), which are described in EPA method 610 with fluorescence detector. PAHs show strong hydrophobic nature and they can be often adsorbed on the surface of HPLC flow line especially resin-made parts. To avoid this phenomenon during auto-concentration, we employed "dilution-concentration" device, which provided reliable recovery and repeatability. Typical performance parameters of this system with fluorescence detector were validated.

Real water samples were collected then BPA, E2 and the PAHs were determined at ng/L levels with excellent repeatability and sensitivity by using proper detection way respectively. Hence, such switching techniques can be used for trace level analysis of environmental contaminants, carcinogens and additives in varied samples with complex matrices. These techniques may therefore assist in sample clean-up, target compound concentration, separation etc. An appropriate choice of a sensitive detection system can afford to carry out ng/L and sub-ng/L levels analysis with ease and required sensitivity.

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MO 030

HPLC analysis of isocyanate aerosol in workplace

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Workplace atmosphere is an important distribution of various pollutants, resulting from the handled products and concerned processes. The fate of these pollutants in the air (transport, deposition, degradation), is determined by their distribution between atmospheric particles and gas phase (Lohmann *et al.* 1998). Harmfulness of particles in workplace atmosphere and the disease hazards that they present are related to their chemical nature and size. The production of one kilogramme rigid polyurethane foam releases nearly 7362 milligrams of solid particles in air.

In this work, we used the personal aerosol sampler CIP10-R (Courbon *et al.* 1988) and filters for evaluation of alveolar (Gorner *et al.* 1996) and inhalable fractions of particles during clean of casts after injection of the polyurethane foam (PU). HPLC was used for detection of MDI in different post.

Obtained Results reveal that MDI in alveolar fraction prevail the total collected particles, this can be allotted to the process used for cleaning and to the quality of formulated foam.

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MO 031

Development and calibration of a passive sampler for N-nitrosodimethylamine (NDMA) in water

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N-Nitrosamines such as N-nitrosodimethylamine (NDMA) are organic compounds of contemporary interest in environmental waters, including groundwater, wastewater and potable water, due to their potent carcinogenicity in laboratory animal studies and probable human carcinogenicity. Heightened awareness of their properties and prevalence has led to increased scrutiny of water with Maximum Contaminant Level (MCL) goals ranging from 2 ng L⁻¹ in California to 9 and 10 ng L⁻¹ respectively in Canada and Australia and quantification limits down to low ng L⁻¹ levels.

USEPA Method 521 [1] for the analysis of N-nitrosamines in water is a coconut charcoal-based solid phase extraction method. This work investigated the use of the charcoal specified in this method as a passive sampler sorbent for NDMA and stipulates its use for other nitrosamines.

The suitability of coconut charcoal as a sorbent phase for NDMA has been previously investigated. However, this involved simple batch experiments. To employ it in a passive sampler, calibration data such as sampling rates (RS L.day⁻¹) and sampler-water sorption coefficients (KSW) applicable to environmental conditions must be available for target compounds. In addition, these calibration characteristics should be independent of ambient concentrations. The aim of this study then was to develop and calibrate a passive sampler for NDMA using coconut charcoal as a sorbent phase.

MO 032

'Wa-shi' (Japanese paper) fabrics as adsorbents

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A traditional Japanese paper, "Wa-shi" has been strained from bark of some tree or shrub. The word "Wa" means Japanese, while "shi" means paper to describe paper made by hand in the Japanese traditional manner. Wa-shi is mainly utilized in traditional arts, such as Origami or Shodo.

Recently fabrics were made using Wa-shi. Some special method realized various types of Wa-shi fabrics and commercialized by a Japanese company, CURETEX [1]. One of the recent topics of Wa-shi fabrics was socks, which were utilized in space by a Japanese female astronaut to proof moisture retention as well as deodorization.

The detailed mechanism for above characteristics has not been elucidated yet, but Wa-shi is simply made from cellulose, therefore, adsorption ability is probably possessed. In addition, textile forms and/or knitting things are really suitable for fast adsorption of environmental water. In this paper, we wish to show fundamental adsorption ability of the fabrics of Wa-shi (Japanese paper) as environmental clean-up device. As the result rather fast adsorption for a dye was observed in spite of loose textures.

MO 033

Mass balances and passive dosing of polychlorinated dibenzo-p-dioxins in a cell based bioassay

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Persistent organic pollutants are ubiquitous environmental contaminants and pose health risks. Cell-based bioassays could be cost-efficient and ethical alternatives to animal testing in chemical risk assessment. However, hydrophobic organic chemicals may appear less potent than they really are because their bioavailability is reduced by sorption to growth medium and vials. In the present study we use the CAFLUX assay, which is specific for chemicals binding to the aryl hydrocarbon receptor and detects chemicals with a dioxin-like activity. For a series of polychlorinated dibenzo-p-dioxins we have assessed bioavailability and partitioning between medium components and cells and demonstrate how the partitioning affects the cellular concentrations with more than 99% of the chemicals sorbed to medium components thus reducing the sensitivity of the assay. We also demonstrate how to overcome this limitation with a novel dosing technique where the polychlorinated dibenzo-p-dioxins are desorbed from a polymer.

MO 034

Passive dosing as a tool to recreate the environmental mixture composition of HOCs in an aquatic toxicity test

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The general aim of this study was to explore the potential of passive dosing for testing the toxicity of a PAH mixture that recreates the mixture composition found in seawater from a coastal area of Spain, the Bay of Algeciras. First, solvent spiking and passive dosing were compared for their suitability to determine the acute toxicity to *A. franciscana* nauplii of several PAHs at their respective solubility limits. Second, passive dosing was applied to recreate the seawater mixture composition of PAHs from a Spanish monitoring program, and to test the toxicity of this mixture at different levels. HPLC analysis was used to confirm the reproducibility of the dissolved exposures concentrations for the individual PAHs and mixtures. This study shows that passive dosing has some important benefits in comparison with solvent spiking for testing hydrophobic organic compounds (HOCs) in aquatic media. These include maintaining constant exposure concentrations, therefore higher reproducibility and a relative increase in toxicity. Passive dosing is also able to faithfully reproduce real mixtures of HOCs, such as PAHs, in toxicity tests, reproducing both the levels and proportions of the different compounds. This provides a useful approach for studying the toxicity of environmental mixtures of HOCs, with a view to investigating their toxicity but also for determining safety factors before such mixtures result in detrimental effects.

MO 035

Speciation of PAHs in stormwater

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Partitioning to particulate matter and dissolved organic carbon (DOC) has a large influence on the transport and fate of hydrophobic organic chemicals in aquatic environments. In this study a newly developed method for studying speciation of hydrophobic organic compounds, called passive dosing, was used to measure partitioning of fluoranthene in samples of stormwater runoff. The freely dissolved concentration of 14C-fluoranthene in the samples was controlled by passive dosing from a pre-loaded polymer phase and the total sample concentration at equilibrium was measured. This method reveals the free fraction of fluoranthene in the sample as well as the enhanced capacity of the sample to carry fluoranthene relative to pure water. Free fractions of fluoranthene in the stormwater samples varied from below 15 % in the first sample from each event to 20-50 % during the last part of the events. Enhanced capacities of the stormwater samples for fluoranthene transport ranged from 2-23 relative to pure water. In the stormwater samples partitioning to the organic part of the suspended solids, measured by loss on ignition (LOI), dominated partitioning compared to partitioning to DOC. Even though samples were taken at different events and at different times during the events, partitioning to LOI remained constant with KLOI = 154000 ± 3000 L/kg. These results can be used in stormwater quality modeling and assessment of the efficiency of stormwater treatment systems.

MO 036

Application of passive samplers for monitoring of cyanotoxins in drinking water

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Massive proliferations of toxic cyanobacteria in drinking water reservoirs represent a serious human health hazard requiring effective tools for assessment and management of health risks resulting from eventual contamination of drinking water with cyanotoxins. Passive sampling is very perspective approach which could be effectively utilized for purposes of environmental and regulatory monitoring of cyanotoxins. Although passive samplers had been successfully developed and/or evaluated for the most prominent cyanotoxins including microcystins, so far they have been only rarely used in research and monitoring of cyanotoxins.

This study is an example of our recent attempt to further extend the application of passive sampling techniques in the field of cyanotoxin research and regulation and especially to introduce this approach to drinking water treatment industry for control and management of cyanotoxins. In this study, we employed passive sampling alongside traditional techniques to monitor cyanobacteria and microcystins in two different drinking water reservoirs and adjacent drinking water treatment plants in the Czech Republic. During the sampling period Jul-Nov 2011, passive samplers were biweekly deployed/replaced and grab samples collected from open reservoirs and from different stages of water treatment process (raw water, flocculation, clarification, filtration, final treated water). Phytoplankton species composition and quantities were determined microscopically as well as by submersible fluorimeters. Microcystin concentrations were determined in both grab samples and exposed passive samplers by HPLC-DAD and/or immunochemically (ELISA). The poster will compare data obtained from both sampling approaches (passive/grab sampling) as well as discuss and demonstrate potential and limitations of passive sampling techniques for monitoring of cyanotoxins in drinking water, drinking water quality control, evaluation of efficiency of cyanotoxin removal during particular steps of the drinking water treatment process, and human health risk assessment. This study should facilitate future applications of passive sampling techniques in water quality monitoring practices.

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MO 037

Field evaluation of chemical probes to determine the fate of persistent organic pollutants in soil

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Persistent organic pollutants (POP) such as polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB) usually enter the atmosphere either in their gaseous form or sorbed to carbonaceous and/or other organic matter containing particles. Long-range atmospheric transport and deposition (wet and dry) lead to a ubiquitous distribution of POP in the environment. POP accumulation in low temperature environments such as the polar regions and mountains is described as cold trapping. In addition, a fractionation of volatile compound mixtures can be seen toward the poles which is probably due to climate-controlled desorption, evaporation and re-deposition processes ("hopping"). It was hypothesized that especially at high altitudes of mountains remobilized contaminants may accumulate and contaminant patterns may change, in order of the contaminants physico-chemical properties. In our study we used PAH and PCB as model compounds to study climate and altitude triggered fractionation processes in detail under field conditions. In a remote alpine valley in the European Alps and in a valley in the Black Forest Mountains (Germany) test sites at various altitudes were equipped with chemical probes. These consisted of a porous ceramic tube (pore size ca. 5 nm) which were filled with various materials (quartz sand, peat and charcoal) to mimic different desorption scenarios from sorbents with varying sorption capacity. The materials were spiked with defined contents of PAH and PCB (20 mg/kg and 1 mg/kg) and the chemical probes were buried in soil in a maximum depth of 5 cm below surface. After several months the tubes were analyzed for evaporative PAH and PCB losses. After a total study period of 1 year, we found for quartz sand losses of volatile compounds such as naphthalene or phenanthrene close to 100% within the first 9 months at all altitudes. Fluoranthene showed a slight decrease of loss with increasing altitude which is in line with a expected fractionation. For stronger sorbents, e.g. charcoal and peat, losses were much smaller and for some tubes even an increase of the loading was observed. Higher-ring-PAH showed no loss or the results were within the analytical error. The PCB with high volatility showed 100 % loss after a 9 month period of deployment, while PCB with lower volatility showed a decreasing loss with increasing altitude. This result could be confirmed from chemical probes analyzed after 12 month of deployment.

MO 038

Seasonal and temporal trends of airborne POPs around a waste incineration plant by passive sampling methods

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High-volume active samplers have been conventionally used for the environmental monitoring of persistent organic pollutants (POPs) in air. However, these systems have several drawbacks, such as high cost or power need. Consequently, alternative methods, such as passive samplers, are being developed, as semi-quantitative measures complementing active systems. Due to the low cost and easy handling, polyurethane foam (PUF) disks are especially attractive. PUFs seem to be a useful tool to compare the state of pollution in specific areas, as well as for seasonal and spatial distributions of local POPs. In 2010, an environmental monitoring program of the municipal waste incinerator (MSWI) in Tarragona (Catalonia, Spain) was initiated by measuring the immission levels of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs) and polychlorinated naphthalenes (PCNs) by means using passive samplers. In order to establish seasonal and temporal trends, four campaigns (between March 2010 and September 2011) were carried out. PUFs were deployed for 3 months at 8 different locations around the facility. Levels of PCDD/Fs ranged from 0.007 to 0.038 pg WHO-TEQ/m³, being very similar to those previously reported in the scientific literature. Regarding PCBs, the concentration of dioxin-like congeners was comprehended between 1.38 and 3.75 fg WHO-TEQ/m³, and the average levels of the 7 environmental marker PCBs was 44.1, 38.9 and 30.2 pg/m³ along the three first studied campaigns. PCN levels presented very similar levels through the program, with values ranging 6.45-6.95 pg/m³. While no temporal and seasonal patterns were noted in the airborne concentrations of POPs, the current levels of PCDD/Fs, PCBs, PCNs in air near MSWI are below or similar to those reported in other references sites, and typical of semi-urban areas.

MO 039

Can car cabin air filters be a useful a sampling medium for air pollution monitoring purposes?

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This study presents for the first time results of gas-chromatographic chemical analysis of car cabin air filters (CCAFs) collected from typical UK cabs from the city of Manchester, United Kingdom. As a first step, this study focuses on polycyclic aromatic hydrocarbons (PAHs), the most important category of semi volatile organic pollutants associated with traffic emissions.

Concentrations of ΣPAHs in CCAFs ranged between 2760 and 10800 µg/filter, with benzo[b]fluoranthene, benzo[k]fluoranthene and indeno[123-cd]pyrene being the most abundant PAHs. Benzo[a]pyrene (BaP) ranged between 315 and 740 µg/filter, accounting regularly for 5-15% of the total PAHs. Atmospheric BaP (and other PAHs) concentrations were estimated by using different scenarios for ventilation rates, and duration of filter's exposure and it was shown that the BaP levels of the air that CCAFs collect can exceed the guideline value of 1 ng/m³ up to 100 times.

Calculation of PAHs molecular diagnostic ratios shows the predominance of traffic related emissions.

We conclude that CCAFs are an invaluable alternative to conventional air sampling methods, featuring unique advantages, that are a) the exposure estimation of car passengers, riders, pedestrian or road-professionals, b) unlimited sample size, ideal for screening of the air quality, especially for compounds that tend to partition on particles, c) provide with an average, time weighted urban air pollution assessment. The main disadvantage of this method is the uncertainty associated with accurate estimations of atmospheric pollutants concentrations, due to lack of knowledge of the exact amount of air filtered, and the fact that vehicles are used also for trips to less polluted places and/or the fact that also the recirculation of car cabin indoor air is undergoing filtration.

MO 040

Observations on flame retardants (FRs) in indoor environments in Istanbul-Turkey

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Due to the several indoor sources of flame retardants-such as upholstery textiles and foam paddings in furniture and mattresses, thermoplastics for computers, TVs, electrical components and cables-indoor air is a very important exposure route of flame retardants (FRs). As they are less subject to photolysis and atmospheric dilution in indoor environments, they show increased air concentrations. Like outdoors, data of POPs indoors are sparse or lacking in Turkey. In this study we investigate PBDEs and non-PBDE novel flame retardants in houses, offices, electronics shops and upholstery+matress shops indoor air and dust samples. The air samples were collected by using indoor air passive samplers. Dust samples were collected by vacuuming a 2 m² area for 4 minutes. The main objectives of this study were a) utilization of polyurethane foam disc (PUF) passive sampling technique for indoor environments, b) to start the database on concentrations of flame retardants in indoor air and dust in Turkey, c) to investigate the congener profiles and partitioning of flame retardants in indoor environments, d) to evaluate flame retardants exposure through indoor air and dust pathway. For these purposes, the planned study is in progress and samples analysis is being carried out at the present.

MO 041

Comparison of four modeling approaches to describe chemical uptake by passive air samplers

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Over the past decades, various passive air samplers (PASs) have been developed to monitor semivolatile organic compounds (SVOCs) in air. Uptake in PAS occurs by molecular diffusion from air to a passive sampling medium (PSM), such as polyethylene (PE), polymer-coated glass (POG), polyurethane foam (PUF), and XAD-resin. Unlike in PE or POG-based PAS, where SVOCs accumulate in thin layers in contact with air, PUF and XAD are porous materials. The Whitman two-film model, which was originally developed to describe mass transfer between air and water, is often used to describe chemical transfer from air to the PSM. This model requires that "in the main body of either liquid or gas [3DOTS] the concentration of solute in the fluid is essentially uniform at all points." While this assumption may be satisfied when modeling chemical uptake by thin, non-porous PSM such as PE and POG, it is unlikely to be valid for thick, porous PSM such as PUF and XAD. Chemical transfer within such PSM only occurs in the gas filled pores, which limits the transfer kinetics because only a low fraction of the SVOCs is in the gas phase. Recent experimental evidence confirms that SVOCs do not distribute uniformly within the PUF and XAD, which therefore directly contradicts the assumption of the two-film model used to describe chemical uptake by PUF- and XAD-PAS. The two-film modeling approach for PAS is also not able to explain the sampling rate decrease that has been observed during the initial uptake phase of a few calibration studies involving PUF-PAS. In this study, in order to seek an alternative approach to modeling chemical uptake by PUF- and XAD-PAS, we compare the two-film model with three other approaches that are commonly used to describe intermedia mass transfer of chemicals. These approaches either (1) describe uptake within the PSM using an effective diffusivity of chemical transfer within porous media assuming an average diffusion length within the PSM, or (2) assume the existence of two types of binding sites within the PSM which contribute to fast and slow uptake, or (3) are based on Fick's diffusion law for chemical transfer in air and in porous media.

MO 042

Layer by layer self-assemble acetylcholinesterase and gold nanoparticles on glassy carbon electrode for sensing organophosphate pesticides

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Organophosphates(OPs) are highly toxic compounds to the vertebrates, which can be absorbed readily through the skin, mucous membranes, gastrointestinal and respiratory tracts. With the increasing awareness of environmental protection and health concerns, there is a growing public concern about the accumulation of OPs in food products and water supplies.

An sensitive amperometric biosensor for the determination of OPs based on Layer-by-layer (LBL) self-assembled acetylcholinesterase (AChE) and gold nanoparticles(AuNPs) on glassy carbon electrode(GCE) is described. The GCE is modified with AuNPs by electrostatic interaction between AuNPs and cationic poly(diallyldimethylammonium chloride) (PDDA). AChE is immobilized on the negatively charged AuNPs surface by alternatively assembling a PDDA layer and an AChE layer.

The unique sandwich-like structure (PDDA/AChE/PDDA) on the AuNPs surface formed by self-assembling provides a favorable microenvironment to keep the bioactivity of AChE. Owing to the inherent conductive properties of AuNPs, the immobilized AChE exhibited a higher affinity to its substrate and produced detectable and fast response. Electrochemical impedance spectroscopy of the AuNPs/PDDA/GCE and PDDA/[AChE/PDDA]2/AuNPs/PDDA/GCE indicate that AuNPs promote electron transfer and AChE is immobilized on the electrode surface successfully. The sensor performance, including inhibition time and pH, was optimized with respect to operating conditions. Under the optimal conditions, the inhibition rate (I %) of methyl parathion towards the immobilized AChE was proportional to the logarithm of the concentration over the range of 4.6[GREEKX] 10⁻⁵ to 5.3[GREEKX] 10⁻³ M with a detection limit of 7.6[GREEKX]10⁻⁶M. The biosensor has been applied for the determination of methyl parathion in spiked vegetable samples.

MO 043

Spatially resolved distribution of chemicals on equilibrium passive samplers for the elucidation of modes of sorption

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Equilibrium passive samplers are widely used in environmental monitoring programs as they are relatively easy to use and concentrations in air or water, respectively, can be easily calculated using the partitioning constants once chemical equilibrium is established (equilibration times depend on the dimension of the samplers, i.e. the thickness of the sampling media). This approach critically relies on the assumption that sorption on the sampling media is fully controlled by a partitioning process and, thus, the partitioning constant is independent from the concentrations (i.e. linear sorption isotherm). However, recent studies have shown that the partitioning constants on some equilibrium passive samplers (e.g., polyoxymethylene passive sampling devices) are higher when the thickness of the sampling media is reduced. Such observations are inconsistent with the partitioning theory, stimulating the idea that, apart from partitioning into the sampling media, additional interactions occur at the surface of the samplers (i.e., adsorption processes).

In this study, batch experiments were conducted with fluorene as probe compound and polyoxymethylene (POM) and polyethylene (PE) as sorbents. Subsequent to equilibration, the samplers were investigated using Raman spectromicroscopy in order to map the fluorene concentrations among the passive samplers. First results indicate that fluorene can be distinguished from the sampling media, which allowed mapping of the chemicals distribution on the surface of the samplers. Additional studies will focus on cross sections to investigate concentration gradients through the samplers to further elucidate the mode of sorption on equilibrium passive samplers.

EC04P - Novel approaches to addressing metal and metal nanomaterial bioavailability in soils

MO 044

Earthworm influence on metals bioavailability in relation with metal speciation and ecotoxicity

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-Metal-enriched atmospheric fine and ultra-fine particulate matters (PM) are currently rejected in environment increasing significantly ecosystem pollution by metals. Few data are currently available concerning metals impact on environment and human health. In the context of Reach regulation, the study of pollutant fate and impact on the biosphere is required, as well as efficient tools development for sanitary risk assessment. Earthworm is a key organism to assess soil quality. By its bioturbation activities, earthworm could modify metal bioaccessibility and bioavailability in soils (in relation with metal localization and speciation). In this context, several experiments are performed to: (i) Estimate the difference of metals bioaccessibility and bioavailability between polluted soils influence by bioturbation or not; (ii) Determine earthworm influence on metal speciation by studying ingested soils and casts; (iii) Assess ecotoxicology impact of metal from atmospheric PM on earthworms.

-Different PM and polluted soils (historically polluted and spiked soils); two species of earthworms: *L. terrestris* (ecologically relevant) and *E. fetida* (international standard for ecotoxicology tests) will be used for laboratory or field experiments. For the different soils before and after bioturbation: i) metal bioaccessibility will be determined by *in vitro* UBM test; ii) metal phytoavailability will be assessed by the study of plant uptake (lettuce); iii) microscopic (MEB-EDS) and spectrometric (Raman, XRF et EXAFS) techniques will be used to perform elementary maps and determine potential changes in metal speciation (cast study); iv) general parameter like survival, biomass changes, cast production and burrowing activities will be studied to assess metal ecotoxicity.

-Strong influence of particle size and metal speciation on metal bioaccessibility was observed: variation between 20 and 82% according to experimental conditions. Moreover significant ecotoxicology effect on earthworm (using cast-production and biomass change measures) was highlighted.

MO 045

Investigations into the environmental bioavailability of lead in metal-rich granules produced by the earthworm *Eisenia fetida*

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Lead (Pb) is the most common metal contaminant found in the soils of small-arms ranges of US military installations. Currently, risk assessors often assume that Pb is 100% bioavailable for ecological receptors, even though research has shown the modification of Pb bioavailability and toxicity by soil physicochemical characteristics and metabolism by organisms. Many terrestrial invertebrates are capable of rendering Pb toxicologically inactive through the formation of metal rich granules (MRGs). While Pb in MRGs is not considered bioavailable via trophic transfer, little is known regarding the fate of MRGs in the terrestrial environment once released from exposed organisms. The current research characterizes the microstructure of MRGs formed by the earthworm, *Eisenia fetida* and determines the potential for the microbial degradation and remobilization of Pb previously sequestered in MRGs. If liberated by bacterial degradation, sequestered Pb may become bioavailable to invertebrates and their predators.

The direct bioavailability of Pb in MRGs to oligochaetes in soil will also be examined. Earthworms were exposed to soils spiked with 4,000 mg/kg Pb for a six-week period and fractionated utilizing differential centrifugation and digestion to separate whole-animal tissue from MRGs to obtain sufficient MRGs for amending soils for microbe and oligochaete exposures. Once extracted, MRG composition and concentration was analyzed utilizing synchrotron based analysis at Argonne National Laboratories (Argonne, IL). Synchrotron imaging provided multi-channel analyzer (MCA) plots which revealed localized MRG hotspots within the samples, not only confirming the presence of Pb, but also the binding of additional metals such as zinc and iron. Additionally, synchrotron techniques were utilized on cross-sections of earthworms to determine the presence and concentration of Pb across a gradient of dermal and gut tissue. MCA plots of cross sections revealed a wide dispersion of Pb inside the gut wall, suggesting the presence of unbound Pb in addition to MRG formation. The results of the exposure of microbes and oligochaetes to Pb in MRGs will be discussed.

MO 046

Lead bioavailability to soil invertebrates as affected by soil physicochemical characteristics

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The bioavailability and toxicity of lead (Pb) to terrestrial invertebrates may be markedly modified by variations in the physicochemical properties of soil. Other factors that modify the toxicity of Pb include aging and leaching, yet most toxicity data are obtained from toxicity tests conducted with soils freshly amended with metal salts and the relevance of data from such tests to contaminated field soils is unclear. This study compares the chronic toxicity of Pb to *Eisenia fetida* and *Folsomia candida* in seven European soils varying widely in physicochemical properties (i.e., pH, organic matter content, texture, eCEC). Toxicity across all seven soils was compared using soils that were leached and pH corrected after freshly spiking with lead chloride. This was done to exclude the confounding factors of increased ionic strength and decreased pH due to spiking. Three of these soils (Barcelona, Ter Munck, Woburn) were also amended with lead nitrate in 2004 and aged under field conditions for five years prior to conducting laboratory toxicity tests and additionally tested directly after freshly spiking (without leaching or pH correction). These treatments allow for the estimation of leaching/aging factors to extrapolate toxicity from laboratory to field conditions. Highly significant correlations between log EC50 and log eCEC were observed for the *E. fetida* reproduction tests, but toxicity of Pb to *F. candida* was low and not affected by soil properties. Leaching and aging generally attenuated the toxicity of Pb to both soil invertebrates studied. The toxicity data, together with leaching and aging correction factors, provide important information on the bioavailability of lead to invertebrates in soil under realistic conditions.

MO 047

Lead bioavailability and bioaccessibility in sediments from wetlands with different hunting management in Tuscany (Italy)

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Lead (Pb) shot spent over wetlands causes Pb poisoning in waterfowl, which confuse it with grit for grinding function in the gizzard. Although often incorrectly regarded as inert, once dispersed in the environment, Pb shot undergoes a slow transformation into various molecular species that diffuse more readily in the environment and increase the bioavailability of the metal. Waterfowl species, which for their feeding habits probe into the sediment in search of food can steadily be exposed to Pb through the unintentional ingestion of contaminated sediment.

In this study we investigate the relative bioavailability and bioaccessibility of Pb in sediments from two Italian wetlands "Diaccia-Bottrona" (costal wetland) and "Padule di Fucecchio" (inland marsh) where previous studies showed high Pb levels. Sediment was sampled from areas with different degree of Pb shot pellet densities in each wetland, including shooting ranges and hunting estates and natural reserves. The relative Pb bioavailability was evaluated using the Community Bureau of Reference (BCR) sequential extraction procedure, whereas the relative avian Pb bioaccessibility was estimated by using an "in vitro" simulation of the gizzard-intestine system.

Pb partitioning obtained with BCR procedure showed a similar pattern for "Diaccia-Bottrona" and "Padule di Fucecchio". Percentage of total Pb concentration in sediment were less than 6% in the fraction 1 "acid extractable" with exchangeable and carbon bound metal, about 72-80% in the fraction 2 "reducible" with iron manganese oxide/hydroxide associated metal, about 4.25-16% in the fraction 3 "oxidisable" with metal bound to sulfide and organic matter, and about 3.5-18.6% in the fraction 4 "residual" with metal in the mineral phase.

Despite of the similar pattern of BCR partitioning of Pb in the two wetlands, results of "in vitro" simulation showed percentages of bioaccessible Pb in gizzard phase (pH 1.5) two order of magnitude higher for "Padule di Fucecchio" than for "Diaccia-Bottrona" in both the shooting ranges (14% and 0.18%, respectively) and natural reserve areas (10.6% and 0.10%, respectively).

Due to the non-acidic conditions of the intestinal phase (pH 6.5), final Pb bioaccessibility were below of detection limits in both study areas.

According to these results, direct ingestion of Pb shot pellets can represent a higher risk for birds than the unintentional ingestion sediment contaminated by the disaggregated pellets in heavily hunter areas.

EM02P1 - Fate and exposure modelling

MO 049

Evaluation of a molecular fragment-based tool for predicting pp-LFER descriptors of complex and multifunctional organic compounds

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Poly-parameter linear free energy relationships (pp-LFERs) are useful models to accurately describe equilibrium partitioning processes of neutral organic chemicals in technical and natural systems. However, only a limited number of pp-LFER solute descriptors are available so far for complex and multifunctional compounds as well as for environmentally relevant substances. This hampers the application of pp-LFER models for estimating environmental partitioning behaviour of pollutants. The experimental determination of these descriptors is tedious and time consuming. Especially for screening purposes, the ability to predict solute descriptors just based on the molecular structure would be highly desirable.

The commercially available software ABSOLV is an easily accessible and manageable tool that predicts solute descriptors based on a SMILES input. Here we evaluate the performance of ABSOLV to predict partition coefficients using calibrated pp-LFER equations in combination with predicted ABSOLV descriptors. This evaluation is done for various partition processes and a large set of complex, multifunctional organic solutes.

MO 050

Model suite to estimate the air/water partition coefficient of organic compounds at 25°C

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The air/water partition coefficient K_{aw} is one of the key properties in environmental partitioning. A number of estimation models for K_{aw} are available from literature.

However, despite of the number of recently published new experimental data, at least to our knowledge there was no relevant new model published within the last years.

The poster first presents a critical review of the existing models through validation with a huge up-to-date data set. Furthermore, this set has been employed to develop three new models based on fragments, Abraham parameters, and read-across through atom-centred fragments.

The performance of the new models is presented, and the inter-correlation of the model errors for the different new and literature approaches is examined. Bundling all methods in a model suite thus allows for a sophisticated consensus modelling of K_{aw} .

All referred and new models are implemented and publicly available in the public free edition of ChemProp (see <http://www.ufz.de/index.php?en=6738> for details).

The development of these models was partly supported from the EU Integrated Project OSIRIS (contract No. 037017).

MO 051

ChemProp - chemical properties estimation software system

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The software system ChemProp is an essential part of the outcome from the EU Integrated Project OSIRIS (contract No. 037017). It is publically available for free based on a bilateral license agreement (see <http://www.ufz.de/index.php?en=6738> for details).

ChemProp comprises models to estimate compound properties and a database for chemical structures and properties, together with tools to manage compound sets. The QSAR part addresses models for physico-chemical properties with particular remark to partitioning processes of environmental relevance and degradation, environmental fate, ecotoxicological endpoints, and human toxicology. It mainly employs models based on the topological matrix and thus does not require quantum chemistry. Particular remark is given to the applicability domain, model uncertainty, and consensus modelling. The integrated database allows for structure and substructure searching, and addresses tautomerism and stereomerism. For effective data handling, it provides access to data in external resources in combination with the structure search facilities. It is linked to the public OSIRIS ITS webtool (<http://osiris.simppl.com/OSIRIS-ITS/itstool.do>).

The poster presents a general overview on ChemProp, and in particular emphasises new developments achieved since the poster presentation of ChemProp at the SETAC Europe meeting 2011 in Milan.

MO 052

ECETOC TRA: refinement of the OPS model parameters for the estimation of the chemical concentrations in environmental media due to atmospheric deposition

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The concentration of polluting substances in the air determines the intake through inhalation by humans. Deposition fluxes in soil, sediment and water determine environmental exposure which of course also indirectly affects humans.

In this poster, the exposure of water and air will be mainly focused due to deposition processes.

Dry and wet depositions from the atmosphere are estimated using the 2004 version of the ECETOC TRA (Targeted Risk Assessment) integrated tool based on the OPS model (Operational Priority Substances) model. The model is based on several assumptions to make it representative at a European level but does not necessarily systematically yield realistic exposure estimates.

A serie of different substances have been chosen differing by their different volatilities and it will be investigating the possibility and feasibility of refining various parameters of the model such as the source description, the emission height and the heat content of the emission plume, suggested by de Bruin et al. (2010). Deposition fluxes in the soil compartment will be calculated on the basis of these refined model parameters and discussed.

MO 053

Establishing a postgraduate course on exposure modeling for environmental risk assessment

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As part of the environmental risk assessments of chemicals, exposure assessments are required by various EU directives, e.g. those on plant protection products, on biocides, pharmaceuticals and on industrial chemicals. In the European community, the latter category is covered by the REACH legislation (Registration, Evaluation, Authorisation and Restriction of Chemicals), which prescribes industry to perform environmental exposure assessments for all chemicals on the market and report them in Chemical Safety Reports. Prerequisite to this is that expertise in environmental exposure assessment is available for regulators, industry and those who provide their service to industry.

Environmental exposure assessments are performed in a tiered process. That means that assessments start simple and can be refined if necessary. First-tier assessment usually involves multimedia fate modeling to predict the concentrations of the chemical in the environment. Models use mathematical expressions to represent the emissions, the reactions and the transport processes that influence the chemicals' behavior in the environment. The basis of the mathematical representation for the tier-1 assessments in the EU is laid down in the REACH guidances R.10-R.16. Performing exposure assessments, as well as doing solid analyses of their results requires a complex set of skills. Basic understanding of the principles of exposure modeling is necessary to adequately interpret model results, such as predicted environmental concentrations (PECs).

Understanding of the effects of input parameters on model output is essential. This more technical expertise needs to be brought together with the regulatory point of view. Therefore, individuals coping with regulatory aspects of environmental exposure assessment need to be trained in exposure modeling. We aim to establish a post-graduate teaching course that provides a thorough theoretical and practical insight in exposure assessment, using a set of acknowledged teaching materials. The goal of the designated postgraduate course is that participants gain (i) better understanding of scientific basis of exposure assessment under REACH; (ii) the ability to perform solid exposure assessments under REACH; and (iii) the competence to put exposure modeling into perspective. Course materials will be made publicly available.

MO 054

Parameter reliability in kinetic evaluation of environmental metabolism data - Assessment and the influence of model specification

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Currently, the usual way to determine parameter reliability in kinetic models fitted to environmental metabolism data includes a visual assessment, the determination of a relative measurement error level that may explain the residual variance based on a χ^2 -test and a t-test for significant difference of the parameter from zero. However, the kinetic rate constants can not be smaller than zero and therefore the assumption of a normal distribution is not appropriate. Furthermore, molar formation fractions can only take on values from 0 to 1. Therefore, also in this case a normal distribution can not be assumed. On this poster, several possibilities to overcome these problems are discussed.

MO 055

Development, application, and evaluation of models for screening organic chemical exposures to humans

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Chemicals are being evaluated to protect humans and the environment; however, there are few measured data available for the assessments. For the vast majority of chemicals, mass balance and Quantitative Structure-Activity (Property) Relationship (QSA(P)R) models are required to obtain data on chemical partitioning properties, persistence, bioaccumulation, toxicity (hazard), release rates, exposures to ecological receptors and humans, and risk. Uncertainty in chemical evaluations exists whether the data are measured or modelled and the uncertainty in the measured and predicted data for chemical screening assessments can be substantial. There is a need to address, quantify and ultimately reduce uncertainty in chemical assessments. The Risk Assessment Identification And Ranking (RAIDAR) mass balance model was used to screen and rank a database of ~13,000 organic chemicals based on far-field human exposure. The model calculations included screening level uncertainty analyses to estimate the propagation of uncertainty in model input parameters on human exposure calculations. Three exposure metrics were selected here: the concentration in a human based on the actual emission rate estimate (CA), the concentration in a human based on an assumed, consistent unit emission rate for all chemicals (CU), and the intake fraction (iF). All of the endpoints are capable of discriminating several orders of magnitude differences in chemical exposure (CA) and exposure potential (iF, CU). In general, the exposure metrics (moving from iF, to CU, to CA) are shown to have increasing discriminatory power for screening purposes, increasing biological relevance for human exposure-based prioritization, but suffer from increasing uncertainty in their calculation due to reliance on additional model input data with high uncertainty (in particular, human metabolic biotransformation rates and emission rates). Efforts to reduce uncertainty in emissions estimates show reduced uncertainty in exposure estimates in revised screening simulations. The screening results from this holistic exposure assessment method are being evaluated with new and existing (bio)monitoring data and compared with screening results using persistence and bioaccumulation criteria. The approach provides a defensible method for identifying which chemical properties determine actual exposures and research needs to reduce uncertainty in the assessments. Research needs to improve the screening system are also highlighted.

MO 056

World exposure assessment tool

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A flexible modelling platform has been developed to evaluate the potential impact of crop protection chemicals on the environment throughout the world. The tool currently has been configured with scenarios containing crop, soil, and weather conditions for major agricultural areas in Canada, Colombia, the European Union, Norway, the People's Republic of China, and the United States. Scenarios are simulated using fate and transport models that have been accepted for regulatory assessment in the U.S. and the European Union, including the Pesticide Root Zone Model (versions 3.12.2 and winPRZM 4.51), EXAMS, RICEWQ, ADAM (groundwater dilution model), and TOXWA. A key strength of the tool is that scenarios can be added for additional geographical areas with relative ease and the appropriate regulatory endpoints.

MO 057

Uncertainty analysis using a Fugacity-based multimedia mass balance model: application of the updated EQC Model to D5

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Among several multimedia models, the Equilibrium Criterion (EQC) fugacity multimedia model has been widely used for the assessment of the fate, distribution and transport

of a chemical of interest in the environment. Since the model requires partitioning and reactive properties of the chemical as input data, it is very important to understand how much uncertainties of the physicochemical properties affect the model outcomes. Thus, this study focused on the uncertainty analysis of the EQC Level III model for decamethylcyclotrisiloxane (D5) to quantify the confident ranges of mass distribution, persistence and intermedia transport rates based on pre-determined dispersion factors of physicochemical properties of the chemical. For the uncertainty analysis, the Monte Carlo simulation technique was employed using Oracle Crystal Ball as an Excel Add-in program to a new spreadsheet platform of the EQC model.

The uncertainty analysis showed that once it is released or vaporized to air D5 stays in the compartment until it is removed at a relatively fast rate via OH radical oxidation and advection, resulting in relatively short overall persistence and no intermedia transport from air to other compartments. These model outcomes are not virtually affected by the uncertainties of physical properties of D5. On the contrary, when D5 is released to water, a major fraction is distributed in sediment due to sorption of the compound to organic substance in sediment where the degradation rate is very small. In this case, the model outcome is predominantly influenced by the uncertainty of KOC.

Under a realistic emission scenario (94.5% to air, 0.8% to water and 4.7% to soil), the 95%-confident ranges of the mass distribution in air and sediment are 47%~78% and 19%~49%, respectively, whereas the overall persistence varies from 3.2 to 5.8 days with the same confidence. The variances are predominantly affected by KOC.

Overall, the EQC model results are strongly dependent on the mode of emission. Since the mass distribution in sediment is sensitive to the emission to water, accurate measurement of KOC and $t_{1/2, Sed}$ is expected to warrant a better understanding of the fate, distribution and transport of D5 in the environment.

MO 058

Multi-scale, multimedia modeling with Pangea - Local to global evaluation of the impacts of a distribution of coal power plants

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A large share of environmental impact and risk assessments of chemical emissions has been performed locally or regionally. However, it is usually not known how global impacts associated with these emissions do compare to local impacts. The present research focuses on the evolution of the intake fraction - the fraction of an emission that is ultimately taken in by the population - from local scale to global scale.

A review of existing models shows that currently published and operational models - ranging from single medium high resolution transport models to low resolution multimedia multi-pathway models - are neither able to properly reflect local to global impacts of emissions of pollutants, nor are they flexible enough to be adapted to specific locations or grid geometries. We already demonstrated innovative features of a new model called Pangea, aiming at addressing these needs, in a single-source context based on emissions of various types of plants located in the north-east of France. The present research provides a statistical result based on the study of more than 500 power plants located around the World, simultaneously demonstrating the flexibility of Pangea.

A set of multi-scale grids covering all the relevant media and refined around each source (coal power plants) are built. These grids allow Pangea to evaluate and compare local to global impacts of each source using a standard multimedia fate/transport and multi-pathway population exposure approach, but spatialized specifically for this study. Substances taken into account were chosen to represent broad categories of known environmental pollutants.

Results obtained so far for a few sources indicate that for volatile pollutants (e.g. Benzene) less than 10% of the overall population intake might happen within a 100km radius from the source, and hence that a local modeling (e.g. local risk assessment) of such emission might capture only a small fraction of the overall impact. We will present a statistical results on the comparison of local to global evolution of the integrated intake fraction. In other words, we will discuss statistically the fraction of the global intake that is captured by a local modeling, for a distribution of coal power plants (at their true locations) that covers the globe.

MO 059

Prediction of environmental and biological degradation half-lives with quantitative structure activity relationships

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Due to a general lack of measured information, predictive methods are required for degradation half-lives in environmental media and in biota to assist in chemical hazard and risk screening. Overall degradability of a chemical in the environment, calculated by the combination of chemical partitioning and environmental media-specific degradation half-lives, is a key determinant of chemical exposure and therefore potential risk. Depending on the partitioning properties, chemical usage, and mode of emission a chemical may be distributed differently in the environment which will alter its overall rate of degradation due to the differential rates of degradation in various physical compartments of the environment (i.e. air, water, soil and sediment). Chemical exposure potential to humans and ecological receptors is also dependent on food web structure, partitioning properties, route of exposure, and differential biotransformation rates of various biota comprising aquatic and terrestrial food webs. In order to interpret the overall role of chemical degradation on exposures, a multi-media multi-pathway mass balance model is required. Using RAIDAR, a multimedia mass balance exposure model linked with aquatic and terrestrial food webs, overall persistence and human and ecological exposures are evaluated as functions of chemical degradability in diverse environmental compartments and organisms. Quantitative structure-activity relationships (QSARs) are developed to cover as many different environmental media and biota as the current data availability allows. A novel algorithm to develop QSARs simultaneously for multiple environmental compartments and organisms is applied, and allows for a detailed analysis of the relationship between chemical structural features and exposure potential.

MO 060

Partitioning of organochlorines in storage lipids, membrane lipids and proteins in lean fish

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Lipids are the dominant force in determining organic contaminant accumulation in aquatic organisms because hydrophobic organic chemicals (HOCs) are believed to partition mainly into lipid phase. Therefore, lipid normalized tissue concentrations have been used to reduce variation in tissue residues between different species and aid in understanding of contaminant toxicity and distribution in food webs. However, partitioning of contaminants between different lipid classes is not very well known. Lipid composition contributes to variation in bioaccumulation potential and neutral storage lipids are thought to be the most important lipid class for HOCs. On the other hand, polar membrane lipids are the target for many HOCs toxicity and knowing the concentration in membrane lipids would greatly improve the risk assessment of HOCs. Using PDMS silicone as a partitioning phase, we have applied equilibrium partitioning approach to estimate HOC distribution in storage lipid, membrane lipid and protein fractions for carbon 14 labelled PCB-153, PCB-77 and PCP. The method is tested with the extracted native PCB contaminated pikeperch oil, and measures of lipid class and protein contents in fish. PCB distribution was dominated by storage lipids (70-78%) while membrane lipids (19-23%) and protein (2-6%) had lower share. The results showed the importance of chemical partitioning to protein fraction in lean fish. The results give new important insight of estimating toxicity by using chemical body burden as a dose metric.

MO 061

Simulating ionogenic chemical fate, bioaccumulation and exposure with RAIDAR

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Globally, chemicals are being evaluated for potential hazards and risks to ecological receptors and humans. When measured data are not available, multimedia mass balance models and Quantitative Structure-Activity Relationships (QSARs) are needed to estimate chemical concentrations in the environment and organisms. Fugacity-based multimedia models have been used extensively for simulating the environmental fate and bioaccumulation of neutral organics; however, exposure models are limited for ionogenic organics. It is estimated that approximately 50% of the 149,000 substances submitted under REACH are ionogenic. The lack of integrated tools for the evaluation of data-poor, ionogenic substances for the complete exposure pathway (i.e. emissions, to fate and transport, to bioaccumulation in aquatic & terrestrial food chains) reduces confidence in chemical assessments for substances that are subject to ionization in the environment such as many personal care products and pharmaceuticals. The Risk Assessment, Identification And Ranking (RAIDAR) model includes linked fugacity-based multimedia fate and bioaccumulation sub-models, thus providing the capacity to evaluate exposures to humans and ecological receptors in a "holistic" manner. The general objective of this project is to revise and evaluate the RAIDAR model for the simulation of ionogenic chemicals for screening-level assessments. A secondary objective is to develop strategies to address chemical property data gaps so that the model may still be used under data-poor conditions. This presentation outlines the model revisions and explores the fate, bioaccumulation and exposure potential of ionogenic chemicals released to the environment. A hypothetical set of ionogenic chemicals are used to simulate how changes in chemical partitioning properties and dissociation constants affect chemical distribution in the environment and thus exposure potential to selected ecological receptors of interest (i.e. fish, piscivorous bird). Simulations are also conducted for current ionogenic chemicals of interest. These simulations highlight data needs for future research to improve model predictions to reduce uncertainty in the assessment of ionogenic chemicals.

MO 062

Predicting the oral uptake efficiency of chemicals in mammals: extension to the hydrophilic range

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Environmental risk assessment requires models to estimate bioaccumulation of untested compounds. So far, most mechanistic bioaccumulation models have focused on very lipophilic compounds, and only few included also more hydrophilic compounds. As shown by pharmaceutical studies, the membrane permeation of polar compounds is not sufficiently reflected by the octanol water partition coefficient (KOW). Therefore, additional descriptors are needed to mechanistically estimate membrane permeation. Our aim was to develop a model to estimate oral uptake efficiency of pollutants via ingestion in mammals for polar and nonpolar compounds over a wide KOW range of pollutants with focus on polar compounds. The model was based on mechanistic processes and thus included the advective transport of the pollutant through the gastro intestinal tract, the diffusion from the food through the unstirred water layer, through the membrane and into the blood. The diffusion through the membrane was split into an inner and outer membrane resistance, where KOW reflected partitioning from the water into the outer membrane, and hydrogen bond donor strength reflected the

partitioning from the outer into the inner membrane. The model was calibrated to two data sets which were merged: uptake efficiency of environmental pollutants measured in different mammals during feeding studies, and a pharmaceutical data set with human oral absorption efficiencies. The new model estimated uptake efficiency for compounds with logKOW ranging from -10 to +8, and estimation was improved for polar compounds by accounting for the bilayer structure of the membrane. Including the inner membrane resistance improved RMSE especially for the compounds with KOW<0 from 28.3 to 18.3, while also overall performance improved from 18.4 to 14.0. Therefore, the new model provides a tool to estimate uptake efficiency for new compounds based on sound mechanistic processes.

MO 063

A standardized contact transfer method for assessing soil-to-clothing exposure to soil-sorbed chemicals

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Exposure to chemicals, including chemical warfare agents (CWA), can present a potential Contact Hazard even when the compounds are sorbed onto soil. Previous studies have investigated levels of CWA transferred from contaminated surfaces utilizing a malleable latex material (dental dam; DD) as transfer substrate; however DD is typically inconsistent in compositions, and not reliable for toxicological investigations. Measurements of CWA on standard Army Combat Uniform (ACU), plus toxicological effects of CWA, have been reliably established for some CWA and ongoing-testing continues for others; but need still existed for a reliable transfer-exposure method for assessing the exposure potential for chemicals sorbed onto soil. We have established a method for reliably determining Contact Transfer of compounds from soil directly onto clothing, utilizing standard mass (x Gravity) as a standard force to produce a standard measure of exposure potential. We initially determined the effects of contact time and applied mass (DD); then in benchmark investigations, we established the efficacy of CWA extraction from ACU (and DD), and Contact Transfer of CWA from soil onto clothing (ACU). A 4"-diameter circular swatch of ACU material was selected, similar to surface areas that may contact soil at knee or elbow locations; standard contact was created by placing the ACU swatch directly onto the soil-surface one minute after CWA dissemination, then covering the swatch with a 4"-diameter Plexiglass disk to distribute force from a centrally-placed standard mass. Contact Transfer of CWA was determined by solvent extraction of swatches, with subsequent analyses by GC/GC-MS or HPLC/HPLC-MS. Masses 0.250, 0.500, 1.000, 1.500 kg, resulted in significantly greater CWA transfer ($p \leq 0.05$) at ≥ 0.500 kg compared to 0.250kg; therefore the 1-kg standard mass was selected for integration into the method. Although the quantities of soil-sorbed CWA that transferred increased as a function of contact time, the rate of transfer decreased dramatically after 1h; therefore 0.25h was selected for integration into the method. The quantities of CWA transferred from soil surfaces contaminated at operationally important levels from Soil-to-Clothing (ACU) are amounts that are within the range of concern for Soldiers. The standardized Contact Transfer Method provides reliable standard predictions of exposure potential, and in conjunction with toxicity data, for predictions of Contact Hazard.

MO 064

Combined modelling of PAH biodegradation, soil sorption and dissolution from organic phases

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Despite promising microbial degradation of polyaromatic hydrocarbons (PAH) in the laboratory, pilot-scale investigations and observations at the field scale often reveal the formation of a considerable residual fraction of PAHs in soil, which is not further degraded. Non-bioavailability of PAH due to adsorption and sequestration would thus result in residual fractions resistant to microbial degradation, which may increase with time. However, residual concentrations may also be due to kinetic reasons, i.e. the concentrations may be too low to maintain microbial metabolism. For a range of biodegradation experiments, combined modelling of microbial growth and degradation, adsorption/desorption processes as well as dissolution from the organic phase has been carried out. The objective of the present study was to analyse and explain observed processes mathematically.

In experiments with phenanthrene added to solution as microcrystals and the degrader microbe *Novosphingobium pentaromativorans*, fast degradation took place at the beginning, corresponding to a strong initial increase in microbial mass. Experiments with higher initial concentrations revealed slower degradation. An initial set of Monod parameters could be fitted and experimental observations could be explained by a decreasing effective surface area for phenanthrene dissolution of the phenanthrene crystal phase. These effects were stronger with higher initial concentrations (assumed higher coagulation of microcrystals with time). Other experiments considered were done with phenanthrene-spiked soil amendments (radio-labelled ¹⁴C-phenanthrene) in suspension and degrader strain *Sphingomonas* sp. 10-1 (see also Marchal et al. session C07). Biodegradation proceeded rapidly due to high cell densities of degrader bacteria. No bacterial growth, but rather a slow decline was observed. Desorption velocities could be fitted for the experiments, reflecting differences in surface areas of the soil amendments. The modelling revealed that high sorption did not have an inhibitory effect on phenanthrene biodegradation. Indeed, simulations showed that no growth of degrader bacteria was required for degradation, since the initial bacterial numbers were high enough to achieve complete mineralisation within the experiment duration. The evaluation and modelling of new experiments with different soil suspensions and added soil amendments (activated charcoal, biochar and compost) is currently under way.

MO 065

Extended dynamic range, high precision analysis of Polynuclear Aromatic Hydrocarbon compounds by GC-MS

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Polynuclear aromatic hydrocarbons (PAH) are a class of compounds used in the determination of contamination from fuels and oils and also monitored in the environment and food supply as potentially toxic compounds. The concentration range of PAHs in any given set of samples, depending on source, may be in the sub-ppb range up through the ppm range. A methodology is demonstrated which takes advantage of both GC-MS injection techniques and mass analysis techniques to extend the dynamic range of PAH analysis to encompass these concentrations. This is accomplished while maintaining the required levels of calibration, replicate and sample QA/QC (precision and accuracy), improving the productivity of the laboratory across a wide range of projects.

MO 066

Modelling PAH bioaccumulation in *Mytilus galloprovincialis* with a three-compartment model

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Polycyclic aromatic hydrocarbons (PAHs) are important organic pollutants in the aquatic environment due to their persistence and accumulation potential both in organisms and in sediment. With the entrance of PAHs into a clean ecosystem, indigenous aquatic biota may be affected due to the accumulation of PAHs in their adipose tissues. Bioaccumulation of pollutants may occur at each link in aquatic food chains, starting from primary producers to humans consuming various aquatic organisms with their diet. In this study, mussels were selected as biological indicators due to their broad geographic distribution, immobility and low enzyme activity, which cause significant bioaccumulation of pollutants in their tissues. PAH bioaccumulation and depuration in *Mytilus galloprovincialis* under dynamic environmental conditions were described using a three compartment model. First order kinetic equations were used for the description of accumulation and depuration of selected PAHs in mussel tissues. Data were obtained from experiments performed with selected PAHs under constant conditions. In the model, three compartments were defined: (1) mussels, (2) surrounding environment (seawater), and (3) algae (*Phaeodactylum tricornutum*) as food source for the mussels. During the bioaccumulation period, the mussels take up the contaminants both from seawater and with PAH exposed algae. Thus, the model considers dynamic exchange of PAHs between algae and seawater. Experimental data were used to parametrise and calibrate the proposed model for benzo(a)anthracene and phenanthrene. The observed dynamics could be described well for three different PAH concentrations. The model provides a mathematical description of bioaccumulation and depuration kinetics of PAHs in mussel species. For better agreement of simulation results with the experimental data, still a deeper understanding of the processes determining the chemical fate of PAH in the system of seawater, algae and mussels is required to be integrated into the model equations.

MO 067

Mass balance of polycyclic synthetic musks using a new dynamic segmented surface water/soil model

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Polycyclic synthetic musks (PCMs) are used as fragrances in a wide variety of personal care products. Among these compounds, the most important are galaxolide (HHCB) and tonalide (AHTN), used respectively, in Europe during the year 2000, at amounts of 1473 and 358 t. Since the removal of these substances through municipal sewage treatment has been estimated to be not higher than 60%, concentrations ranging from ng/L to µg/L of HHCB and AHTN are often measured in aquatic environments, posing a serious concern for ecosystems given the high potential for bioaccumulation of these chemicals. In this context, determining the exposure levels of ecosystems to these substances becomes a priority and environmental fate models represent an essential tool given the lack of monitoring data. In 2010, a survey along the Molgora River, flowing for about 40 km in a densely populated area in the Lombardy Region, northern Italy, was started. Samples of water and suspended solids were collected seasonally in 7 monitoring stations located up- and downstream of the 3 sewage treatment plants (STPs) present along the river. Seasonality in concentrations was observed, with the highest levels occurring in spring. Moreover, the samples collected downstream of the 3 STPs showed the highest concentrations. In order to investigate the reasons of this variability and to predict future trends, a new dynamic modelling approach (DynANet) based on an existing water-sediment model (DynA) was applied. In DynANet, the implementation of geographic information system (GIS) tools allows the subdivision of a river drainage basin in sub-basins and the resulting river links, classified according to the Strahler stream order, communicate by means of a downstream water flow. The loading data of runoff water and modelled chemical concentration coming from the different sub-basins are provided by the connection with the existing dynamic model SoilPlus, developed to evaluate the fate of organic chemicals in the air, litter and soil compartments and working in a GIS environment. The application of the DynANet model to the case study allowed to compile a preliminary mass balance for HHCB and AHTN and to predict concentration changes under environmental phenomena such as precipitations events, temperature variations, soil use, etc. The measured concentrations and simulated results were compared to the prediction of existing models such as GREATER.

MO 068**Estimating chemical emissions from home and personal care products in Asia**J.E.N. Hodges¹, O.R. Price¹, C. Holmes², R. Vamshi²¹Unilever, Bedfordshire, United Kingdom²Waterborne Environmental Inc, Leesburg, United States of America

Asia's economy is growing significantly with China and India leading the growth. This has led to an increased demand for home and personal care (HPC) products. Information on the use and sales of HPC products is critical to estimating emissions and predicted environmental concentrations (PECs). It has also been demonstrated that by coupling population density data with sales data it is possible to estimate HPC product consumption. To date, most studies have been based in developed markets, where wealth distributions are more homogeneous and HPC products are affordable to all resulting in usage being evenly distributed across the population. In Asia, a population's wealth and ability to buy HPC products is not uniformly distributed, therefore combining information on population density and sales data with a sub-population's ability to purchase HPC products could improve chemical emission estimates. Global datasets were sourced for population and economic activity incorporating population, night-time light satellite imagery and land cover. Coupling these datasets with sales data of different HPC products at a (sub) country level and 'takeoff values', (a population's ability to purchase HPC products based on their per capita GDP), emission estimates were calculated. Results show large variation in HPC product usage exist across Asia due to the variability in population density and wealth. The largest refinement of emission estimates are associated with products requiring greater purchasing power. As wealth even out, for example the per capita GDP is above \$10,000, population density becomes the appropriate method for distributing product use. The potential benefits of this approach include more accurate estimation of chemical emission patterns, which could be used to parameterise exposure models for use in environmental risk assessment and contextualising existing monitoring data.

MO 069**Chemical fate and risk assessment at large scale based on direct and inverse modeling approaches and pan-European datasets**D.T. Marinov¹, A. Pistocchi², M. Trombetti³, B. Galwik³, G. Bidoglio³¹EC, JRC, IES, Ispra, Italy²GECOSistema srl, Cesena, Italy³European Commission, JRC, IES, Ispra, Italy

The chemical fate and risk assessment at large scale is typically performed on the basis of explicit GIS fate models which in turn make use of either a "direct" or an "inverse" modeling approach with the direct being a traditional one. The direct models are based on a priori available information about chemical emissions and are intended to answer the question: "Where do chemicals go after being emitted?". Thus, when data for emissions and substance's physico-chemical properties could be retrieved from existing databases, the model predictions of spatial distribution of chemicals prove to be sufficiently accurate for screening purposes. Oppositely, the inverse modeling deals with the question "where do pollutants come from?" and is based on measurements of chemical concentrations. Then, the inverse models may support large scale assessment, in particular for the surface water quality by estimating chemical emission factors at regional, river basin or continental scale in relation to the population density or another appropriate proxy. This work presents the potential of the European version of MAPPE (Multimedia Assessment of Pollutant Pathways in Environment) direct model to simulate a steady-state spreading of pollutants emitted from point or diffuse sources. The model outcome consists of annual fate maps of chemical concentrations, deposition rates, fluxes to other environmental compartments, and could be used for screening of hazard zones at continental scale with spatial resolution 1 km². In this model exercise the target contaminants were few POPs as PCBs, dioxins, furans, pesticides, etc. In addition the study encouraged the applicability of the inverse modeling technique for pollutants with poor and sparse emission estimates but which were extensively monitored in pan-European campaigns. As examples, the capability of the inverse GIS applications was utilised for backward tracking of PFOS and PFOA emission factors from riverine measurements and to investigate the loading of the widely used pharmaceuticals and personal care products to European rivers.

MO 070**Multimedia assessment of chemical exposure by MAPPE-Global explicit model: a case study of pollutant discharge to Mediterranean Sea**D.T. Marinov¹, A. Pistocchi², M. Trombetti³, B. Galwik³, G. Bidoglio³¹EC, JRC, IES, Ispra, Italy²GECOSistema srl, Cesena, Italy³European Commission, JRC, IES, Ispra, Italy

The work describes a case study of pollutant discharge to Mediterranean Sea by a spatially explicit fate and exposure model with worldwide coverage for Multimedia Assessment of Pollutant Pathways in the Environment (MAPPE Global). The model computes removal rates of a substance with given physico-chemical properties in an evaluative environment composed of atmosphere, soil, inland and sea water, in the form of global maps with a spatial resolution of 1°x1° for air and oceans and 0.08°x0.08° for soil and surface water.

The maps of removal rates enable the computation of concentrations from known emissions in one or more environmental compartments using a simple box model, except for the stream network where a plug flow scheme is adopted. To estimate the chemical removal rates the model accounts the following elimination processes: chemical degradation; advective transport; diffusive transport; and sinking.

However, MAPPE Global does not explicitly compute chemical transport in space, but only the fate of a substance at each location by calculating chemical's mass fluxes that are available for transport outside of cells. In order to resolve this problem an adjustment procedure has been adopted comparing the Global model outcome with the results of the European version of the MAPPE model.

Then, in relation to the Marine Strategy Framework Directive and HORIZON 2020 initiative for Mediterranean Sea, MAPPE-Global was used to estimate of chemical load of a few POPs (as PCBs, Lindane, etc.) from emissions in the surrounding countries of Europe, Northern Africa and Asia. The Mediterranean Sea load assessments also accounted for the contribution of the LRAT by emission sources originated from North America or South East Asia.

MO 071**The impact of including ice and snow in multicompartmental modelling of persistent organic pollutants' distributions and fate**L. Hofmann¹, F. Guglielmo², I. Stemmler¹, G. Lammel¹¹Max Planck Institute for Chemistry, Mainz, Germany²Max Planck Institute for Meteorology, Hamburg, Germany

Persistent organic pollutants (POPs) are multicompartmental substances. Ice or snow compartments have only recently been included in POP cycling in large scale multicompartmental modelling.

The impact of including ice and snow compartments on model-predicted distribution and fate was studied using the global multicompartment chemistry-transport model MPI-MCTM [1], which is based on a coupled atmosphere-ocean general circulation model. 10 fictive years of DDT and γ -hexachlorocyclohexane (γ -HCH) as applied in global agriculture in 1980 fate and transport under present-day climate were simulated with and without the compartments sea ice, land ice and land snow [2-3]. 7-9% of the burden of the surface ocean of γ -HCH and DDT are stored in sea ice. Neglect of the role of sea ice would largely underestimate γ -HCH in surface seawater (-62% globally, -30% in the Arctic), but overestimate DDT in surface seawater (+19% globally, +107% in the Arctic). The compartmental distribution shift exceeds the mass of γ -HCH and DDT stored in sea ice by a factor of ca. 8 and ca. 15, respectively. Ocean surface waters in the sea ice melting zone receive a seasonal (summer) contamination wave which on the regional scale may double exposure.

Within 10 years 3.34% and 1.84% of the global total environmental burden of γ -HCH and DDT, respectively, are stored in glaciers over Antarctica and Greenland. Land snow is primarily present in northern winter and stores up to 0.82% (γ -HCH) and 0.45% (DDT) of the total contaminants' burden. Melting snow causes a seasonal contamination wave with a volatilisation peak and a release of pollutants to underlying soil and vegetation. The neglect of land ice and land snow in the model would underestimate the total burdens of γ -HCH and DDT stored in the Arctic (by 7% and 2%, respectively) and Antarctic (by 44% and 32%, respectively) and would overestimate Antarctic oceanic burden (by 44% and 29%, respectively).

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MO 072**Global fractionation of PCBs**

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PCBs are ubiquitous environmental pollutants which are expected to decline in abiotic environmental media in response to decreasing primary emissions since the 1970s.

A coupled atmosphere-ocean general circulation model with embedded dynamic sub-models for atmospheric aerosols and the marine biogeochemistry and air-surface exchange processes to soils, vegetation and the cryosphere [1-3] is used to study the transport and fate of four congeners (3-7 Cl atoms) 1950-2010.

The geographic distribution of the PCB mixture evolves over time reflecting the sources and sinks' involvement over time. Globally, secondary emissions are on the long term increasingly gaining importance over primary emissions. They are most important for congeners of medium hydrophobicity (5-6 Cl atoms). Congeners' fractionation is characterized both geographically and temporally. It causes enrichment of the lighter congeners and more delayed decreasing levels in high latitudes in response to decreasing emissions. Delivery of contaminants to high latitudes is predicted to be more efficient than previously suggested.

The results suggest that the effectiveness of emission control measures may significantly vary among substances, not only reflected as latitudinal gradients, but also as longitudinal gradients.

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MO 073

Predicting the removal of atmospheric particles by vegetation with a dynamic multimedia model

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It is well known that vegetation is capable of capturing contaminants from air. In particular, plant leaves can act as a biological filter and as a sink for airborne particles and their associated Semi Volatile Organic Compounds (SVOCs) such as Polycyclic Aromatic Hydrocarbons (PAHs), influencing their environmental fate. In order to assess the filtration performance of plants with respect to atmospheric particulate matter (PM) and their associated SVOCs, deposition on vegetation has recently been investigated by a number of different methods (field, laboratory studies as well as modelling approaches). Some authors studied the particulate matter uptake and retention capacity of different plant species, showing that some plant type are much more efficient than other at collecting particulate matter and PAHs contained upon them. Other researches were focused on measuring particle deposition velocities not only for specific tree species but also at different wind speeds. Furthermore different predictive approaches were developed to evaluate PM uptake by trees in urban areas and to estimate dry particle matter deposition on leaf surfaces using species-specific deposition velocity. Nevertheless, the existing multimedia fate models do not generally account for PM mass balance in predicting PM deposition to vegetation and the corresponding chemical flux. In the present study we used a dynamic multimedia fate model (SoilPlusVeg), which incorporates a vegetation compartment, to calculate leaf particle-bound PAHs uptake from air. In this model, formulated in terms of fugacity, the vegetation compartment can be composed by a mono- or multi- specific forest canopy, which interacts with dynamic air and the soil compartments. The model, which incorporates some forest structural parameters such as specific leaf area (SLA) and leaf area index (LAI), was modified to account for some of recent findings concerning the particulate matter uptake by vegetation. Simulations were performed for some high molecular weight PAHs, as they exist predominantly in the particulate phase, for a multi-specific semi-urban wood located in Como. The results were compared to data measured in air, deposition and vegetation in the same location.

MO 074

Climatic, biological and land cover controls on the exchange of gas phase semivolatile chemical pollutants between forest canopies and the atmosphere

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An eco-physiological model of a structured broad-leaved forest canopy was coupled to a chemical fate model of the air-canopy exchange of gaseous semivolatile chemicals to dynamically assess the short-term air-canopy exchange and the influence of biological, climatic and land cover drivers on the canopy capacity of accumulating pollutants from the atmosphere. The chemical fate model accounts for effects of short-term variations in air temperature, wind speed, stomatal opening and leaf energy balance, all as a function of layer in the canopy. The model also includes a new wet deposition scheme that estimates the interception of gases dissolved in rain as a function of depth in the canopy. Simulations showed the potential occurrence of intense short/medium term re-emission of pollutants having Log(K_{oa}) up to 10.7 from the canopy as a result of environmental forcing. In addition, relatively small inter-annual variations in seasonally-averaged air temperature, canopy biomass and precipitation can produce relevant changes in the canopy storage capacity for the chemicals, suggesting that climate change may play a significant role in determining the effectiveness of forests in controlling atmospheric transport of semivolatile pollutants.

MO 075

Assessing and comparing the influences of uncertainty in chemical property data and variability in climate variables on the simulated fate of PCBs

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Legacy persistent organic pollutants (POPs) are of concern due to their persistence, bioaccumulation potential, toxicity and ubiquitous presence in the natural environment. Environmental fate modellers have made numerous efforts to simulate the transport and fate of legacy POPs at different spatial and temporal scales. The success of modelling exercises depends largely on the quality of model input data for characterizing either the modelled chemicals or the modelled environment, e.g. the physical-chemical properties and environmental (including climate) variables. The purpose of this study is to explicitly assess and compare the influences which are caused by the uncertainty in chemical property data and variability in climate variables. Representative polychlorinated biphenyl (PCB) congeners having a range of physical-chemical properties are selected as study chemicals. The scientific literature was mined to describe the uncertainty in the physical-chemical properties of the selected PCBs, and online climate databases were surveyed to derive the long-term averages and projected deviations for the selected climate variables. The ChemCAN spreadsheet model is reparameterized for Japan and used to test the hypothesis that property uncertainty has more influence on model outcomes than climate variability. The robust statistical tool Oracle® Crystal Ball (Fusion edition, 11.1.1.1.0) is adopted to perform the correlation and sensitivity analysis. Generally speaking, results suggest that uncertainty in physical-chemical property data leads to more pronounced variances in the model predictions than variability in climate variables, i.e., the predicted environmental fate and distribution of selected PCB congeners is more sensitive to uncertain physical-chemical properties than to climate variables. The large uncertainties associated with half-times for degradation in air, water and soil dominate influences on the model simulated fate and distribution of selected PCBs, if compared to climate variables and the physical-chemical properties. Among climate variables, temperature and its projected future deviations is the most influential. We conclude that predicting the influence of climate on chemical transport and fate is challenging due to the fact that climate-related effects on transport and fate are relatively small compared to uncertainties in physical-chemical properties and degradation rates.

MO 076

Environmental fate, latitudinal distribution and long range transport of decamethylcyclopentasiloxane (D5) in the global environment: a model assessment

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Decamethylcyclopentasiloxane (D5) is widely used as an ingredient in the formulation of personal care products, or is present as an impurity in the silicone fluids and rubbers. The global environmental fate, latitudinal distribution, and long range transport of D5 was analyzed by two multimedia chemical fate models using the best available physicochemical properties as inputs and known persistent organic pollutants (POPs) as reference. The global transport and accumulation characteristics of D5 differ from those of typical POPs in three significant ways. First, a large fraction of the released D5 tends to become airborne and is removed from the global environment by degradation in air, whereas known POPs have a tendency to be distributed and persistent in all media. Secondly, although D5 can travel a substantial distance in the atmosphere, it has little potential for deposition to surface media in remote regions. This contrasts with a deposition potential of known POPs that exceeds that of D5 by at least 4 orders of magnitude. Thirdly, D5 has a short global residence time with the majority of the global mass removed within three months of the end of release. Global residence times of POPs on the other hand are in years.

MO 077

Environmental fate of D5 Predicted by the QWASI Fugacity model

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Due to the unique characteristic property of decamethylcyclopentasiloxane (D5), airborne D5 remains in the compartment until it is oxidized by OH radicals. In contrast, when D5 is released to water, multiple processes including volatilization, sorption to sediment, advection and hydrolysis take place competitively. Importantly, the rates of transport and transformation processes are dependent on the local aquatic environment. Since a better quantitative assessment on the fate, distribution and transport of D5 in a real water body system is needed, this study evaluated the environmental behavior of D5 in Lake Ontario (LO) and Lake Pepin (LP) at various environmental temperatures and under steady-state and unsteady-state conditions. Additionally, uncertainty analyses were performed to determine the most probable range of the model outcome and the most influential input parameters using a new Quantitative Water Air Sediment Interaction (QWASI)-Excel model. The QWASI-Excel model predicted that water concentration of D5 decreased for both lakes due to increase in hydrolysis rate with rising temperatures from 1 to 25°C. In contrast, D5 concentration profiles in sediment were different: it decreased gradually from 1.0 to 0.5 ng/g dw in LO whereas it increased from 120 to 280 ng/g dw in LP. D5 mass preferentially distributed to water in LO but to sediment in LP. Mass fraction of D5 increased with temperature for both lakes mainly due to increased K_{oc} value. D5 was removed via hydrolysis and volatilization in LO whereas via advection and hydrolysis (at only high temperature) in LP. Residence time of D5 in water was relatively short for both lakes whereas residence time of D5 in sediment was much longer due to slow degradation. In contrast with a relatively short response time in water, response time in sediment was relatively long.

Uncertainty analyses with Monte Carlo simulation determined that hydrolysis half-life in water and sediment layer depth was the most influential input parameter to environmental behaviors of D5 in LO and LP, respectively. In addition, other factors such as K_{oc}, hydrolysis half-life, solid deposition rate and sediment resuspension rate also contributed to the total variations in environmental behaviors of D5 in LP. The analysis also predicted 95% confidence intervals for D5 concentrations in water and sediment, mass distribution, intermedia transport and transformation rates, residence time and response time in both lakes.

MO 078

Modelling aluminium fate and transport in ground and surface water for Life Cycle Assessment

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In assessment of the fate, transport, and ultimate exposure of metals to humans, correctly accounting for long-term groundwater emissions, e.g., from a landfill, has been challenging. This research offers a new perspective on human exposure to anthropogenic metal emissions to ground and surface water. Existing models, such as the Ecoinvent LCA database have assumed 100% of landfill emissions reach surface water. To accurately reflect geochemical and hydrologic processes, this conservative assumption can be reassessed.

A multi-component model was developed to assess the total intake by humans of emissions to landfills (for aluminum) and directly to surface water (for arsenic from aluminum ore). For landfill emissions, a kinetic precipitation model was developed to estimate transfer to surface water. In surface water, the geochemical model PHREEQC

[1], including an implementation of WHAM VI [2], was used to estimate speciation, as well as the potential for precipitation, sorption, and complexation, while hydrological data at the continental level provided estimates of continental flows and residence times. Information at the national level on drinking water source and treatment were aggregated to the continent scale and included in the model, thus providing estimates of the population fraction drinking treated and untreated ground and surface waters. Finally, speciation was used to assess bioavailability of ingested aluminum to humans. The model shows lower estimates of transport from source to receptor than previous studies, which lacked a precipitation module. Uncertainty in input data and modeling assumptions, which will be discussed, allow the model to be applied to large-scale systems as a comparative tool. For example, the ingested intake fraction for aluminum emissions to landfills is lower than previous models by a factor of 1×10^6 or more, depending on the location. The continent of emission, via its hydrology and the fraction of treated / untreated drinking water, plays an important role.

[1] Parkhurst DL. 1995. "User's guide to PHREEQC--A computer program for speciation, reaction-path, advective-transport, and inverse geochemical calculations." United States Geological Survey. 95-4227.

[2] Tipping E. 1998. "Humic ion-binding model VI; an improved description of the interactions of protons and metal ions with humic substances." *Aquatic Geochemistry*. vol. 4, no. 1. pp. 3-48.

MO 079

Dioxin Source to Ecological Receptor Region HYSPLIT-SV Modeling in Mexico with Atmospheric Measurements 2008-2010: Montes Azules, Celestun and Monterrey

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Instituto Nacional de Ecología (INE) sampled and measured dioxin in the atmosphere at 10 research stations in Mexico from 2008-2010 with the support of the U.S. Environmental Protection Agency, Environment Canada, Health Canada, and the Commission for Environmental Cooperation for North America. We focus in this presentation on three receptor regions with air monitoring stations with variations in measurements that need to be explained: Montes Azules, (ecosensitive biodiverse ecoservice region, land), Celestun (ecosensitive biodiverse ecoservice, land and marine), and Monterrey (urban and agricultural). The Hybrid Single Particle Lagrangian Integrated Trajectory Semi-Volatile experimental research atmospheric dispersion model (HYSPLIT-SV) is employed to account for variations from meteorology under conditions of uncertain source emissions.

HYSPLIT-SV is a dynamic atmospheric dispersion model adapted to include atmospheric chemistry and environmental fate processes including vapor-particle partitioning, degradation (e.g. via hydroxyl radical, ozone, photolysis) and deposition (wet and dry; gas and particle). Source-to-receptor simulations produce estimates of the efficiency of transport to a receptor as air concentrations and deposition, as fractions, which we call Air Transfer Coefficients [ATC]. ATCs integrate the effects of meteorology and environmental fate processes from individual sources to selected receptors. ATCs enable the development of estimates of source-to-receptor transport under conditions of changing and uncertain emission scenarios.

Secretary of Environment and Natural Resources (SEMERNAT) and INE are in the process of developing a dioxin emission inventory in cooperation with the United Nations Environmental Program (UNEP) under the Stockholm Convention. The UNEP dioxin toolkit provides emission factors in aggregate TEQ, but not by congener. Dioxin modeling must be conducted on a congener basis, since congeners vary in physical-chemical properties that determine environmental fate processes. For this presentation, we estimate congener emissions from congener profiles based on U.S. and Canada measurements. We focus on the major point sources that have been best characterized at this time in Mexico. HYSPLIT-SV modeling output provides data that can be used to rank the importance of each source contribution to the loading to ecological receptor regions useful for efficiently targeting mitigation by policy makers.

MO 080

Mathematical modeling of point source pollutants fate and transport in the Little Akaki River, Ethiopia

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Computer models are becoming increasingly important tools in every environmental management aspects. A general one-dimensional water quality model has been developed for one of the most industrially concentrated and polluted rivers in Ethiopia - the Little Akaki River. The study covers a total of 27.4 km length along the river where pollution issues are significant. The spatial and seasonal variation of hydro-geometric properties that affect mixing and transport of chemicals in the river were estimated by correlating the river hydro-geometry in a power law model. For this purpose, 20 locations were systematically selected and field data were collected for five months. The river has been conceptualized as 1370 equal length completely mixed computational elements, and a functional representation that includes mass transport and hydrodynamic equations were written for each computational elements. The general one-dimensional model equation was solved using the numerical technique by finite volume spatial discretization and finite difference temporal discretization. Computational solution procedures were prepared by writing a computer program code using the MATLAB programming platform (version 7.0). BOD and DO were selected as water quality indicators, and sensitive model parameters that affect fate and transport of DO and BOD in the river were calibrated. The performance of the calibrated model in predicting data not used during calibration was also estimated and the result was in good agreement with these targeted water quality data. The applicability of this model as decision making tool for pollution control activities was also assessed and the result is encouraging. Recommendation, which takes into account the existing water quality problem in the study area, has been suggested for improvement and wider application the model.

MO 081

A study of the relevance of regionalization and archetype approach for aquatic freshwater ecotoxicity

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The importance of regionalization for the aquatic freshwater ecotoxicity impact category has been evaluated at different resolutions (at a watershed, country and continental scale) using multimedia fate and exposure models such as IMPACT World, Europe single zone and Europe spatial models.

A first comparison of water fate factors between the IMPACT Europe single zone model vs. the IMPACT Europe spatial model has been performed considering both a uniform emissions and emissions in each specific watershed. Moreover three watersheds with short, medium and high residence time of water to the sea have been selected to analyze the importance of chemical properties vs. watershed specific properties to identify the key parameter(s) influencing the fate. The water residence time was used as the basis for defining 2 watershed archetypes, i.e. upstream (with water residence time >0.1 [yr]) and downstream of a lake (water residence time <0.1 [yr]). The fate factors of chemicals emitted into water calculated with this latter approach have been compared to the spatial model and differences with the a-spatial approach discussed.

The results of IMPACT World and Europe showed that a-spatial models might overestimate the chemical fate and characterization factors for fresh water ecotoxicity up to a factor 5 when compared to a spatially differentiated model for unknown emission location (i.e. assumed being uniformly emitted compared over the whole model surface). When the emission location is known, a spatially differentiated model can improve the model accuracy up to 2-3 orders of magnitude, because of its ability to accurately predict the water residence time to the sea (or out of the system) depending on the emission location. Is therefore spatial differentiation always required? The answer depends on the physico-chemical property of the chemical: only for persistent chemicals the water residence time plays a key role in determining the chemical fate for freshwater ecosystem. Highly degradable or volatile chemicals for example would disappear before being advected out of the system. This support that, for persistent chemicals, the country/ regional differentiation is relevant.

MO 082

Mixing zones modeling in the water framework directive: comparison between 'discharge test' and 'CORMIX'

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ARCHE, Gent, Belgium

The Priority substance Directive 2008/105/EC has defined EQSs for 33 priority substances. Compliance with these EQSs should be achieved at the point of discharge or at least at the edge of the mixing zone in the receiving waters. Considering this, a "Tiered Approach" has been developed to assess whether compliance with the EQS values is achieved. The approach starts with simple investigations but could end with very detailed assessments on the mixing behavior and concentration profile of effluents in the surface waters. In contrast to lower tiers, higher tiers require an estimate of the extent of EQS exceedance that can be used to define a mixing zone. This may be achieved using a range of tools such as "Discharge Test" or "CORMIX". The aim of this presentation is to discuss the application range of both "Discharge Test" and "CORMIX". "Discharge Test" is a freely available model developed to assess the acceptability of mixing zones resulting from discharges into surface waters at Tier 2 level. This model is based on Fischer equations and requires a limited set of parameters and allows for quick modeling of effluent concentrations from single port submerged pipes only. Moreover, it ignores the importance of boundary interaction in the near field and the possibility of density current formation after initial jet/plume mixing. "CORMIX" is a USEPA supported model that can be used for Tier 2 and higher. It is a mixing zone expert system that classifies momentum and buoyancy of the discharge in relation to boundary interactions to accurately predict all different mixing behaviors. "CORMIX" is a well-validated model that requires just a few more input parameters. CORMIX takes into account all the weaknesses pointed out for "Discharge Test". "CORMIX" was not designed to present less worst case results, it was designed to provide the most accurate representation of the actual physical mixing process given a set of ambient and discharge conditions. The main conclusion is that "Discharge Test" is easy and free and can be used for Tier 2 assessments of single port submerged discharges. If more accurate modeling is required or if "Discharge Test" does not cover the discharge parameters, "CORMIX" could be used.

MO 083

Development of GREAT-ER 3.0, an entirely Open Source software for river and sediment exposure modeling

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GREAT-ER (Geography-referenced Regional Exposure Assessment Tool for European Rivers) is a GIS-assisted computer model for risk assessment and management of chemicals in river basins. Since the release by ECETOC of the original model in 1999 (GREAT-ER 1.0) a number of regional projects have been initiated by various organizations with the aim of exploring and expanding the different applications of the model. To facilitate this process, the model system was reimplemented with a modular architecture and a database back-end in 2003 (GREAT-ER Desktop and GREAT-ER Web).

Over the years, the GREAT-ER model has served a worldwide user community, and currently more than 170 different scientific papers refer to the development or application of the model.

Under the sponsorship of the CEFIC-LRI programme the package has now been updated again as GREAT-ER 3.0 (2011). Most notably, the database has been replaced by the Open Source Software PostgreSQL. For the first time, the entire system can be installed without any software licence fee, and the scientific community is welcome to analyse and enhance the Free Software GREAT-ER. On the model side, GREAT-ER 3.0 now also includes a sediment extension, and the possibility to model lakes as part of a river basin.

Information on GREAT-ER and updates on different projects initiatives can be found at www.great-er.org.

MO 084

Case study using the tiered approach for mixing zone modeling in the water framework directive

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The Priority substance Directive 2008/105/EC has defined EQSs for 33 priority substances. Compliance with these EQSs should be achieved at the point of discharge or at least at the edge of the mixing zone in the receiving waters. Considering this, a "Tiered Approach" has been developed to assess whether compliance with the EQS values is achieved. The approach starts with simple investigations but could end with very detailed assessments on the mixing behavior and concentration profile of effluents in the surface waters. In contrast to lower tiers, higher tiers require an estimate of the extent of EQS exceedance that can be used to define a mixing zone. This may be achieved using a range of tools such as "Discharge Test" or "CORMIX". The aim of this poster is to present a test case using the tiered approach. An industrial site has a surface discharge with a flow rate of 1,000 m³/d to a small river characterized by a flow rate of 18,000 m³/d. Concentration of the contaminant of concern in the effluent ($[CoC]_{eff}$) is 50 µg/L which exceeded the EQS of the CoC in the receiving water of 20 µg/L. A point source is present, a contaminant with an EQS is present in the effluent and the $[CoC]_{eff} > EQS$ therefore triggering the initiation of the second tier. The Process Contribution (PC) is 2.63 µg/L representing 13% of the EQS after complete mixing. This is higher than the allowed 4% and therefore higher tiers should be initiated and an estimation of the extent of the mixing zone is required. Surface discharges are best modeled with CORMIX requiring input data on the discharge characteristics, effluent, and receiving water. Due to the importance of the near field interactions, both the geometry and position of the discharge has to be well defined. In this case study, the discharge is located on the left bank pointing perpendicular to the river with a local depth at discharge of 0.4 m and a slope of 5 degree. The discharge channel is 0.3 m wide and 0.3 m deep. CORMIX simulations show that at approximately 0.6 m downstream of the discharge point the $[CoC]$ drops below the EQS. A proposed extend of the mixing zone is described in the guidance as $10 \cdot W_{river}$ which in the test case would be 20 m. Therefore the $[CoC]$ will be below the EQS in the proposed mixing zone of 20 m and compliance is achieved.

MO 085

Source apportionment of chemicals under the WFD

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New Environmental Quality Standards (EQS) are being set under the Water Framework Directive (WFD) for a wide range of substances. Generally, the concentrations for these EQS are lower than existing values. However, the sources of many of these substances are wide ranging, particularly for metals and nutrients, with multiple diffuse and point source inputs into the aquatic environment. Where an EQS is exceeded, Programmes of Measures (PoMs) may be required to reduce inputs to the aquatic environment subject to considerations of technical infeasibility and disproportionate costs. The WFD states that compliance should, as a priority, be achieved via source control, applying the 'polluter pays principle'. To apply effective PoMs it is therefore necessary to accurately quantify sources of chemicals discharged to the environment. A review of available models has demonstrated that there are no available national scale source apportionment models. UK Water Industry Research (UKWIR) in collaboration with the Environment Agency and SEPA funded the development of a model for future river basin planning. The project comprised a number of key tasks: (1) specification and securing of national datasets, (2) generation of databases for different sector inputs (point and diffuse), (3) upgrading of the SIMCAT water quality model for monthly outputs and in-river partitioning for metals, and (4) the development of a GIS-Simcat interface for data processing and results visualisation.

The model allows prediction of loads of copper, nickel, lead, cadmium, mercury, zinc, PAHs, nitrogen, phosphorus and DEHP to a 1 km² resolution for urban runoff, highway runoff, sewage effluent, storm tank discharges, combined sewer overflows, agricultural runoff from livestock and arable land, atmospheric deposition, septic tanks, mine water discharges, natural background erosion and industrial inputs. A series of detailed catchment scenario investigations were carried out on the Wear, Tame and Hampshire Avon catchments in the north, midlands and south of England respectively to assess the impacts of diffuse and point source phosphorus inputs in order to develop PoMs that may be considered as part of the second cycle of river basin planning.

MO 086

Persistence of petroleum hydrocarbons in seawater: modeling versus experimental results

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Hydrocarbons are a highly reduced form of carbon which provides a valuable source of energy for microorganisms. Therefore, mechanisms have evolved to degrade them, and nearly all hydrocarbons can be degraded under appropriate conditions. However, degradation of petroleum hydrocarbons in biodegradation studies is often underestimated, mainly due to the physico-chemical properties of these substances. One of these properties is their low water solubility, which results in low bioavailability to microorganisms. Biodegradation data are required by many regulatory frameworks around the world. In the persistence assessment for petroleum substances under the EU REACH regulation, biodegradation data for the major hydrocarbon classes present in these substances were used. In order to screen for persistence, aquatic half-life predictions for representative constituents were calculated using the BioHCwin module of the EPISuite v4.0 model. These predictions allowed narrowing the range of potentially persistent hydrocarbon classes and carbon ranges, thereby optimizing biodegradation testing efforts. To experimentally test biodegradation for the hydrocarbons of interest, a new experimental methodology was used which determined single hydrocarbon biodegradation in seawater. To prevent variability due to water solubility constraints, a passive dosing system using silicone tubing was used to load the seawater with the chosen hydrocarbons. Biodegradation was measured using a respirometer, which performed automated dissolved oxygen measurements in the test vessels and minimized variability due to experimental handling. Except for highly branched structures or structures with quaternary carbons, experimental results showed that in general, seawater biodegradation model predictions were overly conservative. It can be concluded that the combination of modeling results and targeted experimental data provided a complete and robust assessment of the persistence of petroleum hydrocarbons.

MO 087

Field to continental phosphorus fate and eutrophication modelling

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To date, it has been difficult to link agriculture practices with local, regional, and long-distance impacts. Using a milk-producing farm as an example, this work demonstrates the possibility for improved environmental impact analysis based on the integration of site-specific biogeochemical models, local hydrology data, and fate and impact models, linking these from the local to regional scale.

An existing process model (Manure-DNDC [1]) was extended to include phosphorus (P), and a new, global P transport model was developed. Based on site-specific information, the Manure-DNDC model calculates total phosphorus loss from the milk producing farm. Subsequently, watershed specific data is used to estimate the amount of P that reaches the local stream.

Once P is traced to local stream, is routed downstream using a newly developed approach [2] that incorporates advective transport, deposition in waterways due to physical settling and biological uptake, and removal from the water system due to water withdrawal. The environmental impact of these incremental phosphorus additions can be assessed by comparison to existing phosphorus concentrations.

Manure-DNDC was used to simulate the runoff flow, sediment yield, and P loads for two test farms. Predictions showed agreement with validation data within a factor of two, and always within one order of magnitude. The model predictions of the fate of emitted phosphorus show that total ecosystem impact, and the location of that impact, is strongly dependent on the location of emission.

In the case of emission to the Great Lakes, the long residence time of water means that the majority of impacts can occur within 200 km of the point of emission, depending on whether impacts are assumed to occur at all existing P concentrations or whether there is a threshold value. For emissions in the Mississippi watershed, ~25% of impacts occur within 50 km; ~45% occur between 50 and 200 km, and ~30% occur greater than 200 km away

[1] Giltrap DL, Li C, and Sagar S. 2010. "DNDC: A process-based model of greenhouse gas fluxes from agricultural soils." Agriculture, Ecosystems & Environment. vol. 136, no. 3-4, pp. 292-300.

[2] Helmes R, Huijbregts MAJ, Henderson AD, and Jolliet O. Submitted. "Spatially explicit fate factors of freshwater phosphorous emissions at the global scale." International Journal of Life Cycle Assessment.

MO 088

ENM fate in freshwater through adaption of USEtox (tm)

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Engineered nanomaterials (ENMs) have in recent time received substantial attention, both in scientific and consumer circles, as these materials are introduced to a steadily increasing number of consumer products. This has led to environmental concerns on how this new material class behaves in the environment, at which concentrations

organisms are exposed to the materials and what effects these materials may have on the environment. In relation to metal-oxide engineered nanomaterials (ENMs), as is the general case for ENMs, many environmental aspects are still unknown and/or hence not properly scientifically mapped. One approach that has not been given much attention in relation to environmental assessment of ENMs, more precisely the fate, exposure and effect modelling of metal-oxide ENMs is the application of adapted characterization modelling (ACM) and hence application of characterisation models designed for single (chemical) compound assessment e.g. the USEtoxTM model for characterisation of ENM effect potentials.

The purpose of this study is therefore to evaluate if existing characterisation model such as the USEtoxTM model can be applied for characterisation modelling of ENMs applying the principles of ACM. The primary principle of adapted characterisation modelling relies on the recognition of the fact that nano-materials do not behave like single chemical compounds in the environment. The second principle of ACM relies on the fact that existing chemical characterisation can be applied to model hypothetical representatives for effect causing emissions such as groups of chemicals (i.e. equivalence approaches applied to model mercury). In this study the approach taken was therefore to consider if USEtoxTM characterisation of ENMs is possible and appropriately valid. The characterisation was done by relating nano-material properties to chemical properties and hence model the nano-material as a chemical with representative fate and exposure patterns.

In the case study involatile ENMs (metal-oxides) were characterised in USEtoxTM applying adapted characterisation modelling. The result obtained indicates that with some limitations the approach is considered valid - the characterisation factors are considered uncertain relating to several facts such as lack of environmental studies on ENMs making it hard to assess the general environmental behaviour of ENMs and hence relate this environmental behaviour to similar "chemical behaviour".

MO 089

Use of multiple tracers to assess non-stationarity of hydrologic transport at the catchment scale[§]

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In this study, the use of multiple conservative tracers is proposed in order to calculate the arrival time distributions of a solute through hydrologic transport. The variability of the climatic forcing and the heterogeneity of the vadose zone (soil properties, preferential flowpaths, evapotranspiration losses, etc.) acting as a stochastic hydrologic filter suggest non-stationary solute travel time distributions. Several travel-time based models have already been developed, but experimental validation is still lacking for this type of models. In order to establish experimental travel-time distributions, 1) temporally displaced tracer injections and 2) spatially displaced tracer injections are conducted in the hillslope of the Chamberonne river (Lausanne, Switzerland). Tracer concentrations are measured downstream both close from the injection site and at the catchment outlet in lake Geneva. In the first experiment, pulse loads of different tracers are injected at the same spot nearby the stream, in order to emphasize the effect of antecedent soil-water conditions resulting from climatic forcing and hydrologic filtering. The extent of the soil water deficit is the main trigger of non-stationarity in the arrival-time distributions. In the second experiment, the injection site consists of a long rectangular strip perpendicular to the water course, along which pulse loads of different tracers are injected at distances from the stream varying between 5 and 50 meters. In this case, simultaneous injections are emphasizing the effect of heterogeneity in the hydrological pathways under similar soil-water conditions. In both experiments, the use of multiple tracers analytically differentiable is essential in order to be able to identify the original time and location of the tracer injection. This allows to physically measure the influence of the sequence of precipitations and soil heterogeneity on solute transport travel time.

MO 090

Estimation of chemical concentrations in river compartments using a 1D numerical model for contaminant transport

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Exposure assessment is based on a reliable estimation of contaminant concentrations in the environment. In aquatic systems, contaminants are transported by water (liquid phase) as well as by suspended particulate matters (solid phase), complicating the determination of their occurrence, source and fate. We introduce here an efficient numerical model for the prediction of contaminant transport in a river network and present results of two case studies for both dissolved and particulate contaminants. The hydraulic conditions are simulated using a 1D hydrodynamic model (MAGE code), which presents very low computational times and allows for long-time simulation at a regional scale. The algorithm offers the option to represent multi-channel river systems, using a looped mesh, and artificial structures such as dams. The solute transport is computed by the resolution of the advection-diffusion equation, using the ADIS code coupled with the hydraulic model. This code permits to take interactions between chemicals into account. Finally, the transport of particulate contaminants is simulated with the help of erosion/deposition laws (TS code). Thus, sediment stocks and erosion areas can be located, which is of huge interest for the identification of contaminant accumulation areas. This numerical model can predict the fate of chemicals resulting from accidental contamination, as well as chronic contamination. In the first case study, passive tracing data (Rhodamin WT) were used to calibrate the longitudinal dispersion coefficient and to verify the model representation of a solute contamination in the Rhone River, France. The second case study focused on suspended sediment and particulate contaminants (PCBs and Hg) transport, based on data collected during dam flushing operations conducted on the Upper Rhone River. These two case studies demonstrated that the numerical model proposed can be an effective tool for determining concentration, transport and fate of contaminant in the aquatic environment (solid and liquid phases), offering a robust base for environmental exposure assessment studies.

MO 091

Emissions of anthropogenic pollutants in the Alpine areas: the GEMINA project

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Evidence of climate change has been observed at both the global and the local scales. It includes changes in surface temperatures and ice cover in the Arctic, widespread changes in precipitation pattern and amounts, ocean salinity, wind patterns and incidents of extreme weather including droughts, heavy precipitation, heat waves and intensity of tropical cyclones. Climate change influence the status of the ecosystem, that may accordingly be affecting human systems.

In this context, GEMINA, a project funded by the Ministry of Education, University and Research in Italy, aims at creating an interdisciplinary network of experts with the capability of developing climate simulations, analysing the impacts of climate change and providing developing mitigation and adaptation scenarios.

GEMINA includes also the specific objective of analysing the impacts of climate change on the fate and transport of selected anthropogenic pollutants (namely, persistent organic pollutants, POPs) in the environment, in order to identify how climate variables could affect the source-to-sink relationship into remote alpine environments. Several multimedia fate and transport models have been applied to assess the overall POPs' persistence, long range transport and bioaccumulation, and to analyse the effect of climate change on their environmental distribution, as POPs' environmental fate and transport is a function of the climate variables (e.g. temperature, atmospheric circulation, and precipitation).

We will present a case study located in proximity to a source of POPs. The main focus is to define emission scenarios according to the Special Report on Emission Scenarios defined by the IPCC in order to identify the effect of climate change on POPs' environmental behaviour in mountainous regions.

MO 092

Bioaccessibility options for detailed quantitative risk assessment of metals

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The United Kingdom has adopted a risk based approach for the assessment of contaminated land and human health impact. As part of this approach, Soil Guideline Values (SGV) and Generic Assessment Criteria (GAC) have been developed to enable Generic Quantitative Risk Assessment (GQRA). One of the main assumptions in the derivation of these assessment criteria is that 100% of the contaminant present in the soil will be bioavailable or bioaccessible. Although this assumption may be necessary in the preliminary stages of risk assessment to ensure the protection of human receptors, it may result in overly conservative risk assessment. Recommendations for remediation may subsequently be made in situations where remediation is not appropriate.

Over recent years the potential for bioaccessibility data to be incorporated into Detailed Quantitative Risk Assessment (DQRA) has been increasingly recognised. To investigate the potential for the application of bioaccessibility data in DQRA, GAC which incorporate published bioaccessibility data (GAC_{BIO}) have been derived using the Contaminated Land Exposure Assessment model (CLEA). GAC_{BIO} have been derived for selected metals; arsenic, cadmium, mercury, nickel and vanadium for standard residential, commercial and allotment land uses. By comparing GAC_{BIO} to typical contaminant concentrations encountered in UK soils we explore the options for the future application of bioaccessibility data in DQRA.

A risk evaluation of the need to remediate based on the use of literature estimates of bioavailability or of GAC that invoke generic bioavailability values would be difficult to defend. GAC_{BIO} can however be useful in deciding whether to incur the cost of site specific studies of bioavailability. The applicability of GAC_{BIO} can be determined by considering three lines of evidence: history of contaminant formation (natural or anthropogenic); geochemical sequential extraction and physiological based tests. The natural or anthropogenic geochemical processes that led to the formation and current disposition of the contaminants of concern (CoC) at any specific site will give an indication of the likely chemical instability and therefore bioavailability of such CoC. Sequential extraction testing can give an indication of the mineral species with which different fractions of the CoC are associated and hence their likely availability. Finally empirical physiologically based tests can give an insight into the material specific chemistry.

MO 093

Urban versus rural GIS-based distinction for human health impacts in LCA. Application on automotive fuels

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Life cycle assessment (LCA) is frequently used to assess the sustainability of the various alternative fuels that are emerging. However, local impacts, especially human health (HH), are often neglected. This is because they are heavily dependent on the local context and thus difficult to understand. In this study, we introduce two distinctions in order to improve HH impacts in LCA of alternative fuels: urban versus rural and high versus near-ground emissions.

We assess the HH impacts associated with volatile organic compounds (VOC), particulate matter, nitrogen oxides and sulphur dioxide atmospheric emissions (main pollutants emitted by cars). To assess their impacts, two indicators are retained: USEtox (for VOCs) and ReCiPe2008 particulate matter formation potential (PMFP, for the other pollutants). Two systems are studied: internal combustion engine (ICE) cars (using gasoline and diesel fuel) and electric vehicles (represented here by a Renault Fluence ZE using power coming from hard coal and natural gas). To assess the rural and urban parts of atmospheric emissions, a geographical information system (GIS) was developed, allowing knowing the share of urban emissions all along the life cycle of the systems studied. It is then coupled with USEtox distinction between urban and regional emissions. For PMFP, the ReCiPe methodology was coupled with the Ecosense model from the ExternE project, allowing differentiating between urban and rural emissions. For distinction between near-ground and high emissions, the same methodology using Ecosense was applied to PMFP. For USEtox, the characteristic of the compartments, especially the mixed height of the air compartment, were modified. Three conclusions can be drawn from the results: first, applying a rural / urban distinction has a strong effect on USEtox and PMFP results. Secondly, the high / near-ground distinction also modifies the results, especially for ICE cars. Finally, the superiority of one fuel on another is complex to assess, as we did not study all HH impacts (heavy metals emissions and photochemical ozone formation are not assessed here). Further researches should focus on the four following points: extension of the methodology to photochemical ozone creation, extension to the whole car LCA, integration of the results with other impacts and assessment of damages (endpoint level).

MO 094

A new version of input decision for the selection of modelling endpoints for PECgw simulations for harmonisation of exposure assessment

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The EU and national registration processes require the assessment of the leaching potential of an active ingredient and its metabolite(s) of plant protection products (PPP) to groundwater as described in the FOCUS Groundwater reports (2000 and 2009). For groundwater simulations using FOCUS-PELMO 4.4.3 in the framework of the national registration procedure in Germany new recommendations related to Tier 1 and Tier 2 were published by the Federal Environment Agency (UBA) and the German Agrochemical Industry Association (IVA) (Holdt et al. 2011). Input Decision 3.1, an easy to handle MS Excel-tool, was developed to select sorption endpoints for realistic worst case groundwater modelling considering a relationship between sorption behaviour and soil properties e.g. organic carbon content (OC), pH-value or cation exchange capacity (CEC). Furthermore, a procedure has been developed to select degradation endpoints depending on a significant correlation between DT₅₀-values and soil pH-value as well as taking the variability of DT₅₀-values into account. The significance of a correlation between degradation and/or sorption behaviour and soil properties is investigated by the Kendall rank correlation test that is implemented in this tool, too. This method represents a reliable statistical test when only a few values (e.g. min. 3 to 6) are available.

Input Decision 3.1 provides:

- Clearly arranged templates for normalisation of laboratory DT₅₀-values depending on temperature and moisture of soil degradation studies using either default or measured values of soil moisture and/or temperature,
- Statistical evaluation of dependency between DT₅₀ - and pH-values from laboratory soil and field degradation studies and recommendations for the selection of DT₅₀-values and the scenario,
- Statistical evaluation of dependencies between Kf/Kfoc and soil properties and recommendations for Kf/Kfoc and scenario selection.

Key benefits are the simultaneous test of significance of correlations between several substance properties and soil parameters using the Kendall rank correlation test and the derivation of modelling endpoints for DT₅₀ - and Kf/Kfoc-values linked to the recommendation to apply one or two suitable scenario(s) in FOCUS-PELMO 4.4.3 simulation relevant for the German groundwater assessment. The approach presented here may contribute to a harmonisation of exposure assessment for PPP between national, zonal and European level in future.

MO 095

Estimation of soil moisture and soil temperature based on different soil hydraulic pedotransfer functions

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The time-step normalisation procedure as described by FOCUS (2006) can be used to normalise soil field dissipation rates and to derive DT50 value standardised to 20°C and pF = 2. This normalisation procedure decreases or increases individual day lengths by means of daily correction factors based on soil moisture and soil temperature. Hence, soil properties such as moisture and temperature also need to be available in daily resolution. For many soil dissipation studies in the field, these parameters are, however, not readily available. In order to still run a realistic assessment, average daily soil moisture and temperature values can be estimated with predictive models, e.g. PEARL. For the present assessment, van Genuchten parameters are derived from the soil properties of a trial site located in Central Europe. Therefore, three different types of soil hydraulic pedotransfer functions are tested. The respective results will be compared to measured soil conditions to determine a simulation method that represents realistic field data the best. Climate data (min/max temperature, precipitation, global radiation) were available in daily resolution for the complete time-span of the study. Additionally, measured soil moisture (10 cm depth) and soil temperature values (5 cm and 20 cm depth) for 12 data points were available, respectively. Hydraulic pedotransfer functions were prepared based on the HYPRES database (Nemes et al. 2001), the Rosetta database (USDA 2000) as well as the Staring Series (Wösten et al. 1994, Van Genuchten 1980). For each approach a specific PEARL scenario was created using the corresponding van Genuchten parameters. For the PEARL simulations a soil profile of 1 m depth were defined. The profile consisted of five horizons (20 cm each). Soil characteristics were only available for the uppermost horizon (0 - 20 cm) and they were duplicated to be identical in all horizons. The PEARL soil moisture and soil temperature simulations were evaluated for the first horizon (0 - 20 cm) for different depths steps (1 cm resolution). The simulated daily soil moisture and soil temperature data from the respective depths were compared to soil moisture and soil temperature measurements from sampling dates to validate the PEARL parameterisation. Using a goodness-of-fit indicator (sum of squared errors between simulated and estimated volumetric soil moisture), the estimation method that represents measured data from the trial site the best will be determined.

MO 096

Bank filtration simulation model SiMBaFi - a tool to refine the PECgroundwater as part of the environmental risk assessment of medicinal products for human use?

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Model calculations are an important component for the exposure estimation of human pharmaceuticals within their environmental risk assessment following the EMA guideline (EMA/CHMP/SWP/4447/00, Juni 2006). In Phase II Tier A of this guideline the potential entry of a pharmaceutical into the groundwater (PEC_{groundwater}) by bank filtration is calculated by multiplying the predicted environmental concentration of surface water (PEC_{surfacewater}) by a factor of 0.25. This factor does not consider substance specific properties, but originated by expert judgment.

The mathematical simulation model SiMBaFi was developed in a project to replace the multiplier (0.25) by a model based decision matrix taking into account the following parameters: distance between shore-line and extraction well, depth of filter screen, hydraulic conductivity and average extraction rates. The result was a Microsoft Access application which calculates the PEC_{groundwater} for 3 standard flow times scenarios (worst case = 0.15 d, realistic worst case = 5 d, median case = 100 d). The following substance specific parameters are needed as input parameters to determine the concentration in groundwater: sorption coefficient (Kd) and degradation (Dt50). This poster will present the results of groundwater exposure calculations with SiMBaFi for more than 30 pharmaceutical ingredients for which experimental data on adsorption and degradation in a water-sediment system were available. The comparison with the multiplier allows a first assessment if an implementation in the environmental risk assessment as groundwater exposure tool is useful.

MO 097

Biofilms in heterogeneous soils: reactive transport of active substances revisited

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The heterogeneity in soils is influenced by its texture, density, porosity, and the distribution and state of microbial life. Soil physical properties as well as microbial life have an impact on the fate of active substances in the soil environment. Microorganisms occur in soil pores either in suspension or as biofilms which alter the pore geometry while growing. This alteration directly influences the soil water flow field and hence the convective transport of organic substances. Furthermore, the activity field of microorganisms degrading those substances depends on soil structure. We will present an upscaled model which combines spatial stochastic generation of soil structures at pore scale and a process model at column scale coupling fluid flow, reactive transport, and biofilm dynamics. Process models are formulated by a system of coupled partial differential equations which are solved by a Petrov Galerkin scheme (COMSOL Multiphysics); the spatial stochastic process model is based on Gaussian Random Fields (R). The model is applied to investigate the degradation behaviour of pesticides in a range of soils through variation of the degree of heterogeneity. This underlines the significance of the effect of soil structure and microbial activity field on transport and degradation of pesticides.

LC01P - Development in life cycle inventory analysis and modelling

MO 098

LCA studies of biofuels in multi-output biorefineries

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The holistic nature of Life Cycle Assessment (LCA) is becoming an indispensable feature in the assessment of sustainable development of society. It is therefore integrated more

and more in decision making, where especially the Carbon Footprint is the exponent of the increasing popularity of LCA. However, several methodological choices still cause discrepancies in the final results, where the allocation procedure is a key issue. Much can be learnt from the current situation, where different regions propose new Carbon Footprint methodologies, with their own specific methodological choices. This causes problems when incorporating LCA results, such as the greenhouse gas (GHG) savings of biofuels, in legislation. In this work, a case study is executed in a Belgian multi-output biorefinery producing a mix of food, feed and fuel. Results, calculated with a black box and subprocess approach for economical value, mass, energy and exergy allocation, show discrepancies of up to 59% between the same procedure in subprocess and black box allocation, with a factor 4.1 difference within black box allocation approaches and 1.8 within subprocess allocation. The GHG savings of the biofuel versus the fossil fuel equivalent differ from 21 to 56%. When considering a broad waste definition, as is suggested by some methodologies, GHG savings range up to 80%. This work highlights that the subprocess approach is preferred, as this reflects reality better. Furthermore, physical relationships, and especially exergy, are very useful in allocation, as economical values fluctuate in time, and can cause difficulties in finding representative values of intermediate flows in biorefineries.

MO 099

A mini-Delphi approach to consensus

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The Delphi method was originally developed as a forecasting method in which a panel of experts is used in a structured manner aiming to a common understanding. We used a version of the Delphi method to resolve a methodological problem where there is no objectively correct solution: deciding the primary energy factor of industrial waste heat, heat generated as byproduct, and heat produced from waste, when these are used for district heating in Sweden. In this context, the aim was not to find a correct answer but to take advantage of the structured Delphi procedure, including its psychological effects, to reach consensus. Our approach was a mini-Delphi: a one-day workshop with 12 participants, representing different types of stakeholders. After an introduction to the methodological approach and to the mini-Delphi approach, the participants discussed the primary energy for the heat source in three groups to improve their understanding of the issue. Each participant then individually put a sticker on a numerical scale to represent the primary energy factor that the participants felt appropriate for the heat source discussed. When all participants did not immediately agree, the participants with the extreme values were asked to justify their decision. All participants were then allowed to revise their decision. Only one such iteration was made for each heat source due to the time constraints of the workshop. The approach yielded consensus on that waste heat should carry zero, and that heat as a byproduct should carry only primary energy corresponding to the increase in primary energy demand of the industry selling the heat. The workshop also converged on the view that waste that should not be recycled should carry zero or very little primary energy when used as fuel. Similar exercises can be performed to search for consensus in other methodological issues such as allocation of emissions from multifunction processes.

MO 100

The Cereal Unit allocation as a new allocation procedure for agricultural life cycle assessments

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Background

For agricultural life cycle assessments (LCA) several different allocation approaches are currently used. This leads to a broad range of results. In addition, parts of the environmental burden might not be accounted for, if individual products are co-produced in the same agricultural system but used in different sectors, e.g. wheat grain used in bakeries and wheat straw used in biofuel production. The users of the individual co-products (baker and biofuel producer) typically do not know of each other and as a result the allocation methods for their individual LCAs are not aligned to each other. This can lead to a situation that the total environmental burden is allocated inadequately.

Material and methods

Specific requirements for a suitable allocation method for the agricultural sector are defined, which were used to identify the Cereal Unit (CU) as a promising parameter for an agricultural allocation procedure.

Results and discussion

Currently available allocation methods do not satisfy all requirements for the wide range of agricultural products. Therefore, a new allocation approach based on the Cereal Unit is suggested. The Cereal Unit has been developed since decades for purposes of agricultural statistics and is optimized continuously. It is based on physical, chemical and nutritive properties. Using the Cereal Unit conversion factors, almost all agricultural products can be allocated based on one common parameter. Exemplary results will be shown, among others, for wheat, rapeseed and sugar beet.

Conclusions and recommendations

The Cereal Unit allocation approach allows using one common allocation procedure within agricultural LCAs by meeting the requirements of this sector. This approach could help to solve agricultural allocation problems and might lead to more robust LCA results for services and products originated from agricultural raw materials. We recommend investigating the applicability of the Cereal Unit as allocation approach for agricultural LCAs.

MO 101

Life cycle analysis (LCA) applied to process, integration of process modelling in the building of life cycle inventory - example of a bio-polymer production process

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Life Cycle Assessment (LCA) methodology is a well-established analytical method to quantify environmental impacts, which has been mainly applied to products. However, it also has the potential as an analysis and design tool for processes [1]. One of the interesting identified challenges of LCA applied to processes is the consideration of the operating conditions in the building of Life Cycle Inventory. It allows taking into account unit process as complex systems instead of black boxes and considering the environmental impact of the used operating conditions. This could be then interesting in multi-objective optimization (e.g. technical, economic and environmental) of processes and help the stakeholder in decision making process at the time of scale up.

This work is based on the case study of an existing pilot process for the production of a biopolymer from wheat straw and bran. An original production process, based on twin-screw extrusion and ultrafiltration processes has been developed. A first cradle-to-gate LCA has been lead in order to help the chose into different options for unit processes and then to identify the hotspots of the process.

Then ultrafiltration process have been modeled using Excel flowsheet, and twin-screw extrusion was studied throughout experimental design. Both of these tools provide inventory datas for several operating conditions and have been coupled to LCA with the aim to obtained results about the environmental impacts of each process unit depending on its operating conditions.

This approach gives new perspectives in the fields of LCA applied to process industry: instead of using the methodology as an assessment method coming at the end of the process design, these one is rather integrated at the early stage of process conception with other parameters like economics and technicals.

MO 102

Exergy analysis and LCA - a design for environment approach of energy conversion processes

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LCA has been applied widely to design for environment (DfE) of energy conversion processes. It covers the environmental impacts from fuel consumption and the construction, operation, maintenance and disposal of plant components. However, due to the methodological scope of LCA, the environmental impact is related to the product of the energy conversion process, for example to the amount of electric energy generated by a power plant. Especially the functional interdependencies between the process components are not known due to the lack of a harmonized physical base of all modelled plant components.

This gap can be closed by an exergy analysis which enables the allocation of environmental impacts to exergy streams as thermodynamical basis for the overall process. To minimize the overall environmental impacts of an energy conversion process, it is necessary to consider all sources of environmental impacts and understand their formation.

A new methodological approach has been developed based on the combination of exergy analysis and LCA. The so called exergoenvironmental analysis assigns environmental impacts to exergy streams. It identifies the environmentally most relevant process components as well as possibilities for the optimization of plant components. As a case study, an energy conversion process consisting of a high temperature solid oxide fuel cell (SOFC) integrated with an allothermal biomass gasification has been analyzed.

The investigation reveals the components which have the greatest environmental impacts caused by their thermodynamic inefficiencies and their life cycle (component-related) respectively. It is shown the distinctions between the results of the LCA and the exergoenvironmental analysis, as well as the influence of the exergy destruction on its environmental impacts.

MO 103

Hybrid approaches in life cycle assessment

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With the ultimate goal to assess the Greenhouse gas emissions of the livestock supply chain in Italy, different life-cycle based methodologies were reviewed with the aim to apply the most appropriate one. Input-Output Assessment (IOA) and Life Cycle Assessment (LCA) are two of them, which have both been applied for this specific aim. Both these methodologies have their limitations, and to try to solve some of them, Hybrid methodologies have emerged, resulting in a more complete assessment. The Hybrid methods have the advantage of incorporating the details of a product perspective given by LCA, with the completeness of the economy-wide accounting of IOA. Therefore, theoretically they are considered a promising approach for linking the micro (product) with the macro (economy-wide) level, which is the core of our goal.

Several Hybrid methodologies have been proposed, with not always a clear distinction among them. We identified three main categories, namely Tiered Hybrid Analysis (THA) applied by among other Treloar et al. (2004), Input-Output Hybrid Analysis (IOHA) reviewed by Joshi (2000) and Integrated Hybrid LCA (IHLCA) developed by Suh and Huppes (2005).

IOHA is the most applied followed by THA and IHLCA, in the area of energy, fuel, forest and waste. Hybrid methods have been also used for the comparison of different life cycles as organic and conventional farming techniques, and for the environmental assessment of regions and countries. When compared, Hybrid methods often show a higher emission level than LCA or IOA, explaining why they are often considered as a more complete assessment. However, this higher value can also be caused by double counting

and errors in data handling. In the same time, when comparing IHLCA to IOHA, the different methods have shown differences in results depending on the input assessed. A review of the case studies in which these approaches have been applied is presented, together with their advantages and disadvantages. Suggestions of potential applications and improvement possibilities are also discussed. If Hybrid methods are a relevant substitution to LCA and IOA, is probably too early to say. Even if some of the methods have been more applied than others, there is still many uncertainties in the results with the potential to improve them.

MO 104

Multi-demand modeling in LCA - the assessment of household consumption in Swiss communities

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Environmental consumption analyses mostly focus on countries and whole economies, because for this level economic input-output tables are available that allow for environmental extended input-output analysis, and the top-down approach guarantees that all environmentally relevant sectors are covered by the analysis. The national impacts from those studies are then often divided by the number of inhabitants to reflect average impacts per capita. However, neither do these average results allow for analysis of distribution of environmental impacts within a society, nor can they serve a basis for decision-support in a legislative processes. Only a bottom-up approach is able to fulfill such requirements.

We developed a model that is capable to assess environmental impacts of individual households by means of life cycle assessment (LCA). As this approach is heavily computational intensive, we restrict the spatial scope of the analysis to communal level. Three separate models for housing, mobility and nutrition demand, determine the consumption of synthetic households. These households, although synthetically generated, reflect in their entirety the statistical census data of Switzerland. The consumption data of all households is compiled in a final demand matrix. Each column of this matrix represents the final demand vector of one household. The elements of each vector describe the demand for certain reference products from the life cycle inventory (i.e. ecoinvent v2.2). As there are several thousand households in an average Swiss community, the final demand matrix consists of several thousand columns. The multi-demand LCA yields the characterization results for each household. As the households are geo-referenced, we are able to visualize of the characterization results on a map.

A case study shows that environmental impacts are not distributed equally over the evaluated community. The Gini-coefficient, a measure of statistical dispersion, for CO₂ eq. emissions is 0.35 for housing and 0.66 for mobility. This suggests that environmental impacts from housing are generated more equally than environmental impacts from mobility. The model is a promising approach to characterize the environmental performance of communities based on their inhabitants, to show graphically where highly emitting households are located, and to support local decision-makers by indicating households with big leverage potential for the reduction of environmental impacts in their community.

MO 106

LCI model based on linear programming for the optimization of regional waste management systems

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Waste management systems are commonly expected to meet numerous (and sometimes conflicting) objectives, e.g. to maximize environmental protection and resource efficiency, to minimize monetary cost, to achieve social acceptance, among others. At the same time, the degrees of freedom and type of decisions to be taken in waste management are context-driven and dependent on a variety of factors, e.g. consumption patterns, availability of technology, infrastructure and capital, and on policies and regulations. The optimization of the environmental impact of waste management considering all the aforementioned limitations and constraints imposed by the regional context is hence a challenging task that requires advanced decision-making tools.

To tackle this challenge, a systematic tool based on a rigorous mathematical programming approach was developed. The optimization problem was posed in mathematical terms as a linear programming (LP) model, comprising two main sets of equations. The first includes mass and energy flow-based process models of common treatment options, including both dedicated waste treatments and co-processing activities. These models enable the quantification of the direct emissions and the resources consumed according to the amount and type of waste to be treated and the technologies available. The second set of equations determines the life cycle inventory (LCI) of inputs and outputs associated with the system operation. The life cycle inventory can be further translated into the associated impact using a damage assessment model.

The model output is the optimal allocation of waste and resources to the available or planned waste treatment infrastructure. This allocation should ensure a given required functionality (both in terms of waste treatment and industrial productivity) while conforming to the constraints imposed by the regional context. The capabilities of the approach presented are illustrated through a case study. Numerical results highlight the importance of considering multiple objectives to avoid shifting burdens between impact categories. In addition, the environmental relevance of shadow prices, i.e. the marginal environmental impact of tight constraints, is discussed as an approach to analyze sensitivities and identify opportunities for improvements. The ultimate purpose of our tool is to guide policy makers and practitioners towards the adoption of waste management patterns with improved environmental performance.

MO 107

Material flow management of biogenic municipal waste: assessment of different technology options by means of LCA

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Currently, the German law on the collection and utilization of waste (Kreislaufwirtschafts- und Abfallgesetz (KrW-/AbfG)) is in the process of amendment to comply with the European waste legislation. One of the main goals is to ensure that any recyclable material from households is to be collected from 2015 on, including biogenic municipal waste.

Generally, biogenic municipal waste can be burned, composted or fermented. Currently, in administrative districts that do not collect biogenic municipal waste separately, the waste is burned in municipal waste incineration plants and the generated electricity and heat are supplied to public. In administrative districts with a separate collecting system the biogenic municipal waste is composted. Composting plants are the backbone and state of the art for further processing of biogenic municipal waste in Germany. The product of these composting plants is sold as fertilizer or soil enrichment to horticultural, agricultural and landscape gardening companies, retail sector or private consumers. Since most of the plants are 10 to 15 years old, they need to be upgraded. A technical possibility of upgrading includes the generation of biogas from waste. The digestate could be either used to generate compost or be burned to generate electricity and heat. Generating compost could result in an increased potential to convert energy from waste, without interfering in the established material flows of biogenic municipal waste.

The objective of the poster presentation is to present, assess and compare from an environmental point of view the current state of the art of technologies to ferment biogenic municipal waste and to use the residue as compost for German requirements. The system boundary of the assessment includes the collection of waste, the fermentation and the distribution of energy and compost.

The environmental assessment of the technologies is based on a comprehensive literature analysis. The findings of the literature study are used to develop a life cycle inventory for each technology. Furthermore a draft assessment of the environmental impacts is discussed.

The results are part of the project BioEnergieDat, whose goal is to provide a database with harmonized, validated LCI data concerning provision of bioenergy under German framework conditions. The database shall simplify assessments in the bioenergy sector and thereby accelerate decision-making processes in the government and for companies.

MO 108

Life cycle assessment of flexible power plants based on difficult fuels

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Due to the increasing share of solar and wind power generation, the load management needs flexible options to manage fluctuations in power supply as well as in power demands. To handle these fluctuations, a new kind of power plant is in development at Karlsruhe Institute of Technology. This power plant consists of a moving grate which is fairly insensitive towards the fuel input and is fired with several biogenic fuels. Additionally, a pulverized-fuel burner is implemented which is co-fired with coal/biomass and can vary the power generation within several minutes. The biogenic fuels are residues which have some disadvantages like low heating values or low ash melting temperatures. Nevertheless, these fuels are so far mostly unused and are available on a regional scale. The power plant has a planned total output power of 20 MW.

For this power plant, a modular LCI model has been implemented. To compare the environmental impact of this power plant concept with other options for power generation, there has to be a suitable reference system. A common reference system is the electricity mix of a country. However, to cope with a power plant concept which aims to fulfil base load and peak load demands simultaneously, a comparison with an average load structure is not a suitable option. Either, the power plant concept with the flexible burner system could be evaluated by contrasting the concept with a typical base load power plant (e.g. lignite) and a typical peak load power plant (e.g. natural gas). However, the results of such a comparison could be misleading, since the power performance of both concepts differs by a factor of 10 to 50. Or, since this power plant is still under development and will not be available until 2018-20, the future types of electricity production should be considered as well. So, to identify a suitable reference system, current as well as future ecological (environmental impacts), economic (profit) and political (e.g. independency in fuel imports, CO₂ reduction targets) aspects have to be incorporated.

The objective of the poster presentation will be

- to discuss criteria to identify an appropriate reference system
- to identify the appropriate reference system, and
- to compare the new power plant concept with other power plant concepts, by selecting different scenarios for current power plants as well as for future power plants.

The results will be presented and the environmental impacts of the new power plant concept will be discussed.

MO 109

Carbon flows from land use changes in LCA on bioenergy as a function of biomass demand and spatial allocation of land

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LCA is designed to calculate environmental impacts in relation to the functional unit, based on the assumption of a linear correlation. In the case of bioenergy, rising demand may lead to land transformation and induce additional carbon flows to the atmosphere, which are not linearly coherent. International environmental policy strategies pursue

ambitious goals regarding GHG-reduction by the bias of increasing biogenic end energy supply targets for 2020-2050. Nevertheless, the impacts of soil and vegetation carbon pools due to land use change might compromise GHG-emission savings along the entire process chain. Employing representative and prospective bioenergy scenarios for Germany as a case study within the framework of our research revealed that land use change and consequent GHG-Emissions are not correlating linearly with the level of bioenergy demand. The nonlinearity is partly accounted for by the fact that various soil and above ground vegetation contains different amounts of carbon pools. Moreover GHG-Emissions due to land use change depend on the type of land transformation that occurs and the productivity of the particular land. Hence, land use should not be modelled as a linear function of agricultural or resource output with increasing bioenergy production targets within the scope of LCA. More importantly the carbon emissions released to the atmosphere due to land use change partially overcompensated the savings by the replacement of fossil fuels. For a reliable assessment of land use change based on a specific bioenergy demand it is vital to assess the investigation on a spatial explicit level. On that account we consider coupling LCA with the land use model LandSHIFT, which is a model for simulations of spatially explicit large-scale land-use change dynamics and their environmental impacts and describes the interplay between anthropogenic and environmental system components as drivers for LUC. The model input is a set of exogenous drivers such as population and production of agricultural commodities. The model output is a time series of high resolution raster maps of the changing land use pattern that can be processed as input for LCA respectively. Based on the land use patterns that LandSHIFT generated for different bioenergy production targets we calculated the carbon flows and investigated the correlation between bioenergy demands, resulting land use change and carbon flows for German scenarios.

MO 110

Capturing spatial heterogeneity using GIS: an LCA case study of pressure management in geologic carbon sequestration

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Life cycle stages for emerging environmental impact mitigation technologies require assessments within a real-world context in order to accurately predict economic, environmental, and social impacts in LCAs. Unlike a product in circulation, emerging technologies have yet to interact with markets, environments, laws and cultures. Because LCA conclusions can strongly influence decision makers, it is critical that regional heterogeneity is captured in data used for LCA metrics and for simulating market mechanisms. Geographic Information Systems (GIS) provide an opportunity to capture regional variations. Our case study is the Lawrence Berkeley National Laboratory LCA of pressure management for geologic carbon dioxide capture and sequestration (CCS). The objective of this study is to evaluate the regional variability of economics, environmental impacts, and risks of a pressure management technique involving brine extraction. We select three saline aquifers targeted for CCS from different geographic regions in the USA to assess brine extraction and disposal scenarios. GIS databases are queried for data from those three regions to perform network analysis. Initial calculations were performed assuming ten GW-size coal fired power plants were injecting 90% of their CO₂ emissions into a saline aquifer CCS site with an annual injection of nearly 90 million tonnes of CO₂ and an annual brine extraction of nearly 200 million gallons. We find that impacts and the potential commercial value of brine vary substantially between regions due to differences in brine composition and in market data. Water residing in the three saline aquifers spanned a range of temperatures and compositions. Economic value was sensitive to regionally specific electricity providers, salt and mineral markets, and water scarcity. Net values for management scenarios ranged from a cost of \$50/tCO₂ to a return of \$50/tCO₂. The unexpected finding that pressure management could mitigate the cost of CCS in certain regions of the USA would not have been recognized if national averages were used in calculations. While averages may be appropriate for upstream or downstream sectors in an LCA, national average values may not be reliable to the order of magnitude required for scenario modeling. The quantification and evaluation of economic mechanisms and regionally specific market data using GIS provides a more spatially resolved alternative to current national-scale input/output economic sectors in LCA.

MO 111

On strong and poor arguments for carbon capture and utilization: an LCA perspective

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Carbon Capture and Utilization (CCU) is most often motivated using environmental ad-hoc criteria like CO₂ balances, fixation amount and fixation duration. In this work, we provide the rigorous integration of these criteria into the Life Cycle Assessment (LCA) methodology. A major driver for CCU is its climate change mitigation potential. In CCU, carbon dioxide (CO₂) is captured from large-scale CO₂-emitting sources such as fossil fueled power plants. In contrast to Carbon Capture and Storage (CCS), where the CO₂ is stored in geological formations, in CCU, the carbon is re-used as a feedstock for fuels or chemicals. The capture and subsequent utilization of the CO₂ promise reductions of both greenhouse gas (GHG) emissions and fossil fuel depletion. Up to now, CO₂ balances for the utilization process have been used as a metric to account for the amount of CO₂ fixed in carbon-containing products [1]. However, this metric does not provide any information on the actual CO₂ emissions reductions. The evaluation of these reductions must account for the entire life cycle from cradle to grave. In particular, upstream processes such as the CO₂ capture process have to be included; therefore, the use of Life Cycle Assessment (LCA) is mandatory. Yet, potential pitfalls and methodological limitations exist in the application of LCA to CCU: the CO₂ capture effect is easily misvaluated; the CCU system is inherently multi-functional; and the important criterion of CO₂ fixation duration is not considered in a static, attributional LCA. This work shows how the pitfalls and limitations can be overcome to achieve a sound assessment of the environmental impacts of CCU.

MO 112

Utilization of CO₂ captured in coal power plants for photocatalytic methanol production - ecological implications

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One way to reduce climate-related CO₂ emissions from coal fired power plants is carbon capture and storage. Another possibility could be the use of CO₂ captured as a secondary feedstock for a photocatalytic conversion to methanol. Such a process is developed within the research project "Solar2Fuel" (funded by the German Federal Ministry of Education and Research), clarifying technical requirements, economic conditions and ecological effects. The conversion envisaged takes place within a photocatalytic reactor using dye-sensitized catalysts, which encourage the overall equation CO₂ + 2 H₂O + solar energy → CH₃OH + 1.5 O₂. This study evaluates the energetic and ecological prospects of the photocatalytic concept (Solar2Fuel concept). Additionally, the Solar2Fuel concept is compared to two other concepts: first, conventional power generation without CO₂ capture and conventional methanol production by natural gas reforming (reference concept); second, power generation with CO₂ capture and storage as well as conventional methanol production (CCS concept). Different options for CO₂ capture, MEA-scrubbing and membrane based separation, are investigated. Life cycle assessment (LCA) method is an appropriate tool to determine the overall ecological effects of the process chains of the concepts. Thereby, two functional units are used to analyse the concept (1 kWh electricity and 605 g methanol produced in a photocatalytic and conventional way). Results in terms of environmental impacts are discussed in detail. Even though ecological impacts differ for the different CO₂ capture options, all results show that Solar2Fuel reduces CO₂ emissions and therefore, the global warming potential in comparison to the other concepts. Furthermore, the primary energy demand and the acidification potential are reduced by the Solar2Fuel concept. With respect to the eutrophication potential no carbon capture but the reference concept is recommended. Regarding to the human toxicity potential Solar2Fuel concept can reduce emissions if membranes are used to capture CO₂. In contrast, the CCS concept can only reduce greenhouse gas emissions in comparison to the reference concept. All other environmental impacts increase by the CCS concept, with exception of the acidification potential of the MEA-scrubbing option in comparison to the reference concept.

MO 113

Life cycle risk assessment (LCRA): description of this methodological proposal and a case of study

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The life cycle of a product is generally characterized by the main following stages: Raw materials acquisition, Manufacturing, processing and formulation, Distribution and transportation, Use, re-use, maintenance, Recycle and Waste management. Considering the life cycle thinking in a risk analysis approach requires the adjustment of the classic risk analysis methodology. In order to build up this new methodology called Life Cycle Risk Assessment (LCRA), we relied on the Life Cycle Assessment (LCA) methodology, which allows the assessment of the potential environmental impacts throughout the life cycle of a system. Once these adjustments made, this LCRA new methodology is explained and applied to two energy pathways for transportation sector: hydrogen (produced from the biomass) and gasoline pathways.

The life cycle thinking is not taken into account in the traditional method of risk analysis. To integrate this fundamental concept to the risk analysis methodology following the model of the LCA (Life Cycle Assessment) methodology (ISO 14 040, 14044 and the ILCD Handbook), we made some adjustments to the risk analysis methodology. Each of the LCA's four steps has its counterpart in terms of LCRA (Life Cycle Risk Assessment). The variations between these two methodologies reside on two key steps of the LCA: the inventory (step 2) and the assessment (step 3). The inventory collects data whose nature is different between the two tools. For LCA, collected data are matter and energy flows; these data are qualitative and quantitative ones. For LCRA, the data collected are only qualitative since it is an inventory of dangerous situations. The assessment step consists of three sub-steps: classification, characterization and valuation, and allows a conversion of inventory data into results of impact / risk levels. For LCA, the conversion of inventory data is performed by a calculation using characterization factor. For LCRA, this conversion is done qualitatively by rating and prioritizing risks. However, the goal of sub-step classification is the same in both tools because it links the inventory data and the impacts/risk to be assessed. Just like the flow identified by a LCA that can contribute to different categories of impacts, dangerous situations can cause different types of accidents. Therefore, limitations and hypotheses should be established to make the LCRA methodology usable and relevant in view of the objectives and the applicability of the expected results.

MO 114

Adapting life cycle assessment for multi-criteria analysis of a complex system: case study of urban mobility

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Urban mobility is identified as one of the most CO₂ emitters in France: transport represents 34% of CO₂ emissions in France in 2006 (ADEME 2006), including 25% of urban trip emissions. Some previous studies compare transport modes (Finkbeiner & Al. 2006), or assess CO₂ emissions that are related to urban mobility on a real case

(INSEE 2011). But literature is lacking about multi-criteria analysis of urban mobility in the general case. In particular, Life Cycle Assessment has not been largely used to qualify urban mobility impacts despite its possibility to evaluate the environmental impact (ISO14040, 2006). Nevertheless, the complexity of the system “urban mobility” is a first difficulty to the evaluation, especially concerning the definition of goal and scope. The main topic of this poster is to find out a way to complete and adapt LCA in order to enable the evaluation of a complex system under the case study of urban mobility. We propose an approach based on the System Analysis Design Technique (SADT) that allows a clear and complete definition of the “urban mobility” system. Then the possibility to include more societal indicators beyond the environmental ones (such as noise, satisfaction of consumers, time travel, costs etc) will be studied. The final aim is to provide a configurable dynamic system to evaluate different scenarios of urban mobility. The first results consist of a complete definition of the system that is based on a segmentation of urban mobility into sub-systems that constitute the “goal and objectives” step in LCA. This decomposition prepares to the next step of LCA, in which modal splits will be aggregated with elementary assessments of modes to obtain a multi-criteria analysis for several scenarios.

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MO 115

LCA of land-based freight transportation: including accidents in LCIA

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In this paper we performed an LCA of 12 land-based freight transport chains starting and/or ending in Switzerland. In the impact assessment, the ReCiPe method was used, extended with a method for the assessment of accidents. For each transport chain, all realistic transport modes were assessed (road, rail, and intermodal). The number of damaged persons and severity of injury were retrieved from statistical data and accident protocols for each mode of transportation. In order to translate this information to the Human Health endpoint category of the ReCiPe method, the number of damaged persons were weighted with weight and duration of the according injury type. The results show that in rail transport damage from accidents was negligible (<1% of the total human-health impact), while in road transport this share was between 20% and 36% of the total damage to human health. Since accident data on background processes was missing, the analysis could only be performed for the operation phase of the vehicles. Nevertheless, the relevancy of accident data in traffic operations could clearly be demonstrated for road transport indicating that accidents should be routinely considered in LCA studies in order not to miss impacts of high relevance.

Please see extended abstract for detailed information.

MO 116

Trends in transport-related human health impacts from life-cycle emissions and road accidents in Europe

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The analysis combined human health impacts generated from life cycle assessment with those generated from accident statistics. The aims were to compare the European trends in road safety with those of vehicle efficiency and emissions, and this in the context of the uncertainty surrounding the determination of emissions-related impacts. From an LCA perspective, the use of road vehicles causes emissions from the up and down-stream stages of the vehicle and vehicle fuel life-cycles as well as during use, and by applying conventional LCIA methodologies it is possible to achieve an impression of potential human health impacts quantified using the DALY (Disability adjusted life years). As a very different form of human health impact, the occurrence of road traffic accidents follows clear trends such that the consequences are an unfortunate aspect of motorized road use. Road accidents are accurately recorded and there is a large and readily available body of official statistics. The trends in both emissions and road accidents are, however, showing clear declines as vehicle engines become cleaner and road and vehicle safety increases, even as the overall number of registered vehicles and passenger kilometers in Europe continues to rise. For a calculation of DALY one requires not only years of life lost (YLL) but also years of life spent suffering disability (YLD) - the latter being appreciably more difficult to quantify with regard to the consequences of both emissions and accidents. Results of this initial study show not only similar orders of magnitude for impacts related to emissions and accidents but that, depending on the perspective applied to the characterisation of emissions-related impact factors and the current trends for both impact types, the impacts of accidents could still be higher.

MO 117

LCA of age-related environmental impact for biogenic hydraulic fluids

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Biogenic hydraulic fluids, based on synthetic esters, have an excellent environmental profile in the unused state, so that they are typically classified as ‘not hazardous to water’. During storage at room temperature and tribological application, occurring chemical and toxicological changes take no account in the classification of lubricating oils until now. The ageing and oxidation stability gets increasing importance, since it determines the service life of lubricants in tribological systems in addition to the storage time. With an increased use of biogenic hydraulic fluids in environmentally sensitive areas, thus the need for an appropriate monitoring and assessment approach as part of a Life Cycle Assessment (LCA).

A lubricating oil mixture, based on high oleic sunflower oil, was monitored in an adapted life cycle for biogenic hydraulic fluids. For the detection of ecotoxicological effects, water soluble fractions (WSF) of oil samples with a real effect concentration of 100 g/L were prepared. Ecotoxicological testing was performed in standardised bioassays using 24- and 96-well microplates for algae and bacterial growth inhibition tests. Biodegradability was determined with the optimised test system “O₂/CO₂-Headspace Test”.

After tribological application in an ageing test bench (48-192 h) there was only an increasing toxicity for the algae growth inhibition test as a function of time in contrast to the unused fluid with EL₅₀-values ranged from 80 % to 5 % (192 h). The ecotoxicity of hydraulic fluids increased depending on storage conditions. Storage at room temperature accelerates this process compared to 4°C. Compared to ecotoxicological behaviour by ageing during storage and use, a readily biodegradability within 28 days (≥ 70 %) was determined as it is demanded for the environmental label “Blue Angel” for biogenic hydraulic fluids.

Due to the quantification of metal content with ICP-MS in used hydraulic fluids and resultant WSF as well as the determination of the aqueous available metal content, there is a better interpretation and distinction of age-related aquatic ecotoxicity. The additional characterisation of conditional-use changes in ecological characteristics allows a comprehensive assessment and is still largely unknown, but an important aspect in terms of LCA for biogenic hydraulic fluids. This methodological approach thus allows the feedback of the desired product properties on the production method.

MO 118

Hydrotreated vegetable oil production in Spain: compliance with Directive 2009/28/EC in terms of GHG emissions savings

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Oil hydrotreating units in refineries are aimed at reducing the sulphur content of mineral fuels in order to get compliance with standard specifications. Moreover, this process is one of the best available technologies to obtain biofuels from vegetable oil at present. This study presents the GHG emissions of the biofuel HVO (Hydrotreated Vegetable Oil) obtained from vegetable oil by means of co-processing with conventional fossil fuel in hydrotreating facilities of two crude-oil refineries, using the LCA methodology. Results were compared with a mixture (in the same shares) of FAME (Fatty Acid Methyl Ester), a biofuel obtained by means of transesterification of vegetable oil, and mineral diesel. Results highlight that the HVO mixed with mineral diesel has at least the same environmental performance compared to a mixture of FAME and mineral diesel. GHG emissions savings compared to standard diesel from Renewable Energy European Directive 2009/28/Ec are exposed. In addition, sensitivity analysis have been conducted in order to assess the influence in results of different data sources and different allocation processes that LCA standards suggest, showing the most relevant hot-spots that influence the improvement of previous results.

MO 119

Life cycle assessment of chitosan-based films

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Conventional plastic films used nowadays for food packaging are based on non-renewable sources, non-biodegradable and impact heavily on the environment. For all these reasons, a new biodegradable packaging material based on renewable raw materials has been developed. Chitosan is the second most abundant polysaccharide found in nature and has non-toxic, biodegradable, and antimicrobial characteristics, which are of great interest for food packaging purposes. Chitosan is mainly manufactured from crustaceans due to the large amount of its skeleton available as a by-product of food processing. In this context, chitosan is an interesting film forming material.

A comparative life cycle assessment between two different food packaging systems has been carried out: a commercial food packaging film based on polypropylene (PP) and a new biodegradable chitosan-based film manufactured in our labs. The environmental load of chitosan-based films in different stages of its life cycle has been studied and compared with the one of conventional PP films used as food cover.

The functional unit considered in this study is 1 m² of similar thickness packaging film. The studied system includes three main stages: material obtaining, film manufacture, and end of life. The data relating to PP packaging films were obtained from Ecoinvent v2.0 database, developed. The life cycle inventory for chitosan powder from crustacean shell was taken from literature and film manufacture data was measured directly by our research group in the lab. The EcoIndicator 99 method was selected for comparison of the films.

Obtained results showed that PP film has a higher impact than chitosan-based film in carcinogens and fossil fuels impact categories. The environmental burden associated to carcinogens is mainly due to the end of life stage, while the impact related to fossil fuels is owing to the extraction of PP. On the contrary, the categories in which chitosan-based film has a higher environmental load are respiratory inorganics, land use and minerals. Environmental load associated to respiratory inorganics is mostly related to the

acetic acid used in film manufacture and, in a more significant way, to the hydrochloric acid used in the raw materials extraction, which is also responsible for the impact in minerals category in the raw materials extraction stage. In addition, the main responsible for land use is the glycerine used in the film manufacture stage considered as a by product from biodiesel.

MO 120

Environmental indicators, as a result of the Life Cycle Assessment application for the Mexican corrugated cardboard industry

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Considering the present situation in Mexico, the environmental protection and the responsible use of the available resources are current needs. The involvement of each of the industries in the country is essential to achieve tangible results for the environmental welfare.

The Life Cycle Assessment (LCA) is used for this study with the objective of making a comparison between the use of virgin, and the use of recycled raw materials in the production of corrugated cardboard, under Mexican conditions. The contribution of applying LCA works to identify different environmental impacts from the individual processes of the complete system.

The identification of the environmental impacts runs in order to consider specific aspects, which would focus on the sustainability of the enterprises involved in the corrugated cardboard manufacturing industry. The aspects identified could be properly implemented in the future as a functional part of an environmental policy proposal.

The results showed that the system of the recycled corrugated cardboard production impacts less in every environmental impact category analyzed, than the virgin corrugated cardboard production. However, in Mexico, it is a system that can be optimized through specific activities; especially in the manufacturing and transportation processes, where the greater environmental impacts were located.

MO 121

GHG emissions comparison of tissue paper from virgin pulp vs. recycled waste paper

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Pulp and paper manufacturing industry is the fourth largest greenhouse gases (GHG) emitter and is responsible for around 9% of the total global CO₂ emissions from manufacturing sectors. The increasing trend of paper consumption is one of the principal drivers behind the emissions from the sector. Therefore, policy measures which ensure not only the reduction in consumption of paper but also the increasing use of recycled waste paper, the implementation of cleaner production practices and the use of fibres from sustainable sources are of main importance. The aim of this work is to compare the environmental implications of tissue paper produced from virgin pulp (VP) vs. recycled waste paper (RWP). By doing so, this work informs decision-makers both at company or national levels on the main driving forces behind emissions generation and suggests necessary actions to decrease pollution. The main questions which this work addresses are: how the choice of raw materials for the VP and RWP processes influences the life cycle GHG emissions of tissue paper production? what are the main drivers behind the se emissions? which is the share in emissions generation of the direct material and energy requirements or of transportation? All these aspects are analysed by considering all the stages involved in the life cycle of tissue paper production and identifying the most relevant processes that have significant contribution to the global GHG emissions of the product. Our results show that the energy demand in the form of electricity and steam is relatively higher in the case of RWP process than VP process, if only the manufacturing stages are considered. However, different picture comes out when the comparison is based on the entire life cycle of the product. The GHG emissions from the VP process are about 24% higher than the RWP process. This implies a saving of 424 g eq CO₂ for each kg of tissue paper produced from RWP instead from VP. GHG emissions from pulping process alone, 470 g eq CO₂ per kg of tissue paper, were about 3 times higher than emissions from waste paper collection and transportation. According to our results, replacing recycled waste paper for virgin pulp has environmental benefits.

EP02P - Endocrine disrupting chemicals: recent developments

MO 122

Combination of Planar Chromatography with in vitro bioassays as a tool for effect directed analysis

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The identification of environmental contamination with a (specific) biological activity is demanding due to the vast number of compounds which are released in the environment by humans. In recent years various strategies and methods were developed for an effect directed analysis which in general combine separation methods like LC or HPLC with biological assays and a subsequent chemical analysis of fractions which are biologically active.

Beside the complexity of chemical mixtures in the environment, the often low concentrations of contaminants are a further challenge, especially in case of anthropogenic micropollutants with specific modes of action like drugs or endocrine disruptors. In order to overcome these difficulties, environmental samples are usually concentrated by solid phase extractions (SPE). However, potential active compounds which are not bound to the matrix of the solid phase are not concentrated and so the SPE might already result in a separation of the sample. They might be even lost if the flow-through of the SPE is not further characterized.

An alternative to the method described above is the use of planar chromatography (HPTLC) in combination with bioassays. Samples can be easily concentrated on a thin layer plate by multiple sample application and/or a focusing step if a multi development method is used for the chromatography. The challenge of this strategy is to perform bioassays for the detection of specific effects directly on the surface of the thin layer plate. Therefore, these bioassays have to be adapted for this new application.

The combination of thin layer chromatography with the luminous bacteria test for the detection of acute toxic effects is already well described and frequently used. In contrast, studies showing the coupling of HPTLC with specific bioassays like the Yeast Estrogen Screen (YES) are scarce. The objective of the presented work is to develop and optimize techniques for the combination of HPTLC with various specific bioassays and to test the hypothesis that such an approach is a useful tool for the effect directed analysis of environmental samples.

MO 123

Development of a multi-component detection method for with UPLC-tQ-MS/MS to identify steroid hormones in CALUX positive surface water samples

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Surface waters, such as the rivers Rhine and Meuse, are important sources for the drinking water supply in the Netherlands. Dutch drinking water companies therefore intensively investigate their water sources for the presence of emerging contaminants and their fate during treatment processes. One of the classes of concern are compounds with endocrine disrupting, e.g. estrogenic, activity. Nowadays, natural and synthetic steroidal estrogens are known as the primary causative agents of estrogenic activity in the aquatic environment. With the development of new bioassays, it has become possible to investigate also the activity of other classes of steroid hormones and compounds with comparable or antagonistic activities. Many hormone-like compounds are excreted naturally or are used as pharmaceuticals and might thus enter the environment via similar routes as estrogens. Indeed, using CALUX bioassays for progestagenic, androgenic, estrogenic and glucocorticoid receptor activation, the presence of especially glucocorticoid activity in Dutch surface waters and glucocorticoids in waste water was recently reported. Now that activity is found, methods are needed for the identification of the responsible compounds. Due to the low environmental concentrations, an analysis sensitivity is required that is currently only feasible by target analysis methods. We developed a highly sensitive multi-component analysis method for over 40 natural and synthetic steroid hormones in water, including estrogens, androgens, progestagens and glucocorticosteroids. The method uses Ultra Performance Liquid Chromatography separation followed by tandem Mass Spectrometry detection (UPLC-MS/MS). Due to the narrow bore (i.d. 2.1 mm) column packed with small C18 particles (size 1,7 µm), improved separation power and detection limits and reduced analysis times are achieved in comparison with conventional LC-MS. The method was applied to samples from Rhine and Meuse and results were compared with results from the CALUX analyses of hormone-like activity in samples from the same locations.

MO 124

Development of simultaneous analysis technique for phthalate di- and mono-esters using online-SPE and high speed mass spectrometer

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Simultaneous analysis technique for trace amount of phthalate di- and mono-esters was developed and applied to aqueous samples. Phthalate esters are used as plasticizers and often reported to have endocrine disrupting activities. When phthalate di-esters are taken up into the body, they are metabolized into mono-esters and excreted to urine. Since both di- and mono-phthalates are likely to exist as mixture in the environmental water and wastewater, it is important to develop a simultaneous quantification method for both forms. Recently some researchers reported the advantage of using on-line SPE system couple with LC-MS/MS determination which gives easy and high throughput analysis. This technique also provides greater reproducibility of pretreatment processes. To achieve high throughput analytical methods, not only the on-line SPE system but also high speed mass spectrometer is required. Phthalate esters have various homologues with different alkyl side chains. Positive ionisation is more suitable for phthalate di-esters while negative ionization for mono-esters. The mass spectrometer used in this study were capable of high speed MRM (500 ch/s) and polarity switching (15 ms). This presentation reports simultaneous analysis of the phthalate di- and mono-esters using online-SPE system and simultaneous positive/negative electro-spray ionization.

MO 125

Synthesis and identification of novel metabolites of polybrominated diphenyl ethers in human blood

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Hydroxylated polybrominated diphenyl ethers (OH-PDBEs) are exogenous, bioactive compounds that originate, to a large extent, from anthropogenic activities, although they are also naturally produced in the environment. In the present study nine new authentic OH-PBDE reference standards and their corresponding methyl ether derivatives (MeO-PBDEs) were synthesised and characterised by NMR spectroscopy and mass spectrometry. Seven of the prepared authentic reference standards were thereafter identified in a pooled human blood sample. The identified OH-PBDEs were 3-hydroxy-2,2',4,4',6-pentabromodiphenyl ether, 3'-hydroxy-2,2',4,4',6-pentabromodiphenyl ether, 3-hydroxy-2,2',4,4',5-pentabromodiphenyl ether, 3-hydroxy-2,2',4,4',5,6'-hexabromodiphenyl ether, 3'-hydroxy-2,2',4,4',5,6'-hexabromodiphenyl ether, 3-hydroxy-2,2',4,4',5,5',6-hexabromodiphenyl ether and 4-hydroxy-2,2',3,4',5,5',6-heptabromodiphenyl ether. An additional seven OH-PBDEs were identified in the pooled human blood sample, of which one OH-PBDE, 4'-hydroxy-2,2',4,5,5'-pentabromodiphenyl ether, has not been identified in human blood before. The identification was performed using gas chromatography-mass spectrometry (GC-MS) recording the bromine ions m/z 79, 81. The identification was supported by the peaks relative retention times (RRTs) compared to authentic references on two GC columns of different polarities for the hexa-, and heptabrominated OH-PBDEs, and three different GC columns for the pentabrominated OH-PBDEs. The OH-PBDE congeners most likely originate from human metabolism of the flame retardant polybrominated diphenyl ethers due to the relatively high concentrations of PBDEs in the same human blood sample and the strong metabolic relationship between OH-PBDEs and their parent PBDEs.

MO 126

Isolation and identification of ligands for the goldfish testicular androgen receptor in chemical recovery condensates from a Canadian bleached kraft pulp and paper mill

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This study builds on a series of investigations characterizing substances in kraft mill chemical recovery condensates that depress sex steroids in fish. Here, incubations of goldfish testis androgen receptors (AR) with condensate extracts were used to investigate the potential role of androgens in hormone depressions. Condensates contained variable levels of AR ligands, with the highest amounts in nonpolar extracts of filtered solids prior to solid phase extraction (SPE). High pressure liquid chromatography (HPLC) fractionation recovered the majority of activity in one fraction, with ligands detected in three additional fractions. Gas chromatography mass spectrometry analysis of the most active fraction confirmed the two most abundant components as the diterpenes manool and geranyl linalool. Manool exhibited a relative affinity for the AR that was 300 fold less than testosterone and accounted for 26% of total filtered solids activity. Geranyl linalool exhibited no affinity for the AR. Three additional diterpenoid families were tentatively identified as principal components of the three other androgenic HPLC fractions. Compared to condensates, final effluent had 3000 fold less androgenic activity, with <1% attributable to manool. Putative androgens previously associated with mill effluents (androstenedione and androstadienedione) and progesterone were not detected; however, additional condensate diterpenes suspected as androgens were identified in final effluent. This study is the first to confirm nonsteroidal cyclic diterpenes as androgenic at pulp mills. A major in-mill source of these substances was identified, and the role of androgens in mill effluents affecting fish reproduction was reinforced.

MO 127

Thyroid hormone disruption in effect-directed analysis - An endpoint of growing concern

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Thyroid hormones (THs) play an important role in the maintenance of a normal physiological state. Chemicals in the environment that interfere with the ability of the thyroid hormone system to function normally could have devastating effects on individuals, as well as on whole populations. Serum transport proteins are important in regulating total hormone levels by influencing the transport capacity of the TH, which are lipophilic compounds poorly soluble in the blood. TDCs can bind to transport protein and hence replace the natural THs; hindering the THs to reach their target organs.

In Effect-Directed Analysis (EDA), various bioassays have been implemented that have an endpoint related to endocrine disruption. We have used the radioligand TTR-binding assay to direct our fractionation for the identification of unknown TDCs in environmental samples. In addition, to assess what classes of environmental toxicants are capable of interfering with the TH system, an inventory of the literature describing compounds with reported potency to bind to the TH transport protein TTR was made. Compounds that are known to influence the thyroid system (e.g. hydroxylated PBDEs and PCBs, triclosan, DDE, TBBPA) all have structural similarities with the natural hormone Thyroxine (T₄), i.e. an aromatic ring with a hydroxyl-group and adjacent halogens. But more recently, also polyfluoroalkyl substances were shown to have moderate TTR binding potencies. The presence of functional groups together with the length of the fluorinated carbon chain was a crucial factor for binding to the TTR binding site. This shows that more compound groups than the phenolic aromatic halogens are capable of disrupting the thyroid hormone system.

Using GC-MS, 43 compounds were tentatively identified in the sediment and 57 compounds were tentatively identified with LC-HRMS. In total, 25 of these compounds were purchased and tested in the TTR-assay: only 4 compounds showed a response. For the sediment fractions, only a very small part of the response could be explained by the presence of the tested compounds. In order to enhance the identification of these unknown compounds that can bind to TTR and hence may be disruptors of the thyroid hormone system, it is necessary to develop identification strategies using e.g. high resolution mass spectrometry and compound lists and mass spectrometric libraries need to be further developed.

MO 128

Estrogenicity of 75 European waste water effluents evaluated by in vitro assay

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Various natural and synthetic contaminants present in urban waste waters have been shown to possess estrogenic activity, and many questions related to the risks of these compounds are not fully resolved. Under environmental conditions, steroidal hormones appear to be primarily responsible for adverse effects observed in fish downstream of waste water treatment plants (WWTPs). However, instrumental analyses of steroidal estrogens often fail in detection of their actual concentrations in complex environmental matrices such as waste water effluents. In the present study MVLN in vitro assay was used to evaluate estrogenicity of 75 European waste water effluents. The samples originated from the FATE-SEES 2010 pan-European monitoring campaign coordinated by the European Commission's Joint Research Centre (JRC) in Ispra, Italy. Estrogenic activity was expressed as 17 β -estradiol equivalents (EEq). Twenty seven sample extracts showed significant estrogenic activity higher than the detection limit >0.5 ng/L EEq with values ranging 0.53 to 17.9 ng/L EEq. The highest activities have been detected in WWTPs at some of the major European capital cities indicating the importance of this contamination source. Our study provides some of the first EU-wide snapshot data on the estrogenicity of WWTP effluents, and demonstrates suitability of in vitro bioassays as effect-based monitoring tools.

MO 129

Assessing the endocrine activity of sediment samples from Laguna Lake, Philippines, using the LYES and H295R assays

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Due to the increasing use of diverse chemicals by modern society, nearly every living space is affected by numerous known and unknown chemicals of anthropogenic origin. Since the early 1990s there is increasing concern about the exposure to chemicals that have the potential to interact with the endocrine system and associated physiological functions in human and wildlife. The present study was conducted to further investigate the level of contamination of sediment samples from Laguna Lake. The said lake is the second largest freshwater lake in Southeast Asia and the largest lake in the Philippines. It is also a member of the Living Lakes Network since 2001. Based on preliminary results, the present study selected sediment samples of two different sites for fractionation and effect-directed analysis: Central Bay (S5) and East Bay (S8). While the watershed around the Central Bay is urbanized and industrialized, that around East Bay is basically residential and agricultural area. In this study the LYES-Assay (Yeast estrogenic screen assay assisted by enzymatic digestion with Lyticase) was performed to screen for estrogenic active fractions in sediment samples from Laguna Lake. As a further investigation the crude extracts of five main sites have been tested in the H295R-Assay for their potential to disrupt steroidogenesis pathways. The sediment samples have been extracted by using an accelerated solvent extraction method (ASE 300, Dionex Corp., Sunnyvale, CA, USA). The fractionation of Central Bay and East Bay extracts was conducted using an automated multistep fractionation method developed by Lübcke van Varel et al. (2008). In the sediment samples from East Bay four fractions showed a significant endocrine effectiveness at the one fold concentration (fraction 11, 15, 16, 18). The estrogenic activity ranged from 8.43 $\pm$ 4.37 ng/L at fraction 18 to 10.79 $\pm$ 5.28 ng/L at fraction 15. Fraction 16 also revealed a significant endocrine effectiveness at the 1/2 fold concentration of 12.59 $\pm$ 9.91 ng/L. Only one fraction indicated a significant endocrine potential from sediment samples of Central Bay. Indeed, fraction 18 revealed significant endocrine effectiveness at the 1/8 fold concentration of 8.80 $\pm$ 2.29 ng/L up to 27.32 $\pm$ 18.39 ng/L at the one fold concentration. In the H295R-Assay all crude extracts showed an increase of the 17 β -estradiol production. It ranged from 589.62 $\pm$ 116.31 pg/ml E2 at Central Bay (S5) to 1833.57 $\pm$ 146.24 pg/ml E2 at South Bay (S10).

MO 130

Effects of 4-nonylphenol and/or diisononylphthalate on THP-1 cells: impact of endocrine disruptors on human immune system parameters

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The aim of the present work was to investigate the link of two endocrine disruptors compounds (EDCs) and the human immune response through a study of their effects on the THP-1 human cell line which was used as a model for macrophages. We used two EDCs, diisononylphthalate (DiP) and 4-n-nonylphenol (NP) alone or in combination in order to evaluate the effects of these compounds on several parameters of the immune response: cytokine secretion, phagocytosis and the putative implication of the estrogen receptors by studying the level of MAPK activation. NP and DiP strongly reduced phagocytosis and modify cytokine secretions. Indeed, THP-1 cell exposures (i) to 5 and 10 μ M of combination of NP and DiP induced an IL-8 level in the medium respectively 28.9 and 45% higher than level obtain for control (untreated cells), (ii) to combination

of NP and DiP at 10 μ M induced an increase of IL-1 beta level in comparison to the control level, (iii) to combination of NP and DiP induced an increase of TNF-alpha level whatever the concentration of EDCs tested (between 0 and 10 μ M). Last, THP-1 cell exposure to NP, DiP alone or in combination (2 μ M for each condition) induced a decrease of ERK1/2 phosphorylation in comparison to ERK1/2 phosphorylation level of control. Moreover, THP-1 cell treatments by ICI-182780 (an estrogen receptor antagonist) suppressed the EDCs effects on ERK1/2 phosphorylation level which indicates an estrogen receptor-dependent pathway. These results suggest that EDCs have the ability to alter the human immune function, maybe by interfering with endocrine balance.

MO 131

Rapid determination of bisphenol A in water by amperometry using a tyrosinase biosensor

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Recently, Bisphenol A (BPA) has received considerable attention because of its interfering the endocrine system of wildlife and human, increasing cancer rate, etc. In this work, we prepared a novel electrochemical biosensor for the rapid determination of BPA.

A 10 μ L aliquot of 1.0mg/mL the treated multiwall carbon nanotubes (MWNTs) solution was cast on the surface of a glassy carbon electrode (GCE), dried in air. Gold nanoparticles were electrodeposited onto the MWNTs modified electrode by amperometry at 1.5V for 5min from a fresh colloidal gold solution which was prepared with 0.01% HAuCl₄. The modified electrode was denoted as Au/MWNTs/GCE. A 7 μ L aliquot enzyme solution containing tyrosinase (Tyr) and Silk fibroin was then dropped on the surface of the Au/MWNTs/GCE, allowed to dryness at room temperature. This enzyme activity was approximately equal to 40U on each electrode surface. All electrochemical measurements were performed with CHI650c electrochemical analyzer. A bare or film modified GCE with a diameter of 3 mm was used as working electrode, a platinum wire as auxiliary electrode and a saturated calomel electrode (SCE) as the reference, respectively.

The SEM image of the Au/MWNTs/GCE showed that the diameter of the Au nanoparticles dispersed on the MWNTs was about 10 nm. In the presence of molecular oxygen, the tyrosinase can catalyze the oxidation of BPA to o-quinones. The experimental results of cyclic voltammetry showed that the reduction peak current increased with the BPA added. MWCNTs and gold nanoparticles possessed excellent synergistic electrocatalytic effect to enhance the electron transfer rate. The amperometric cathodic current is proportional to BPA concentration in 0.1M pH 7.5 phosphate buffer solution over the range from 0.2 μ M to 8.0 μ M with a correlation coefficient of 0.9991 and a detection limit of 0.1 μ M. The method was applied to the determination of BPA in real water samples. The experimental results showed the biosensor possessed high sensitivity, good selectivity and stability towards the determination of BPA.

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MO 132

Evaluating endocrine activity in plant leaf extracts: temperature and solvent choice influence androgenic, oestrogenic and progestogenic activity in vitro and in vivo

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The goal of this research is to characterize the *in vitro* and *in vivo* activities of plant-derived endocrine active compounds (EACs) and to evaluate potential effects of relevance to fish reproductive health. In contrast to mammalian species, there is a scarcity of knowledge on the importance of plant-derived EACs for fish and aquatic life. A scientifically reliable assessment of the potential impacts of synthetic EACs on fish health therefore depends on a holistic assessment of cumulative aquatic exposures to EACs of specific modes of action pertinent to fish reproduction (especially androgens, oestrogens and progestins). Currently, leaf material was collected from three woodland and marsh sites in south west England and processed for screening using a battery of (anti)estrogenic, (anti)androgenic and (anti)gestagenic *in vitro* screens (the YAS, YES and YPS assays, respectively). Key factors that have been addressed during the first phase of work are the impact of temperature and solvent on the extraction of dissolved organic carbon (DOC) and consequent responses in the YAS, YES and YPS assays (as both agonism and antagonism). For each plant species sampled, beech (*Fagus sylvatica*), oak (*Quercus robur*) and reed (*Phragmites australis*), ca. 30L of dried leaves were collected from April to June 2011. Leaves were air dried (7d) then stored @ -80°C before homogenization in water for 24 h at 20, 15 or 4°C. The aqueous extracts (approx 2L) were stored at -80°C and subsamples used to measure DOC. Aqueous extracts (10 μ L volumes) were directly screened in the YAS, YES and YPS assays. In addition, aqueous extract subsamples (10 ml) were concentrated by solid phase extraction (SPE) onto a Sep-Pak C18 cartridge which was then eluted with methanol. The samples were dried under vacuum before being resuspended in ethanol. The concentrated extracts were then also screened in the YAS, YES and YPS assays. Extraction temperature had a marked proportionate effect on aqueous DOC values. For the reed aqueous extracts, there was a DOC-related response in the YES assay. For the beech and oak extracts, there were variable responses in the various yeast assays. The information generated in this study will be used to support further characterisation of EACs in plant leaf material using a range of *in vitro* screens and *in vivo* fish test protocols. This work is funded by the CEFIC Long-range Research Initiative (RfP reference EMSG55).

MO 133

Utilizing a functional, sensitive, and specific cell-based assay for screening potential endocrine active chemicals

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Endocrine active chemicals are thought to produce endocrine disruptive effects in humans and wildlife by interfering with steroid hormone signaling. Environmental contaminants and industrial pollutants that interact with estrogen and androgen receptors can profoundly affect normal development and hormonal homeostasis and are suspected in numerous health and reproductive problems in both humans and wildlife. Regulatory mandates requiring endocrine disruption risk assessment for consumer products present major obstacles to current testing procedures which are expensive, time consuming, and require large numbers of animals. These obstacles can be overcome by automating procedures to assess endocrine activity *in vitro* using high content cell-based assays as a pre-screen and such *in vitro* screens for potential endocrine active chemicals (EACs) reduce animal testing by categorizing and prioritizing chemicals based on their effect on endocrine receptor activity.

Two cell-based assays were used to efficiently test chemicals for their ability to activate GFP-tagged steroid receptors estrogen receptor alpha (ER α) and androgen receptor (AR). These biosensors form nuclear foci in response to stimulation that can be easily quantified by automated fluorescence imaging (high-content). Additional outputs for individual cells are simultaneously measured resulting in direct assessment of compound toxicity and comparison to positive controls, thus providing insight into the dynamics of receptor activation while simultaneously monitoring cell cycle perturbations and toxicity. Compared to currently validated *in vitro* endocrine disruption assays, these cell-based functional assays resulted in higher specificity and sensitivity (>80%) against a panel of compounds that included pesticides, phytoestrogens, and plasticizers. Bisphenol A was detected by the ER α assay at ~66 ppb, which is more sensitive than the current allowable intake limits from several regulatory agencies. The assays were developed following EPA and ICCVAM guidelines for endocrine disruption assays and provide functional *in vitro* determination of receptor activity, resulting in a more thorough assessment of the potential for *in vivo* endocrine disruption.

MO 134

Development of an *in vitro* model for screening EDCs and deciphering the specific target sites along the HPG axis

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Currently, the vast majority of studies on endocrine disrupting chemicals are based on *in vivo* approaches. The intrinsic complexity of the neuroendocrine and endocrine systems in *in vivo* system often makes it difficult to decipher the target sites and action mechanisms of endocrine disrupting chemicals (EDCs) in specific organs. As such, studies using *in vitro* systems are required to provide information complementary to *in vivo* studies, and to provide important insights into the action mechanisms of EDCs in specific organs. We have successfully developed primary cell cultures (pituitary, ovarian follicular and testicular cells) in the marine medaka (*Oryzias melastigma*), a universal model for marine environmental study. Using these *in vitro* systems, we have performed a series of experiments to elucidate the effects of different EDCs at different levels of the medaka HPG axis, particularly on steroidogenesis in the gonads. To validate these systems, individual cell types were challenged with environmentally relevant concentrations of common EDCs including polychlorinated biphenyl ethers (PCBs), 4-nonylphenol (NP) as well as hypoxia. The expression levels of various steroidogenic genes (including *cyp19a*, *cyp11a*, *3bhsd*, *20bhsd*, *star*, etc.) were analyzed by quantitative real-time RT-PCR. Among the steroidogenic genes studied, aromatase (*cyp19a*) was responsive to almost all treatments, suggesting that it is a major target site for endocrine disruption. The results obtained from these *in vitro* studies were compared to those obtained from *in vivo* exposure of whole fish as well as from H295R, a human cell line. Although different systems did not generate identical expression responses to the same chemical exposure on all parameters, the results were largely comparable. Our results suggest that medaka primary cell cultures not only serve as a useful tool for screening EDCs, but also provide a platform for deciphering the specific target site of EDCs along the HPG axis and their action mechanisms.

MO 135

TTR binding of metabolite extracts from biological matrices: identification, quantification and TTR binding potency of co-extractants from microsomal extracts

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PCB and BDE metabolites are retained in tissues, particularly in blood, and are considered to be the main cause of several observed POP effects. Thus, transport protein binding of metabolites coming from plasma and/or from biologically activated extracts becomes of paramount importance within risk assessment. However, efforts to apply TTR binding methods to biological matrices have encountered insurmountable technical problems or required copious procedures to succeed. Free fatty acids interact with transport proteins like transthyretin (TTR), and with the hormone thyroxine (T4) interfering with the competitive binding and ultimately preventing the analysis. However, it is not exactly known which are the co-extracted compounds from various biological matrices that present a problem for the competitive binding analysis. We aimed to identify and quantify the co-extractants from a biological matrix and their potency to interfere with the TTR competitive binding analysis to understand the problem dimension and enable its future solution. Incubations of rat liver S9 fraction were extracted according to published methods and co-extractants were identified and quantified through GC/MS analysis after silylation. Saturated fatty acids (SFA), non-saturated fatty acids (NSFA) and cholesterol were identified as the prevalent co-extractants. At current dilution factors, co-extractant concentrations ranged from 8-26 μ M of SFA, 2-5 μ M NSFA and 0.2-1.4 μ M of cholesterol. The potency of identified co-extractants was determined in a 96-well plate using the competitive displacement of the ANSA fluorophore (K_a = 1.5 μ M) from TTR. Although less sensitive (T4 EC50 = 300 nM), this method is high

throughput and it does not require radioactive ligand or expensive equipment. SFAs partially displaced the fluorophore from TTR with an average EC50 of 1.6 μ M, while NSFA completely displaced it with an average EC50 of 1.0 μ M. Given the typical co-extractant concentrations, the fatty acids in the extract are the most likely cause of the interference. Due to their physicochemical similarities, complete separation of POP metabolites from fat has been only possible through derivatization and acid clean-up, which will render the metabolites unsuitable for biological testing. As further dilution will weaken the detection limit, a method to selectively remove the fatty acids is currently under validation.

MO 136

Tetrabromobisphenol-A disrupts thyroid hormone receptor alpha function in vitro: use of fluorescence polarization to assay corepressor and coactivator peptide binding

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Thyroid hormone receptors (TRs) recruit corepressor or coactivator factors to the promoters of target genes to regulate their transcription. Corepressors such as nuclear hormone receptor corepressor (NCoR) are recruited by unliganded TRs, whereas coactivators such as steroid receptor coactivator-1 (SRC1/2) are recruited when triiodothyronine (T3) is bound to TRs. These coregulator proteins interact with the ligand binding domain (LBD) of TRs via short, conserved peptide sequences that can be used to probe the conformational changes induced in TR LBD by TR ligands. Recombinant LBD of the human TR 1 isoform (hTR 1 LBD) was produced as a fusion with glutathione S-transferase, and used to develop assays based on fluorescence polarization to quantify the binding of either NCoR- or SRC1/2-derived fluorescent peptides to the hTR 1 LBD. Under predefined conditions, T3 induced a dose-dependent decrease in NCoR peptide binding, and a reciprocal dose-dependent increase in SRC2 peptide binding, in both cases at similar 50%-effective doses. The TR agonists triiodothyroacetic acid and thyroxine were also effective in preventing NCoR peptide binding and increasing SRC2 peptide binding, whereas reverse-triiodothyronine was less efficient and the biologically inactive thyronine had no effect on either process. These experiments validate cell-free assays based on the use of binding of corepressor or coactivator peptide probes, as measured by fluorescence polarization, for investigating the conformational changes of TR 1 LBD induced by potentially TR-interfering compounds. Both these methods were used to elucidate the mechanism of the disrupting effects of tetrabromobisphenol-A (TBBPA) on the hTR 1 LBD conformation related to the transcriptional activity of the receptor. TBBPA is a flame retardant that is released into the environment, and is a suspected disrupter of thyroid homeostasis. The present results indicate that TBBPA did indeed interfere with the ability of the hTR 1 LBD to bind both NCoR and SRC2. TBBPA behaved similarly to T3 in promoting the release of NCoR from LBD, whereas it failed to promote LBD interactions with SRC2. However, it did reduce the T3-induced interactions between LBD and the coactivator peptide. This study therefore suggests that TBBPA in the micromolar range can affect the regulation of transcription by both the apo- and the holo-TR 1, with potential disruption of the expression of genes that are either up- or down-regulated by T3.

MO 137

The other endocrine activity: in vitro screening of sewage treatment plant effluents for retinoid, thyroid and vitamin D receptor agonists

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In the past, immense scientific and regulatory attention has been paid to environmental chemicals interfering with steroid receptors, especially to estrogenic and anti-androgenic compounds. Doubtlessly, focusing on androgens and estrogens is important since they are key players in reproduction and development. However, besides androgen and estrogen receptors there is a plethora of transcription factors controlled by other than steroid hormones. To elucidate potential effects mediated via non-steroidal pathways, environmental toxicology needs to widen the focus to effects mediated via other hormone receptors.

In this context, we employ Yeast Two Hybrid assays with thyroid receptor (TR), retinoid X receptor (RXR), vitamin D receptor (VDR), and retinoic acid receptor (RAR) in order to characterize the endocrine activity of 78 effluents sampled at 51 industrial and municipal sewage treatment plants (STP). To avoid the loss of active compounds, water samples were analyzed without further treatment, e.g. filtration or solid phase extraction (SPE).

STP effluents did not induce TR, RXR, or VDR activity. Compared to that, 54 of 78 effluents (69%) significantly activated RAR. This retinoid activity was very potent: Expressed relative to the endogenous ligand all-trans retinoic acid (ATRA), we detected a mean of 241 ng L-1 ATRA-EQ and a maximum of 2160 ng L-1 ATRA-EQ. Since other studies analyzed solid phase extracts of STP effluents and detected a much lower activity, we evaluated the suitability of several SPE methods to concentrate the RAR agonists. Since none of the procedures was able to extract significant levels of RAR activity we conclude that the causative chemicals are poorly extractable and thus might be missed when analyzing SPE extracts solely.

In a more general context, our data indicate that the investigated STPs are a source of chemicals that activate the retinoic acid receptor. Since the responsible chemicals are so far unidentified, potential *in vivo* consequences remain to be evaluated. However, in the light of the known teratogenicity of some retinoids, an identification and further toxicological characterization of these 'emerging' chemicals is imperative.

MO 138

The endocrine disrupting effect of hypoxia on pituitary cells

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Hypoxia is an endocrine disruptor which can affect the synthesis and balance of sex hormones and thereby impairs reproduction of fish. However, the precise mechanisms of endocrine disruption remain unclear. Moreover, effects of hypoxia on reproduction in higher vertebrates such as mammals remain largely unknown. Gonadotropin releasing hormone (GnRH) is known to play a pivotal role in vertebrate reproduction. Previous studies have suggested that suppressed expression of GnRH and/or GnRH receptor (GnRHR) along the Hypothalamus-Pituitary-Gonad (HPG) axis could be an important mechanism contributing to reproductive impairments in hypoxic fish. Given that the HPG axis and genes controlling steroidogenesis as well as the sex hormones are highly conserved across vertebrate groups, the endocrine-disrupting effects of hypoxia found in fish may also occur in mammals including human. The aim of the present study is to determine whether hypoxia would affect the expression of GnRHR gene (one of the key genes along the HPG axis) in mammalian pituitary cells. A mouse pituitary cell line, LbetaT2, was used as an *in vitro* model to test the hypothesis that hypoxia can affect the expression of GnRHR in pituitary, thereby affecting reproduction in mammals. LbetaT2 cells were incubated for 24, 36, or 48 hours in a hypoxic chamber with 2% O₂ and an identical set of cells were incubated under normoxic condition to serve as controls. The expression level of GnRHR mRNA was subsequently determined by SYBR Green-based real-time RT-PCR. Furthermore, the effect of hypoxia on transcriptional activity of GnRHR gene in the LbetaT2 pituitary cells was determined. A 1.2 kb fragment of mouse GnRHR promoter was cloned into pGL3-Basic vector and transfected into the LbetaT2 cells. The transfected cells were put into hypoxic chamber with 2% O₂ and incubated for 24, 36, or 48 hours. The promoter activity determined from hypoxic cells was compared with that determined from normoxic cells. The effects of hypoxia on GnRHR gene expression and promoter activity in the LbetaT2 pituitary cells will be discussed in this presentation. Our current study represents the first important attempt to decipher the effects of hypoxia (an endocrine disruptor) on mammalian reproduction.

MO 139

Gammarus pulex responses to short-term exposure towards endocrine disruptors and pharmaceuticals in waste water

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Gammarus pulex is one of the most frequent and abundant freshwater amphipod in European streams, playing an important key-role in the aquatic food web and detritus cycling. Moreover, *Gammarus pulex* is sensitive towards pollution. Endocrine disruptors and pharmaceuticals are important chemicals in the effluent of urban waste water treatment plants. Traditional purification steps cannot completely remove these so-called micropollutants.

Gammarus pulex has been used in the fully automated real-time based Multispecies Freshwater Biomonitor[TRADEMARK] (MFB) to detect rapidly low concentrations of different micropollutants released in municipal waste water studying behavior (locomotion, ventilation) and survival. Moreover, the removal of the ecotoxicological potential in different treatment steps was recorded in short and long-term exposures.

MO 140

Do Bacillus thuringiensis israelensis products affect the endocrine system?

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Mosquitoes are well known as vectors for many pathogens, such as malaria (*Plasmodium falciparum*). Global warming and an increasing formation of potential breeding habitats favor the global distribution of mosquitoes and pose a high risk to human health. Therefore, potent agents for the proper control of mosquitoes are indispensable. However, use of synthetic insecticides - such as DDT - bears the risk of causing serious damage to wildlife. As a consequence, there is need for alternative insecticides that effectively control pests while not impacting the environment. Over the past three decades a protein from *Bacillus thuringiensis israelensis* (*Bti*) has been successfully used as a biological control agent against mosquitoes. *Bti* is supposed to be ecological friendly, and thus, is used in ecological sensitive habitats. A recent study, however, has detected estrogenic activity in the *in vitro* Lyticase-supported Yeast Estrogenic Screen (LYES assay), after exposure to four different *Bti* formulations. Three formulations have shown significant estradiol equivalent quotients (EEQs) up to 22.8 ng/L. Subsequent studies revealed that the estrogenic activity was caused by the active substance rather than the compounds of the formulation matrix. To fully understand the mechanism by which *Bti* interacts with the endocrine system, further investigations are necessary.

The aim of this study was to analyze two *Bti* fermentation products, one of which has been proven to be active in the LYES assay, for additional endocrine potentials including disruption of steroidogenesis. *Bti* products of concern were extracted at two different temperatures (30 °C / 60 °C) using liquid pressure extraction. Extracts were then analyzed in the H295R steroidogenesis assay, an *in vitro* screening assay using a human adrenocortical carcinoma cell line. We focused on major hormones of the adrenal gland - such as estradiol, testosterone, androstenedione, progesterone, 17-hydroxyprogesterone and 21-hydroxyprogesterone - using LC-MS/MS and commercial ELISA kits to quantify hormone concentrations. Preliminary results indicate that both products have no significant effects on hormone production. Furthermore, we are currently

verifying the results from the LYES assay using a breast cancer cell line (MVLN). Both products showed an increase of human estrogen receptor (hER) binding at the highest concentration during a pilot experiment.

MO 141

Intersex in *Scrobicularia plana*: transcriptomic analysis reveals novel genes involved in endocrine disruption

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Successful reproduction is a vital process for the survival of any species. Xenobiotics that interfere with normal male and female gonad development will alter the population structure. In contrast to the extensive studies performed on vertebrates, there has been little information available regarding the gonad transcriptomes of molluscs. Our previous analyses on mussels revealed that a large number of genes showed sexually dimorphic expression in the gonads of adult and juveniles.

UK populations of the common estuarine bivalve *S. plana* are considered to be inherently gonochoristic and their extensive distribution, sedentary lifestyle and deposit feeding habit make *S. plana* a potentially valuable monitoring species. In this context it is interesting, therefore, that this species has been observed to display intersexuality (feminisation of males), including following exposure to known endocrine disrupting chemicals (EDCs).

In order to identify and characterize putative effects of endocrine disrupting chemicals (EDCs) on gonad development and function of *S. plana*, we isolated candidate sex-specific transcripts using a SSH (Suppression Subtractive Hybridization) technique. The current paper describes some important and unexpected observations on the expression of sex related mRNA in these clams.

MO 142

Multi generational and individual effects of endocrine disruptors on growth and reproductive endpoints using the copepod, *Eurytemora affinis*

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The watershed area of the Seine estuary is under high anthropogenic pressure due to high urban density, to an intensive industrial activity (mainly chemical industries) and also agriculture. Consequently, the Seine estuary is one of the most polluted area in the north of Europe. In addition, most of chemical compounds present in the Seine estuary are persistent threatening the health of organisms living in this environment. Troughs the generation, organisms are perpetually exposed to chemical and biological molecules. The evaluation of the quality of the Seine estuary has become a major issue in the last years, in particular on endocrine disrupter. The present work is to develop ecotoxicological tools for identifying reproductive and growth disrupting at the sub-individual, individual and populational level of a calanoid copepod species, *Eurytemora affinis*.

To meet our objectives, we applied parallel handling multi-generational protocol and a bioassay based on embryo-larval growth and reproductive endpoints. At first after one generation of acclimation, ovigerous females of copepods were exposed to a various chemical compounds (PCB126, 4-NP, methylfarnesoate) at a sub-lethal concentration then two generations were followed. For each generation, clutch size, individual number and stages were estimated. In parallel, new hatched of nauplii were exposed for 6 days to the same chemical molecules. Every 2 days mortality, growth and chitobiase activity were followed.

The results obtained from the experiments showed that the first generation is very sensitive to effects of contaminants. However, subsequent generations as well as lower juvenile stages seem to be much less sensitive. Following the results, it appears that copepod *Eurytemora affinis* is capable to adapt to its environment.

MO 143

Photosynthetic and proteomic responses of the marine diatom *Thalassiosira pseudonana* to triphenyltin exposure

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Triphenyltin compounds (TPTs) have been widely detected in the marine environment because of their widespread applications as antifoulants (e.g., on ship hulls and submerged mariculture facilities) and biocides against fungal diseases. However, little is known about its toxicity towards marine autotrophic species. Marine diatoms, as globally important primary producers, are responsible for 20% of global carbon fixation. It is, therefore, important to investigate if TPTs can pose hazard to marine diatoms. The marine diatom *Thalassiosira pseudonana* was the first chosen eukaryotic marine phytoplankton as model species for whole genome sequencing, and its available genomic information enables toxicologists to investigate the algal response to environmental contaminants at molecular levels. This study was designed to elucidate molecular toxic mechanisms of TPTCl on *T. pseudonana* by using an integrative approach of measuring physiological end-points (i.e., growth inhibition and photosynthetic response) and molecular responses (i.e., proteome expression). Through the standard growth inhibition test, we found that the 96-h EC50 value was 1.90 µg/L (95% CI: 0.96 - 3.78 µg/L). Using the pulse amplitude modulated (PAM) fluorometry, the 96-h EC50 values for photosynthetic parameters were founded to be 2.67 µg/L (95% CI: 2.50 - 2.84 µg/L) and 2.62 µg/L (95% CI: 2.44 - 2.82 µg/L) for $\Phi P0$ and ΦP , respectively. The results also showed that increasing exposure concentrations of TPTCl led to reduction in photochemical quenching (i.e., decreased $\Phi P0$ and ΦP values). This dwindling trend of both $\Phi P0$ and ΦP indicated that electron transport from photosystem II (PSII) to photosystem I (PSI) was hindered. Proteomic response of *T. pseudonana* is currently being investigated using two-dimensional electrophoresis and advanced mass spectrometry. As anticipated, the results of the proteomic analysis will be essential for revealing the toxic mechanisms of TPT on the diatom and identifying a suite of protein biomarkers as exposure and/or effect indicators.

MO 144

Mechanisms of action of the neural disruptor group 'selective serotonin reuptake inhibitors' in *Daphnia magna*

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Selective serotonin reuptake inhibitors (SSRIs) are known to increase offspring production in *Daphnia magna*. This study tested the hypothesis that the increase of serotonin post-synaptic activity by SSRIs changes the perception of the food environment and switches life-history responses towards higher food level: females reproduced earlier, producing more but smaller offspring. *D. magna* reproduction tests, respiration, feeding and survival-starvation assays and studies of lipids, proteins and carbohydrate levels of unexposed and exposed females to the SSRIs fluoxetine and fluvoxamine and the 5-HT serotonin receptor antagonist cyproheptadine. Factorial life-history experiments and reproductive assays showed that exposure to SSRIs increased juvenile development rate, clutch size and decrease offspring size at low and moderate levels of food rations. These effects were reverted by the presence of cyproheptadine, indicating that 5-HT function was essential to the SSRIs effects on *Daphnia* and linking them to the pharmacological effects of SSRIs in humans. Respirometry and survival assays and biochemical analyses on lipids, proteins and carbohydrate levels showed that SSRIs increased oxygen consumption rates and decreased carbohydrate levels in adult females. These changes did not effect survival under starving conditions, but they significantly affected the capacity of the exposed animals to survive under anoxic conditions. These results suggest that SSRIs increased aerobic catabolism in *D. magna* making exposed individuals apparently more fitted exploiting food resources under normoxic conditions, but at the cost of being more sensitive to low oxygen levels, a common situation in natural environments.

MO 145

Effects of chronic exposure to gemfibrozil on aquatic organisms *Pseudokirchneriella subcapitata*, *Daphnia magna*, *Moina macrocopa*, and *Oryzias latipes*

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Gemfibrozil is a commonly used lipid regulator in humans. Gemfibrozil has been frequently detected in the aquatic environment, and suspected for adverse ecological consequences not only because of frequency of detection but also its high log K_{ow} value (4.76). In this study, we conducted the chronic toxicity tests using freshwater algae *Pseudokirchneriella subcapitata* (72 hr), cladocerans, *Daphnia magna* (21 d), *Moina macrocopa* (7 d) and, 132 d long fish toxicity test using *Oryzias latipes*, and report the toxicity information that could be used for risk assessment of gemfibrozil in freshwater. In *P. subcapitata* test, the 72 hr EC₅₀ was 5.27 mg/L (95% confidence interval of 4.77-5.62). In *D. magna* test, survival was reduced significantly at 5mg/L. In fish test, the body length of 30-day post hatch (dph) juvenile fish was significantly decreased at the gemfibrozil concentration of 3mg/L. When the mRNA expressions of several mRNAs related to endocrine disruption were measured at 30 dph juvenile fish, significant up-regulations of vitellogenin1 (vtg1) and vtg2, and cyp19b mRNA levels were observed in the juvenile fish at 3mg/L, suggesting effects on steroidogenesis and estrogenicity of gemfibrozil in fish. Histological observation at 50 dph revealed the presence of tubular basophilia and tubular vacuolization in kidney of the exposed fish. Our results demonstrate that long-term exposure to gemfibrozil resulted in adverse effects of survival and reproduction in cladocerans and fish. Mechanisms of endocrine disruption in fish warrant further investigation.

MO 146

Endocrine disruption effect of chronic exposure to mefenamic acid on aquatic organisms *Daphnia magna*, *Moina macrocopa* and *Danio rerio*

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Pharmaceuticals are trace contaminants of growing importance in aquatic ecosystem due to their physiologically active nature and their potential for impacts on non-target species. Mefenamic acid (MFA, CAS No. 50-78-2) is a non-steroidal anti-inflammatory drug (NSAIDs), which has been widely used for analgesic, antipyretic and anti-inflammatory purposes. We evaluated the acute and chronic toxicity of mefenamic acid employing several aquatic organisms, including two cladocerans, *Daphnia magna* and *Moina macrocopa*, and a teleost, *Danio rerio*. The 48 h acute median effective concentration (EC₅₀) of *D. magna* was determined at 17.16 mg/L. In 21 d chronic toxicity test, *D. magna* showed significant changes in reproduction related characteristics after the exposure. In the early life stage test using *D. rerio*, there was a significant decrease of larval survival at as low as 1 mg/L of mefenamic acid. In addition, vitellogenin gene expression and VTG protein level were reduced at the highest experimental concentration of mefenamic acid, 1 mg/L. The result of this study indicates that this pharmaceutical may have a potential effect on survival, reproduction and growth of the aquatic

organisms, but the effect concentrations are a few orders of magnitude greater than those occurring in the ambient water environment.

MO 147

The effects of nonylphenol ethoxylates having different ethylene oxide (EO) chain on fish, daphnia, and alga

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Nonylphenol ethoxylates (NPEs) have been used as surfactants in detergent formulations in both of industrial and domestic. NPEs are biodegraded to shorter ethylene oxide (EO) or nonylphenol (NP). NP shows high toxicity to aquatic organisms, and also, NPEs having shorter ethylene oxide (EO) chain have been known to show greater toxicity than longer EO chain. Therefore, it is important to examine the effects of not only NP but NPEs on aquatic organisms. However, there were little toxicity data on aquatic organisms of different EO chains. To examine the different effects of NPEs having various EO chains (NP1EO, NP2EO, NP3EO, NP4EO, NP5EO, and NP6EO) on aquatic organisms, we performed the acute toxicity tests using alga (*P. subcapitata*), daphnia (*D. magna*), and fish (*O. latipes*). Each acute toxicity test was carried out according to OECD test guidelines No. 201, 202, and 203. Chemical analysis of NPEs concentrations in test water were carried out using LC-MS-MS.

In fish acute toxicity test, our results showed that NPEs having shorter EO chain had greater toxicity than that having longer EO chain. The toxicities were observed in range from 1 ppm to 5 ppm in LC50 (96 h). The similar results were shown in alga and daphnia. In the present study, the toxicity of NPEs was shown in all species examined. In further study, we need to compare toxicity among alga, daphnia, and fish for each NPE.

MO 148

Can fish embryos help predict endocrine disruption? An example with genistein

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The plant-derived isoflavone genistein is categorised as a weak oestrogen and the effects of genistein are well documented by many, particularly fish studies. However, these studies required the use of numerous animals, as do all current regulatory accepted tests for endocrine disruption. Hence, alternative methods need to be considered to comply with ongoing changes in regulation to fulfil the 3R's principle and reduce animal testing.

In this context, we studied the suitability of fish embryos for testing and analyzing endocrine disruption, using genistein as a model substance. Fish embryos are not regarded as protected animals even though their use allows effects to be assessed in a whole living organism. The drawback of fish embryos is that they are sexually undifferentiated and conventional endocrine disrupting adverse effects, like gonadal malformations or sex ratio alterations, cannot be assessed. To overcome this obstacle, we used transcriptomics to address suborganismic test endpoints.

We chose two complementing fish species in terms of embryogenesis time and sexual differentiation, zebrafish and medaka. For zebrafish, microarray experiments were conducted, whereas for medaka, gene expression of selected endocrine genes was analyzed by quantitative PCR. Functional gene enrichment analysis of the zebrafish microarray data identified several affected pathways, including pro-apoptotic signaling and the disruption of brain and nervous system development. Hox genes were downregulated. Further, the estrogenic biomarkers *aromatase b* (*cyp19a1b*) and *vitellogenin* (*vtg1*) were already upregulated at low genistein concentrations (EC10), emphasising the high oestrogenic potential of genistein. These biomarkers had also been induced in medaka, additionally to species-specific responses, including *estrogen receptor 2a* gene induction.

In conclusion, the transcriptome response of zebrafish revealed key mechanism of genistein's molecular action, whereas with the medaka data, a more in-depth insight in the estrogenic mechanisms was achieved, making both fish species complementary. Overall, we demonstrated that combining fish embryo testing with transcriptomics emerges as a beneficial and animal free approach for testing endocrine disruption.

MO 149

Effects of the UV filter benzophenone-3 in adult zebrafish (*Danio rerio*) and cleuthero-embryos at environmental relevant concentrations

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Organic UV filters including benzophenone-3 (BP-3) are widely used to protect humans and materials from damage by harmful UV irradiation. These compounds enter the aquatic environment directly from sunscreen application, or indirectly via wastewater. BP-3 has been detected in surface water, wastewater and in biota. To date, still little is known about the fate and effects of this compound. BP-3 and some of its metabolites, which comprise of benzophenone-type moieties, were previously shown to have estrogenic effects in fish *in vitro* and *in vivo*. In our study, effects of BP-3 are evaluated after an exposure of adult male zebrafish (*Danio rerio*) and zebrafish embryos for 14 days and 120 hours post fertilization, respectively. Exposure concentrations of BP-3 are 10, 200 and 600 µg/L, as confirmed by LC-UV. The water analysis indicates that within 48 hours adult zebrafish, but not embryos, transform BP-3 in part to benzophenone-1 (BP-1), an estrogenic compound also used as UV filter. BP-3 and BP-1 are accumulated in fish up to 21 µg/g and 266 ng/g b.w., respectively. We analyse molecular effects by whole-genome transcriptomics (microarrays) and find no significant transcriptional alteration at 2.4 µg/L. At 312 µg/L BP-3, 123 transcripts are altered belonging to different pathways. By applying a target gene expression approach focusing on the endocrine system in the brain, liver and testis of adult zebrafish and in embryos, expression of estrogen receptor, androgen receptor, and vitellogenin are determined by qRT-PCR, as well as key enzymes involved in steroid hormone synthesis, such as the hydroxysteroid dehydrogenases, hydroxylases, aromatases. Transcript and protein level of vitellogenin is not induced up to 312 µg/L BP-3 in adult zebrafish (441 µg/L BP-3 in embryo). Our gene expression analysis data indicate that BP-3 has a low anti-estrogenic and anti-androgenic activity in adult zebrafish and embryos at environmentally relevant concentrations. The analytical chemical analysis emphasizes that effects may be related to a combination of the parent compound (BP-3) and its transformation products including BP-1.

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MO 150

Reversibility of endocrine disruption in zebrafish (*Danio rerio*) - comparison of different effect levels

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Endocrine disrupting chemicals (EDCs) exert effects at very low concentrations and can cause serious problems for the hormonal balance of various organisms. Exposure of wildlife to EDCs is not necessarily continuous, but may often occur in pulses. Consequently for the evaluation of the long-term effects on populations, it is essential to know whether such EDC-related effects are reversible. Three different substances selected for different modes of action were tested for their long-term impact on sex ratio, gonadal development, vitellogenin (VTG) induction and aromatase activity in zebrafish: the androgen trenbolone binds directly and very effectively to the androgen receptor. Ethinylestradiol, a synthetic derivative of estradiol, causes feminization in wildlife and humans. The fungicide prochloraz acts as an aromatase inhibitor by direct interference with the aromatization of androgens to estrogens. All compounds have previously been shown to cause striking effects in zebrafish, but recovery has never been studied in detail. In order to test whether EDC-related effects are reversible, an exposure scenario limited to 60 d was followed by (a) a recovery period of 40 d or (b) continued exposure for another 40 d. Four effects levels were examined: (1) population level: sex ratio; (2) organism level: growth; (3) organ/tissue level: histology of gonads (light microscopy); and (4) molecular level: vitellogenin induction (ELISA) and aromatase expression (RTq-PCR).

Results show clear correlation of effects at all levels, but clear-cut differences between the two different exposure groups. We conclude that endocrine disruption in zebrafish following discontinuous exposure is only partially reversible and may thus have serious implications for fish.

MO 151

Testing chemicals for endocrine activity by 21-day fish screening assay

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Beyond designed properties, plant protection products, biocides, pharmaceuticals and other chemicals may pose undesired impacts on aquatic environment via sewage.

Endocrine Disrupting Chemicals (hormone-like) are toxicants that cause various effects in regulatory functions depending on length of exposure period, age, sex and stage of organism exposed. Testing chemicals for endocrine activity is required by law. For this reason, OECD Guideline for Testing of Chemicals No 230 (Sept 2009) introduced bioassay using endpoint biomarker - vitellogenin (VTG) in fish.

Zebrafish WT AB adults of both sexes were exposed for 21 days to four chemicals: E2, prochloraz, flutamide and propiconazole. VTG biomarker of endocrine activity was determined in whole body homogenates by ELISA and additionally gonadosomatic index (GSI) was reported. Results were analysed by ANOVA statistics. Natural estrogen E2 caused increase of VTG in males and prochloraz (fungicide, aromatase inhibitor) caused decrease of VTG in females. Therefore, positive controls confirmed correct test design. Changes of VTG level detected in female fish exposed to flutamide (nonsteroid anticancer drug) suggest U shaped concentration-effect curve. Decrease of GSI in male fish was also observed. VTG levels were increased in female and decreased in male fish corresponding with concentration of propiconazole (biocide, fungicide) whereas GSI in male fish was increased.

Evaluation of endocrine activity in *in vivo* models requires integration of processes in complex organism. Therefore, implementation of standard screening methods is expected by regulatory institutions and producers of chemicals.

MO 152

A comprehensive study on the toxicity of triphenyltin chloride to the rotifer *Brachionus* sp. at different biological organisation levels

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Phenyltin compounds in particular triphenyltin (TPT) are widely applied as effective biocides for different industrial and agricultural purposes. These compounds have been detected in the marine environment but little is known regarding their toxicity to the marine organisms. This study was designed to comprehensively investigate the molecular, individual and population responses of the rotifer, *Brachionus* sp. upon waterborne exposure to TPT chloride (TPTCl). Our results indicated that the population growth rate of the rotifers was decreased as TPTCl concentrations increased. A 96-h EC50 value for population growth inhibition was found to be 1.96 µg/L, which was ten times lower than the 96-h LC50 value derived from a standard acute toxicity test (i.e., 29.6 µg/L; 95% CI: 27.2 - 32.1 µg/L). At molecular level, gene expressions of twelve heat shock proteins (*hsp*), four glutathione S-transferases (*GST*), one retinoid X receptor (*RXR*) and thirteen cytochrome P450 monooxygenases (*CYP*) were studied for their responses to TPTCl exposure. At 20 µg/L TPTCl, *hsp 90α2*, *GST-O* and *CYP 29* were significantly up-regulated with the relative expression levels to the control up to 32.9, 4.4 and 62.6 folds, respectively. Temporal trends of these three genes were further studied in rotifers exposed to TPTCl over a period of 24-h. The expressions of these genes showed an increasing trend in the first few hours after TPTCl exposure, peaking at 3 h (for *hsp 90α2* and *GST-O*) and 12 h (for *CYP 29*) and then followed by a gradual decline. The overall findings of this study provide a better understanding on toxic mechanisms of TPT-mediated effects in the rotifer.

MO 153

Expression of all-trans and 9-cis retinoic acid receptor (RAR/RXR) genes during the zebrafish early development

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The zebrafish genome encompasses no less than 10 different genes codifying either for retinoic acid (RAR) or 9-cis retinoic acid (RXR) receptors, which participate in the retinoic acid response as well as in many other hormone-dependent regulatory pathways. In this work we analyzed the changes on mRNA abundance of each of these ten genes (RARα, RARβ, RARγ, RXRα, RXRβ, RXRγ, RXRδ, RXRε, RXRζ, RXRη, RXRθ, RXRι) during the first 5 days of zebrafish development. The data shows a transition from maternal to embryonic transcripts during the first 24-48 hpf (hours post fertilization), as well as a differential response of the receptor genes to the exogenous addition of their cognate ligands, particularly during the first 24 h after fertilization. These results suggest that this transitional period between maternal to embryonic mRNA is particularly sensitive to the presence of putative disruptors (retinoids or retinoids), and that the RAR/RXR system is functional throughout the zebrafish leutheromorphonic period. As several emerging contaminants are suspicious of disrupt either the RAR/RXR or other mechanistically related hormone regulatory systems (TR, CAR, PXR, PPAR, and others), the analysis of the retinoid/retinoid response in zebrafish embryos facilitates its implementation as convenient, non-animal model to assess these potentials risks.

MO 154

Sexual endocrine disruption in fish with focus on estrogen receptor antagonists- a fish life cycle test with fulvestrant

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Estrogen receptors represent important interfaces for mechanisms concerning gonadal development and reproduction of fish and therefore target sites for endocrine disruption.

Within the scope of establishing a tiered testing strategy to detect and assess a potential for sexual endocrine disruption in fish, data is needed to detect sensitive windows of exposure. Furthermore, the respective windows of effect manifestation should be identified. Especially for the estrogen receptor antagonists, a data gap is evident.

A full life cycle test was performed at the Fraunhofer IME to detect the population relevant effects of the selective estrogen receptor down regulator (SERD) fulvestrant on zebrafish. The nominal test concentrations were 1.3, 4.0, 13 and 40 µg/L. The test was performed under flow through conditions.

The test was initiated by introduction of fertilised eggs in the test vessels. The fish were kept until they have reached the adult stage. Survival, Growth and reproductive parameters were recorded. Secondly, a filial generation was prepared and effects on the early life stage phase (survival and growth) will be recorded.

A significant effect on reproduction could be observed at 13 and 40 µg fulvestrant/L. The cumulative number of fertilised eggs was found to be reduced.

The results confirm findings from a former study with the anti-estrogen tamoxifen-citrate, suggesting the sexual development as sensitive window of exposure and reproduction as sensitive window of manifestation for the estrogen modulated mode of actions.

A shift towards an increased number of males could be observed for tamoxifen-citrate. This has to be confirmed by dissection and sexing of the fish at end of the FLCT with fulvestrant.

MO 155

Peak exposure versus intrinsic toxicity in fish full life cycle tests with an anti-estrogen

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Environmental exposure is characterised by seasonally and locally fluctuating concentrations due to imission patterns, hydromorphology and dilution by rainfall events.

Thus, the duration of concentration peaks surpassing effect threshold levels has to be compared with intrinsic chronic toxicity data, e.g. by calculating time weighted average concentrations. This is especially problematic when focussing on endocrine effects, as the most sensitive life stages or performances regarding effect setting and effect manifestation may be different. Both have to be covered by exposure and appropriate endpoints. For this reason, fish full life cycle (FFLC) or multi-generation tests are performed in flow-through systems with permanent exposure. These tests are not able to differentiate endpoints according to their susceptibility to short-term exposure and reversibility of effects. We perform FFLCs simulating worst case peak exposure by using static artificial sediment/water systems (270 L) with three life stages of zebrafish (fertilised eggs, juveniles, spawning adults) separated by stainless steel nets. The fish groups including their filial generation are investigated during declining exposure. Measured effects can be related to initial concentrations and to time weighted average concentrations during defined life stages.

There is still a data-poor situation for anti-estrogenic effects. In zebrafish, these are represented by a reduction of fertilisation rate and egg quality (exposed adults) and a sex ratio shifted towards males (fish exposed during sexual maturation). We used fulvestrant as potent anti-estrogen in a flow-through FFLC and a static peak exposure test with artificial sediment to

- confirm or add knowledge to population-relevant effects caused by anti-estrogens,
- compare effect threshold concentrations of peak versus permanent exposure,
- find principles of linking endocrine exposure and effects via bioaccumulation and/or stage-specific time weighted average exposure concentrations,
- assess the relative sensitivity of endpoints depending on exposure duration,
- rank endpoints according to their relevance for population effects.

In the peak exposure test the DT50 in the water column was measured to be approximately 0.5 d. The only effect was a temporary one on fertilisation rate at 632 µg/L (initial concentration) on days 4 to 6. The flow-through test at concentrations of 40, 13, 4 and 1.3 µg/L will be ended by the end of 2011.

MO 156

Characterization of the mode of action of the pharmaceutical clotrimazole on testicular steroidogenesis in zebrafish

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Clotrimazole is a pharmaceutical fungicide that has been recently detected in aquatic environment. This substance is known to inhibit CYP enzymatic activities, including several steroidogenic CYP but little is known about its in vivo endocrine disrupting potency in fish. In vertebrates gonadal steroidogenesis is under the control of the hypothalamus-pituitary-gonad (HPG) axis. The aim of the present study was (i) to assess the effect of clotrimazole on zebrafish testicular steroidogenesis by conducting in vivo and in vitro experiments and (ii) to characterize its mode of action by studying a network of functional target genes of the pituitary-gonad axis by means of Q-PCR. In vivo exposures of male zebrafish to clotrimazole were conducted for 7 (70-150 µg/L) or 21 days (20-200 µg/L) and led to concentration-dependent inductions of steroidogenic gene and protein expressions. In vitro testicular explants were exposed to similar concentrations of clotrimazole, and no effect was observed on transcript levels of steroidogenic enzymes. However, clotrimazole inhibited 11-KT release in the culture medium. This result suggests that clotrimazole does not act directly on testis to regulate the transcriptional activity of these genes. Induction of steroidogenic genes could be interpreted as a compensatory biological response to inhibition of cytochrome P-450 dependent steroidogenic enzymes.

To support this hypothesis, a network of functional genes of the pituitary-gonad axis was used. We showed that clotrimazole induce a cascade of molecular events in pituitary and testes. Transcript levels of genes encoding for pituitary Gonadotropin releasing hormone receptors (GnRH-R) and folliculo- stimulating hormone (FSH) -subunit, as well as testicular FSH receptor and steroidogenic enzymes were induced. All together, these molecular events are consistent with the involvement of FSH in inducing steroidogenic gene expressions to compensate the inhibitory action of clotrimazole on 11-KT synthesis.

Our study highlights the relevance of studying a network of relevant genes of the pituitary-gonad axis to investigate the mode of action of clotrimazole on the endocrine system of fish. Such approach could be extended to other compounds acting as inhibitor of P450-steroidogenic enzymes. The disruption of testicular steroidogenesis raises further concerns about the impact of clotrimazole on reproduction.

MO 157

Reproductive toxicity of methyltestosterone in embryo and adult zebrafish

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In Brazil, the hormone 17 α -methyltestosterone (MT), besides not being regulated is widely used in fish farming to reverse sexual juvenile females into male of tilapia. However, the effects of MT to non-target aquatic species and the environment are largely unknown. Field studies have documented physiologic and developmental abnormalities in feral fish, such as increased vitellogenin (Vtg) concentrations and gonadal intersexuality in male fish. Altered levels of Vtg have been widely used as an effective and sensitive biomarker for the detection of endocrine disrupting chemicals in the aquatic environment. The present work aims to assess the effects of commercial MT (cMT) (90% purity, Toledo, Paraná), as well as its active ingredient 17 α -methyltestosterone (aMT) (99.9% purity, Sigma) in adult and embryos of zebrafish (*Danio rerio*). Adults and embryos of zebrafish were exposed during 4 days to the nominal concentrations of 4, 23, 139, 833 and 5000 μ g/L of both MT products. At the end of exposure adult fish were weighted and dissected to remove gonads and liver for further Vtg. Gonadosomatic (GSI) and hepatosomatic (HSI) indexes were determined in adult fishes. Results showed no significant differences in GSI neither in males or females exposed to cMT or aMT. The HSI was significantly higher in females exposed to the highest concentration tested of aMT and also in males exposed to the lowest concentration of cMT, suggesting that MT might induce hepatic toxicity, as expected since liver is the main organ for androgenic steroids metabolism. Hepatic and gonadal vitellogenin concentrations in adults did not show significant differences with cMT or aMT exposure. However, the Vtg levels in embryos were significantly lower in all concentrations tested of aMT and in two highest concentrations of cMT. These results suggest that MT might induce endocrine disruption in exposed early-life stages of zebrafish. In the other hand, the adult males and females that already have the endocrine system completely developed might be able to cope with MT stress as evidenced by the higher HSI registered. The lower levels of Vtg registered in MT-exposed larvae are in accordance with the reproductive changes caused by application of the hormone in fish farms to reverse sex in larvae tilapia. These results raise great concern about the risk of endocrine disruption caused by exposure to MT products of early life stages of aquatic non-target species.

MO 158

Sub-lethal effects of mixture of compounds in Zebrafish (*Danio rerio*)

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The prevalent use of different compounds in industry, agriculture, health care and other activities lead to an increased release of these compounds to the aquatic environments. Concerning the risk of mixture of these compounds in the environment, it is unlikely that fish are exposed to one single substance each time. There are few studies to evaluate the sub-lethal effects of mixture of different compounds on development stages, molecular physiology, and behavior of fish. Zebrafish, *Danio rerio*, is a suitable model in fish embryo toxicity test (FET) and behavioral studies. To evaluate the toxicity of mixture of compounds, the zebrafish embryos were exposed to single substances, including ethinylestradiol, nonylphenol, benzo-a-Pyrene, beta-naphthoflavone, and silver. The embryonic sub-lethal endpoints were heart rate, pigmentation, and eye development and the aim was to identify the most sensitive parameter. The lowest concentration of each single substance that might cause an effect (ECx) has been considered in joint mixture exposure. Since even the concentration below the threshold, which can cause no effect, might impair fish behavior, the movement of zebrafish larvae after 6 dpf (days post fertilization) has been studied. In molecular physiological level the induction of vitellogenin and CYP1A, in protein and gene expression levels, were measured in adult fish exposed to single compound and joint mixture to demonstrate the endocrine disrupting effects. Due to the interaction between the compounds in the mixture, there might be a cross-talk between estrogen receptor (ER) and Ah receptor (AhR) agonists available in the mixture of compounds that can inhibit or modulate the AhR- or ER- signaling pathways. Further, another sub-lethal effect, the multixenobiotic resistance, can be observed in fish exposed to the effluent of the sewage treatment plants for a long time. The present study has been undertaken to evaluate the sub-lethal effects of mixture of compounds on development, molecular physiology, and behavior of zebrafish.

MO 159

Zebrafish embryos as bioanalytical tools for detection of estrogenic and dioxin-like chemicals in environmental matrices

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Recent studies have reported the use of fish embryos as *in vivo* mechanism-based screening bioassays for the specific detection of biologically active compounds. In this study, we aimed at evaluating the use of zebrafish embryos for the detection of estrogenic and dioxin-like compounds in environmental matrices. Effect on phase I xenobiotic metabolism was assessed using EROD (Cyp1A-like) and BFCOD (Cyp3-like) assays in living embryos; estrogenic activity was measured using a transgenic zebrafish line expressing green fluorescence protein reporter gene under the control of the aromatase-B promoter (AroB-GFP). A panel of standards chemicals was first tested in order to calibrate the bioassays. One significant result was the finding that dioxin-like compounds were strongly active on the BFCOD assay. Then, these bioassays were applied to a multi-contaminated sediment extract, which has been shown to exert multiple toxicological activities as determined by a panel of *in vitro* bioassays. Both the crude extract and 4 fractions of increasing polarity (F1 to F4) and obtained from normal phase solid phase extraction were tested on the bioassays. Overall, crude and/or fractions were active on all endpoints, demonstrating the suitability of the bioassay to detect biologically active compounds. While no estrogenic activity could be detected in crude extract, weak activities were detected in F1, F2 and F3 fractions whereas *in vitro* estrogenic activity was only detected in F2 and F3. F2 and F3 are known to contain estrogenic compounds such as alkylphenols, parabens and steroids (data not shown). EROD and BFCOD gave parallel results, with significant activities in crude, and F1=F2>F3 fractions. Altogether these preliminary data show the suitability of zebrafish embryos-based assays to detect estrogenic and dioxin-like chemicals in environmental matrices and could serve as useful tools for effect-directed analysis approaches. Fundings: P189-ECOP1 and ITN EDA-EMERGE.

MO 160

Evaluation of estrogenic activities of benzophenone derivatives using innovative *in vitro* and *in vivo* zebrafish models

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Benzophenone derivatives (BPs) are used as UV filters in many different products. These lipophilic compounds were recently described as ubiquitous contaminant of different compartments of aquatic systems, including fish. In this study, we evaluated estrogenic activity of ten BPs in a model fish species, the zebrafish (*Danio rerio*), by combining newly developed *in vitro* and *in vivo* models. *In vitro*, three specific zebrafish reporter gene models expressing zebrafish estrogen receptors (ZfERs) in zebrafish hepatic cell line (ZFL) were used. Estrogenic activity of BPs was quantified *in vivo* by using a newly developed transgenic zebrafish line that expresses green fluorescent protein (GFP) under the control of the cyp19a1b promoter. The cyp19a1b gene codes for the brain aromatase B that is under the control of estrogens. Vitellogenin (Vtg), a marker of estrogenic compound, was used to assess the potential estrogenic effect of certain BPs in male fish.

Five of 10 BPs induced an estrogenic activity on the three models: BP2, THB, BP1, 4BP, while BP and 234 BP were non active. We noted difference in BPs ranking depending on the ZfER subtypes suggesting receptor selectivity by certain BPs.

By quantifying GFP expression in live zebrafish, we confirm the estrogenic activity of some BP, i.e., BP1, THB, 4BP and 44'BP, as shown by their ability to induce GFP in a concentration-dependent manner. However, the ranking of BPs as a function of their estrogenic activity assay is different between models. For instance, 4BP was the most estrogenic compound *in vivo* but not *in vitro*. Differences were also noticed regarding the estrogenic activity of BP2. *In vitro*, BP2 has strong activity but had no effect on GFP in cyp19a1b-GFP embryo. However, BP2 induced strongly Vtg in male. The lack of estrogenic activity of BP2 in embryo may be related to difference of biotransformation and/or metabolism of this compound between embryo and adult fish. Our approach allows to characterize the estrogenic potency of emerging pollutants, using innovative *in vitro* and *in vivo* assays within a unique model fish species. The differences observed between assays deserve further studies to characterize the behavior of compounds in the different biological models in term of biotransformation and metabolism.

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MO 161

Endocrine disrupting effects of OSPW and ozonated OSPW on sex steroid synthesis and signalling in fathead minnows

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There is concern about the large volume of oil sands process-affected water (OSPW) produced by surface mining of the oil sands deposits in Alberta, Canada. OSPW has endocrine disrupting effects on exposed fish and *in vitro*. Although ozonation attenuates some of the endocrine disrupting effects of OSPW the effects of ozone-treated OSPW on sex-steroid production and signaling in fish is unknown. In this study, sexually maturing male and female fathead minnows were exposed to either control freshwater, relatively fresh OSPW, or OSPW treated with 80 mL/L of ozone under conditions of static renewal for 7-days. At the end of the exposure period, brain, liver, and gonad samples were collected from each fish. Transcript abundances of 23 genes representing key signalling pathways and functional process of the HPGL axis were quantified by quantitative real-time PCR (qPCR). In male fathead minnow, abundances of transcripts of FSHb and LHb were greater in brains when exposed to OSPW compared to males exposed to freshwater. Ozonation attenuated the effects on abundances of transcripts of these genes. In testis isolated from males exposed to OSPW the abundances of transcripts of FSHR and LHR were greater compared to males exposed to the freshwater. Greater abundances of transcripts of ER α , VTG, CHGL and CHGH in livers from male fathead minnows exposed to OSPW suggested that some component of OSPW acted as an agonist of the ER α that stimulated signalling. In female fathead minnow, abundances of transcripts of FSHb but not FSHb were greater in brains when exposed to OSPW. Ozonation attenuated the effect on LHb but stimulated greater abundance of transcripts of FSHb. The abundances of transcripts of FSHR, LHR and CYP19a were significantly less in ovaries from females exposed to OSPW and ozonated OSPW compared the controls. The abundances of transcripts of AR, ER α , ER β , as well as the estrogen-responsive genes VTG, CHGL and CHGH were less in livers from females exposed to OSPW relative to abundances in livers from females exposed to freshwater. In sum, OSPW has endocrine disrupting effects all levels of HPGL axis in fathead minnows. OSPW has different effects in male and female fathead minnows. The results of varied effects may provide mechanistic basis for results from other studies. Ozonation attenuates effects of OSPW on some endocrine endpoints, and the effect is more prominent in males than in females.

MO 162

Differences in fathead minnow fecundity using tiles with and without trays for fish endocrine assays

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A trial to further evaluate the use of trays with tiles for the FISH SHORT-TERM REPRODUCTION ASSAY WITH THE FATHEAD MINNOW (*Pimephales promelas*) (FSTRA) test. The five-week trial simulated the experimental design of the FSTRA test that has a two-week pre-exposure period followed by a three-week exposure period. No test substance was used in the trials. Fish were divided into two test groups, one with tiles only and the other with tiles and trays. The test compartments followed the FSTRA guidelines and had three tiles per tank and reproductive groups that consisted of six fish (4 females and 2 males). Fecundity was measured daily throughout the five-week exposure. The methodologies used in the trials were those presented in OPPTS and OECD guidelines. The data for the first two weeks of the trial simulated the pre-exposure phase of the assay. The tile only group had a mean fecundity of 30.35 eggs per female per day and a CV of 35.6%, while the tile and tray group had a mean of 36.93 eggs per female per day and a CV of 34.25%. This indicates that for the first two weeks, trays do slightly increase mean fecundity, but both groups clearly exceed the guideline requirement of 15 eggs per female per reproductive day and have similar CVs. The data for weeks 3 to 5 of the trial simulates the exposure phase of the test. The tile only group had a mean fecundity of 19.66 eggs per female per day and a CV of 26.7%, while the tile and tray group had a mean of 27.95 eggs per female per day and a CV of 42.8%. Fecundity in weeks 3 to 5 was greater for tanks having tiles with trays, however, the variability was much higher for trays and tiles which had a much larger CV. Of greater concern was that two of the tanks stopped spawning in the tile and tray group. One tank had 51.8 eggs per female per reproductive day during the first two weeks and zero for weeks 3 to 5, and the other decreased from 17.8 to zero eggs per female per reproductive day. Both of these tanks would likely qualify for inclusion in a test that would greatly confound the interpretation of the results. There also was a greater incidence of injuries with trays than without trays, which is supportive of our concern for injury from the trays and screens in tanks. The use of trays with tiles in fathead minnow reproduction tests does increase fecundity, but increased variability in the last three weeks of a five week trial.

MO 163

Reproduction and endocrine function in Fathead Minnows (*Pimephales promelas*) exposed to Polybrominated Diphenylether (DE-71)

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To determine if DE-71 altered reproduction and endocrine function in fish, the Tier 1 EDSP 21-day Fish Reproduction Screen with fathead minnow (*Pimephales promelas*) was used. Reproductively-active male and female fish were exposed to DE -71 (0 [control], 0.65, 1.3, 2.5, and 5.0 µg/L) via flow-through exposure for 21-d and evaluated for survival, reproductive behavior, and secondary sexual characteristics. Reproductive fecundity and fertilization success were monitored daily. At termination, the status of the reproductive endocrine system was assessed by the gonad-somatic index (GSI), gonadal histology, plasma steroids (estrogen [E2] and testosterone [T]), and 11-ketotestosterone [11-KT]), hydroxysteroid dehydrogenase [HSD], and plasma vitellogenin (VTG). Results indicated that the DE-71 exposure did not affect survival or body weight, but reduced male GSI. Reproductive fecundity, gonad histology, and endocrinological measures of reproductive status were altered. Exposure to DE-71 did not alter plasma E2 and VTG levels in females, however; decreased plasma T levels were detected in both sexes. These results were generally similar to those found in *S. tropicalis* during gonad differentiation demonstrating similar effects of polybrominated diphenylethers on the reproductive system of male fish. Evaluation of HSD activity and the male-only androgen 11-KT will be presented to describe whether the effect may be related to general alteration of early steroidogenesis as measured by HSD, or if DE-71 is more specifically altering androgenesis in male fathead minnows as measured by 11-KT and thus, acting in an anti-androgenic manner.

MO 164

Endocrine disruptive effects of the common feedlot contaminant 17α trenbolone on the Australian Murray River rainbowfish (*Melanotaenia fluviatilis*)

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The effects of Endocrine Disrupting Chemicals (EDCs) are of global concern; however in Australia their potential effects are largely unknown particularly on native species. In Australia, the beef industry is largely dependent on Trenbolone acetate (TBA) implants, a synthetic steroid that is used as a growth promoter. Within the animal's body the administered acetate is hydrolysed to 17β trenbolone and part of this one epimerized to 17β trenbolone. In animal wastes the proportion of the isomeric form α is estimated to be 10 fold higher than β, where these metabolites are known to be very stable. The aquatic environment is potentially exposed via direct discharge, runoff, or both. This study evaluated the androgenicity of 17α trenbolone on the native Australian Murray River rainbowfish, *Melanotaenia fluviatilis*. Four adult female fish and two male fish per tank were exposed for 21 d to three different concentrations of 17α trenbolone. Cumulative egg production, hatchability, larval fitness as well as adult VTG concentrations and histopathology of gonads were assessed. In vivo exposures at the tested concentrations lead to the masculinisation of the female fish at the highest concentration tested. Fecundity of the fish seems to be reduced as well as larval fitness, leading to deformities in new hatched larvae. Gonadal morphology of adult female fish also seems to be affected by the exposures.

MO 165

Effects of towel eluates on Vitellogenin levels and EROD activities in rainbow trout (*Oncorhynchus mykiss*)

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The consumption of textiles in the world is increasing every year. Several chemicals used in textile industry and fabrics may have adverse effects on aquatic organisms. Alkylphenols like Nonylphenol (NP) are one of the widely used chemicals acting as a dispersing agent of color pigments in the textiles with a high amount of outlet in the aquatic environment through industrial and household waste. NP is reported to have estrogenic activity in both in vitro and in vivo studies. Limited studies have focused on the estrogenic activity of chemicals present in towels. To evaluate the estrogenic activity of chemicals present in towel eluates juvenile rainbow trout were exposed to waters from heated towels and "filtered" towels for 6 days. Induction of vitellogenin (VTG) protein, a biomarker of exposure to xenoestrogen, was observed in the fishes exposed to water from heated towel but surprisingly in fishes exposed to "filtrated" towels the VTG levels were suppressed. The possible effects of these exposures on the CYP1A activity (EROD activity) showed a drastic increase of hepatic CYP1A activity in fish exposed to filtrated towels but lower CYP1A activity in fish exposed to heated towel water. It should be noted that textiles contain complex mixture of compounds, possibly containing both VTG and EROD inducing chemicals. There might be Ah receptors agonists in filtered towel water which can induce EROD activity, while they can suppress the induction of VTG. On the other hand, since there is a high EROD activity in this group, ER agonists could not inhibit the induction of CYP1A gene expression. This might be explained as a one-way inhibition AhR-ER cross-talk when the fish were co-exposed to AhR and ER agonists. The difference between the response in heated and filtered eluate exposure may be due to volatile Ah receptor agonist evaporated from the eluate. The present study was undertaken to evaluate the estrogenic activity of towel eluates in rainbow trout and also the effects on EROD activities. The study was conducted as a student project in the Master course Ecotoxicology with emphasis on physiology at the University of Gothenburg, Sweden.

MO 166

Effects of 17-β estradiol on thymus volume and regionalisation in juvenile sea bass (*D. labrax* L.)

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Providing the first lymphocytes during the maturation process of the fish immune system, the thymus is in addition to the head kidney considered to be a primary immune organ. It consists of two different compartments: the cortex and medulla. Immature T-lymphocytes migrate from the cortex into the medullary region during their maturation process. Only mature T-cells appear in the medulla. Contrary to mammals, there is no observable thymic atrophy in adult fish and the organ stays active during the whole lifetime. The critical period in thymic regionalisation lies between 51 and 92 dph in sea bass. The thymus gland is considered to reach adult volume 365 dph. The thymus is particularly susceptible to waterborne pollutants as only an epithelial layer separates it from the gill chamber. In mice it is evident that 17-β estradiol (E2) plays an important role in T-cell maturation, more precisely on the initiation of apoptosis in auto-reactive T-lymphocyte precursors, which occurs at the border between the two thymus regions. As a result of livestock breeding and anthropogenic wastewaters, higher concentrations of oestrogens are present in the aquatic environment, therefore the current study aimed to elucidate the possible impairments of E2, as a model oestrogen, on fish thymus.

Juvenile sea bass (*D. labrax*, 60 dph) were exposed to different environmentally relevant concentrations of E2 (0; 2; 20; 200 ng/L) for 56 days. The head sections, including the thymus, were prepared for histological observations in order to assess thymus volume, region volumes and the ratio between cortex and medulla. No differences in thymus volume or regionalisation between exposed (200 ng/L) and control fish could be observed. Nevertheless, E2 might affect the thymus at a sub-cellular level with consequences for whole organism immunity. For instance, we investigated the expression of different cluster of differentiation (CD) -receptors associated with T-cell maturation in a subsequent study. This research is funded by INTERREG IV A (DIESE, #4040) of the European Union.

MO 167

17-β estradiol impairs head kidney leucocyte phagocytosis in juvenile sea bass (*D. labrax* L.)

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With the detection of oestrogen receptors in immune relevant organs and cells, it seems likely that the functionality of the immune system is also dependent on the concentration of sex hormones in the organism. Knowing that the concentrations of steroid hormones are elevated in the aquatic environment, the question of effects on the immunity of aquatic animals arises. Exposure possibly leads to direct as well as indirect consequences on the mature immune system of adult fish. But more importantly, the development of the relevant immune organs may be impaired in larvae and juveniles.

Phagocytosis is one of the crucial functions of the innate immune system, and is one of the rare, established biomarkers for the immune system in ecotoxicology. Intracellular digestion of pathogens is followed by antigen processing and presentation, and the secretion of cytokines, which activates immune cells and the adaptive immune response. Here we investigate the impact of 17-β estradiol (E2), one of the most potent (xen)oestrogens, on the phagocytosis of head kidney leucocytes from juvenile *Dicentrarchus labrax*. Sea bass, at different developmental stages of the immune system (30 dph, 90 dph, 120 dph), were exposed to 20 ng/L E2 for 35 days. Phagocytosis of head kidney leucocytes was determined by incubating them with fluorescent latex beads and detecting cells with ingested beads via flow cytometry. Composition of cell populations, mortality and phagocytosis were measured for the whole, lymphocyte and monocyte/macrophage populations. While no effect in phagocytotic performance was found in younger animals, we observed a significant difference for whole cell population and lymphocyte phagocytosis in the 120 dph group. Our results suggest that lymphocytes are more sensitive to E2 exposure than monocytes/granulocytes, where only a tendency towards a decreasing phagocytotic activity for exposed fish was found. As sea bass head kidney lymphocytes are mainly B-cells, the obtained results strengthen the observation of phagocytosing B-lymphocytes in teleosts. The impairment of phagocytosis by an environmentally relevant concentration of E2 in nearly immune mature sea bass leads to the question of a potential impact on cytokine release and other B-lymphocyte

functions. Further these results indicate a possible impact on the recruitment of the European sea bass population, given the fact that European estuaries, serving as nursery areas, suffer from higher levels of oestrogen pollution.

MO 168

A non-invasive method based on head morphology to sex mature three-spined Stickleback (*Gasterosteus aculeatus*) in rearing conditions

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The threespine stickleback (*Gasterosteus aculeatus*) is a small-bodied teleost fish which is a major component of shallow water food webs in the northern hemisphere. *G. aculeatus* is a reference species for experimental studies, in particular for the assessment of endocrine disruptors. Sex ratio can bias the outcomes of an experimental study, especially if this study focuses on reproduction or behaviour. Moreover, sex-ratio, by itself, can be a very relevant endpoint in experimental tests. A mathematical model to distinguish mature female and male three-spined Sticklebacks *G. aculeatus* is proposed. This method is based on sexual dimorphism in the head morphology. The discrimination was established on five distances of interest on the head, divided by the standard length of fish. The parameters were estimated based on a training set composed of 102 fish and characterised by an equilibrate sex ratio and validated on a test set composed of 69 fish. Our model permits to balance between the percentage of fish that can be sexed and the percentage of fish correctly sexed. Compared to other available methods to sex *G. aculeatus*, our model is non invasive, not expensive, rapid, replicable, and can be calibrated out of the breeding period.

MO 169

Effects of produced water from an offshore oil-platform; an in vivo study using the three-spined stickleback (*Gasterosteus aculeatus*) as a model species

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There is growing concern over the high levels of anthropogenic contaminants entering the Arctic region. In light of the anticipated expansion of oil exploration and production in Arctic areas, of particular concern is the potential impact of hydrocarbon-related contaminants on the Arctic ecosystems. Several of these compounds are known to exhibit endocrine disrupting properties. The predicted expansion of oil production into Arctic areas would pose a considerable environmental risk, and highlights the need for the implementation of robust indicator species for future biomonitoring programmes.

The three-spined stickleback (*Gasterosteus aculeatus*) is a small teleost fish widely distributed throughout the Northern hemisphere, occurring as far north as Svalbard. This ubiquitous species is increasingly being recognized as an emerging model in ecotoxicology, notably as a sentinel for endocrine disruption. The stickleback can be used as a combined biomarker of both estrogenic and (anti-) androgenic compounds, and with the sequencing of its genome an increasing number of molecular tools are being developed for the assessment of contaminant exposure. The stickleback has the potential of becoming a valuable indicator species to identify and monitor the impact of anthropogenic pollutants in Arctic ecosystem, and be included in future biomonitoring programmes. The objective of this study was to evaluate the effects on stress tolerance in fish after exposure to produced water, using the three-spined stickleback as a model organism. Effects were assessed by quantification of gene expressions (UDPG, CYP1A, VTG, PLA), in addition to measuring the levels of glucose and cortisol.

MO 170

ZnO nanoparticles exposure alters transcriptomic profile in Hydra

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The acute toxicity test for 20 nm ZnO and 100 nm ZnO and comparative transcriptomic profile analysis using hydra exposed to 20 nm ZnO and 100 nm ZnO were carried out. As a result, the LC50 for 72 hours was 8.7 mg/L in 20 nm ZnO exposure and 14.9 mg/L in 100 nm ZnO exposure group. For the microarray experiment we exposed hydra to 20 nm ZnO and 100 nm ZnO as the concentration of 1/50 of LC50 for 12 hours and hybridized those RNAs extracted from the exposed groups with that of control group on the hydra cDNA chips including 17,639 genes. As the results, 137 genes expressions were induced and 106 genes were reduced over 10 fold by 20 nm ZnO exposure. Among them the expression of multidrug-Resistance like Protein 1 CG6214-PM gene increased over 100-fold. In 100 nm ZnO exposure, the expressions of 137 genes were induced over 10 fold and 106 genes were reduced over 10 fold. The upregulated more than 2-fold in both 20 nm and 100 nm ZnO exposures were 886 genes and most of them belonged to cellular processes and signaling category and especially they were related to signal transduction mechanisms. The downregulated more than 2-fold in both 20 nm and 100 nm ZnO exposures were 1006 genes and most of them were related to signal transduction mechanisms. The functional clustering of differentially expressed genes by ZnO nanoparticle exposure showed that those nanoparticles tend to affect the transcription of genes in cellular processes and signaling group in most.

MO 171

Bioaccumulation and sublethal toxicity of 4-nonylphenol in chironomus riparius meigen 1804 (Diptera, chironomidae) larvae: assessment of mentum shape variations by means of morphometric analysis

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Nonylphenol ethoxylates are surfactants widely used to produce oil-soluble detergents and emulsifiers. In the environment these substances are transformed by microorganisms to form more toxic compounds, such as short-chain nonylphenol ethoxylates and 4-nonylphenol (4-NP), well known for their ability to mimic steroid hormones. In this work, we studied bioavailability, bioaccumulation and sublethal toxicity of 4-NP by exposing first to four instar chironomid larvae (*Chironomus riparius*) to a 4-NP-spiked sediment (0.5, 67.1 and 184.6 mg/kg d. weight). 4-NP in pore-water was monitored by Semi Permeable Membrane Device (SPMD). After 20 days exposure, larvae were removed and 4-NP concentrations in chironomid bodies were determined. On the head capsule, sublethal effects were assessed by observing and comparing four mentum shape responses: (1) rate of deformities; (2) the fluctuating asymmetry level via traditional morphometrics; (3) this same fluctuating asymmetry level via geometric morphometrics; and (4) subtle shape variations between the control group and stressed groups by means of geometric morphometrics. The bioaccumulation test revealed that the 4-NP uptake in organisms increased with increasing 4-NP concentration in pore water. Nevertheless no correlation could be drawn with either deformities or both FA indexes. Significant shape variation, corresponding to lateral tooth size increases, were detected at 184.6 mg/kg d. weight.

MO 172

Yolk proteins as biomarker for endocrine disrupters in bivalve molluscs

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Endocrine disrupting compounds (EDCs) are known to cause intersex and altered sex ratios in fish but effects of endocrine disrupters on bivalve molluscs have only been reported a few times. Vitellogenin, the precursor of yolk proteins, is a widely accepted biomarker for endocrine disrupters in fish as it is influenced by changes in circulating 17 β -oestradiol (E2) levels. Yolk-like protein levels in bivalve molluscs have so far been determined by an alkali-labile phosphate (ALP) measurement assay, which is an indirect method for detection of yolk proteins and due to the unspecific character of the assay, it has been discussed whether yolk proteins are present in the hemolymph of bivalves and if production of yolk proteins in bivalves is induced by oestrogens and EDCs. A direct method for detection of yolk-like proteins in bivalves is therefore highly needed and we started development of an ELISA (Enzyme-linked Immunosorbent Assay) for yolk protein in bivalves as we have previously developed ELISAs for yolk protein in several fish species. Yolk protein levels in different bivalve tissues were determined both by ALP and the developed ELISA and a comparison of results obtained from both methods was made. In addition, swollen river mussels (*Unio tumidus*) were exposed to E2 (57, 164 and 512 ng/L) for seven weeks during the reproductive period to investigate if intersex could be induced. Histological examination of the gonads revealed that intersex could not be induced at these E2 concentrations and the presence and quantity of yolk protein in tissue and hemolymph was investigated.

MO 173

Comparison of vas deferens and penis development between the rock shell, *Thais clavigera* (Muricidae) and the ivory shell, *Babylonia japonica* (Buccinidae)

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The characteristics of the development of male genitalia (penis and vas deferens) in imposex-exhibiting female and male rock shells, *Thais clavigera* (Muricidae), were histologically examined using specimens from wild populations and tributyltin (TBT)-exposed females in the laboratory. Vas deferens and penis development was already observed in both imposex-exhibiting female and male rock shells even at approximately 6 months old. A variety of vas deferens morphogenesis patterns were observed in wild adult female *T. clavigera*, and the characteristics were summarised. The immature vas deferens at an initial stage, however, was only observed beneath or behind the penis, and no vas deferens was observed close to the vaginal opening (i.e., vulva) of the capsule gland in TBT-exposed females, which was different from the characteristics of vas deferens formation observed in wild females. Taking into consideration both the observed results from wild female specimens and from TBT-exposed females in the laboratory, the vas deferens sequence (VDS) index for *T. clavigera* was proposed as VDS 1-6. Meanwhile, we also histologically examined development of genitalia in the ivory shell, *Babylonia japonica* (Buccinidae), using 2-year-old shells from wild populations and laboratory-reared juveniles for 0-20 months of age. Differentiation of gonad (i.e., testis and/or ovary) was unclear before 14 months of age, and progressed after 16 months of age. Immature vas deferens, however, was observed in males at 14 months of age, although no penis was observed in them. Formed penis was recognized in almost all males at 16 months of age, although vas deferens was not yet completed. Vagina, bursa copulatrix and capsule gland were developing in 14-month-old females. Albumen gland and receptaculum seminis were also developing in 16-month-old females. Differentiation and development of gonad did not precede the development of genitalia in the ivory shell, suggesting that regulatory mechanism of reproduction might be different between molluscs (prosobranch gastropods) and vertebrates. Moreover, there might be a considerable degree of difference on vas deferens and penis development

between the rock shell, *T. flavigera* (Muricidae) and the ivory shell, *B. japonica* (Buccinidae), namely, even among prosobranch gastropod species.

MO 174

Impact of Roundup® and 17 α -Ethinilestradiol exposure on the liver morphology of bullfrog's tadpoles (*Lithobates catesbeianus*)

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Ecothermic vertebrates have melanocytes on several organs (lung, heart, thymus, and gonads) and tissues (meninges and connective tissues surrounding blood vessels), constituting an extracutaneous pigmentary system. Our results showed that both Roundup® and 17 α -Ethinilestradiol provoked an increase in the liver pigmentation due to the patent increase of melanomacrophages and MMG when compared to their respective controls. The melanomacrophages are pigmented cells with macrophagic activity. They are found in hematopoietic organs and even in the liver of amphibians and have different types of granules in the cytoplasm, besides melanin, that suggest the presence of chemically distinct substances. The functional role of these visceral melanocytes is unknown, but studies in low vertebrates like fishes and also in frogs showed that the melanomacrophages are evolved in inflammatory combat against bacterial and all kind of toxic substance. These cells are present in the liver of the adult amphibians under normal conditions, but present a considerable increase under toxic conditions. This process is so drastic that the change on liver pigmentation due these cells is the first step to analyze the liver response against inflammation. They do not only increase in number, but also form melanomacrophages aggregates (MMG). MMG are supposed to be a natural immunity and physiological response of low vertebrates against several toxic substances, bacterial infection and other antigens. Studies dealing with the effect of the cadmium in adults of *Rana ridibunda* showed the same results obtained here [3]. We also observed invasion of abundant immunity cells among the hepatocytes in exposed tadpoles. According to the authors, the melanomacrophages may act as cell storage of toxic substances. The macrophages are able not only to accumulate metals and philological active molecules, but also are antigen-presenting cells that play a key-role in immunity system of vertebrates. As the mammalian macrophages, the frog melanomacrophages also accumulate hemosiderin by destruction of senescent erythrocytes. In this manner, the increase of the melanomacrophages and MMG in bullfrog tadpole exposed to Roundup® and 17 α -Ethinilestradiol in the livers demonstrate a response to an inflammatory condition, which may jeopardize the development of the individual if in natural conditions the exposure to these xenobiotics are constant and maintained.

MO 175

Validation of the amphibian metamorphosis assay for potential endocrine disrupting chemicals with *Xenopus laevis*

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The new European Union Plant Protection Products Regulation (PPPR 1107/2009) identifies the need to consider whether a substance is a potential endocrine disrupter in aquatic non-target organisms and the current draft of the PPP data requirements refers to three screening assays for ecotoxicological endocrine-disrupting potential. Of these, we describe in detail our experience in the establishment and validation of the amphibian metamorphosis assay (OECD 231) with the African Clawed Frog. In this method, in order to satisfy validity criteria in the rearing phase, conditions necessary to allow tadpoles to develop from fertilisation to development stage 51 as defined by Nieuwkoop and Faber (1994) were established, and individuals selected for the exposure phase and transferred to test vessels. To establish the assay, 400 tadpoles were exposed to a range of levels of the three reference substances, thyroxine (T4) which produces stimulatory effects on the normal function of the hypothalamic-pituitary-thyroid (HPT) axis, sodium perchlorate (which retards development) and iopanoic acid (which affects hind limb development) and levels of each were verified using an appropriate analytical method. At Day 7, 80 randomly selected individuals at each exposure level were removed and assessed (body weight, developmental stage, hind limb and snout to vent length). Exposure continued for a further two weeks and the study terminated on Day 21 when all the remaining individuals were assessed as on Day 7. Following developmental stage matching, 80 individuals were selected for thyroid removal and histopathological analysis.

We found that our results were similar to the ring test results published by the OECD (Series on Testing and Assessment Document Number 77) and make a number of observations on methodology that may improve the reproducibility of these assays.

MO 176

Progestins - potent endocrine disrupters of the female reproductive system

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Progesterone signalling has received very little attention as a potential target for disruption although it is a key regulatory pathway in the development and function of the female reproductive organs. Recent research has shown that progestins (synthetic progesterone) in the environment might pose a threat to egg laying in wild fish. Progestins are extensively used in contraceptives and in other hormonal therapies in human and veterinary medicine. More information on the targets and effects of progestins in adult and early life stages is needed to assess the environmental risks of this type of compounds. The main objective of this study was to determine developmental and reproductive toxicity of a common environmental progestin, levonorgestrel. Using the amphibian model *Xenopus tropicalis* (African clawed frog), effects of levonorgestrel on gonadal differentiation, reproductive organ development, oogenesis (egg development), spermatogenesis and fertility were characterized following developmental (larval) or adult exposure via the surrounding water. The test concentrations ranged from low environmentally relevant at 1 ng/L up to 1240 ng/L. Developmental levonorgestrel exposure inhibited oogenesis and caused a complete lack of developed oviducts which was visible in adult females. As a consequence, the females were sterile. No developmental effect was visible at metamorphosis, directly after the levonorgestrel exposure had been discontinued, implying that a full life-cycle study seems necessary to reveal the consequences of developmental exposure to this type of compounds. Also adult exposure to levonorgestrel inhibited oogenesis, implying impaired fertility. The lowest tested concentration, 1.3 ng/L, increased the proportions of previtellogenic oocytes and reduced the proportions of vitellogenic oocytes in the ovary, indicating inhibited vitellogenesis. No developmental or reproductive effects of levonorgestrel were seen in the males. In conclusion, 1) the female reproductive system was more susceptible to progestin toxicity than the male system, 2) several stages of egg development (germ cell progression into meiosis and vitellogenesis) as well as the differentiation of the Müllerian ducts (precursors of oviducts) are sensitive targets for progestagenic endocrine disrupters.

MO 177

Biotransformation of PBDE-47 by human liver microsomes and Cytochrome P450s and formation of potentially toxic metabolites

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During the past decade both animal and human studies have supported an association between polybrominated diphenyl ether (PBDE) flame retardants and neurobehavioral / neurodevelopmental disorders, particularly following in utero and postnatal exposure. Evidence is also growing suggesting that bioactivation by oxidative metabolism adds considerably to the neurotoxic potential of PBDEs. Thus, there is a critical need to further our understanding of PBDE metabolism in humans. This study conducted a qualitative and quantitative characterization of the in vitro metabolism of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47), the most abundant congener retained in humans, using recombinant human cytochrome P-450s (CYPs) (20 μ M BDE-47) and pooled human liver microsomes (0.1 to 10 μ M BDE-47). Of the 11 human CYPs that were screened, CYP2B6 was the predominant CYP capable of forming six different mono-hydroxylated-BDEs (OH-BDEs), s, including 3-OH-BDE-47, 5-OH-BDE-47, 6-OH-BDE-47, 2'-OH-BDE-66, 4-OH-BDE-42, and 4'-OH-BDE-49. With the exception of 2'-OH-BDE-66, all of these metabolites have also been detected in human blood samples, supporting the similar in vivo biotransformation of BDE-47. Moreover, the analysis by full scan GC/MS of the metabolites of BDE-47 formed by CYP2B6 showed the formation of di-OH-BDE-47 and di-OH-dioxin, a novel metabolite. Kinetic studies of BDE-47 metabolism by CYP2B6 and pooled human liver microsomes found K_m values ranging from 1-2 μ M, indicating the high affinity of CYP2B6 for the formation of hydroxylated metabolites. The prominent role of CYP2B6 in the metabolism of BDE-47 to potentially toxic metabolites will better inform future mechanistic and epidemiological studies investigating the potential of BDE-47 and its' metabolites to produce neurobehavioral / neurodevelopmental disorders.

MO 178

Affect of EDCs on fish communities in the Levuvhu River, Limpopo Province, South Africa

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Contaminants present within aquatic ecosystems worldwide are the result of agricultural applications, urban development and industrial effluents. Current contamination by DDT in the Luvuvhu River, Limpopo Province, South Africa is primarily due to IRS (indoor residual spraying) for mosquito vector control of the malarial epidemic. DDT and its resulting metabolites DDE and DDD may continue to persist in various environmental phases, long after spraying. The characteristically high lipophilicity, chemical stability and very slow biodegradation result in a high uptake or bioaccumulation. Despite DDT spraying for many years, limited data on these concentrations in aquatic ecosystems exists. Fish community structures are widely used to assess the effect of human impacts on aquatic ecosystems e.g. water quality, habitat deterioration and organism health. A study was therefore done to assess the effect of DDT, its metabolites and other pollutants in the water, sediment and biota in the aquatic ecosystem of the Luvuvhu River catchment.

Values for physico-chemical variables were measured to determine water and sediment quality. Available historical water quality data were obtained. Pesticides extracted from water, sediment, and fish tissue were quantified on a gas chromatography mass spectrophotometer (GC-MS). Electronarcosis and seine nets were used for fish sampling from different river habitats. Frequency of occurrence and relative abundance of fish, and non-parametric diversity indices were determined. Relative abundance for fish communities were described employing two statistical models. Selected biomarkers were used to determine sub-cellular effects of EDCs in field sampled fish. Extensive abstraction of water occurs for irrigation, afforestation, community development and extensive rural activities in the in stream and riparian habitat. These activities yielded impacts such as bank erosion, flow modification, water quality changes, bed modification and some solid waste disposal. Pesticide levels were only above existing guidelines at specific sampling sites. Biomarkers showed effects of the contaminants on sub-cellular to organ levels with no effects on organism survival. No changes in fish community structures occurred while exposed to DDT and other contaminants measured in the study. The exposure duration and/or concentrations of pollutants were probably too low to induce any effects. Ecosystem services are therefore not negatively impacted.

MO 179

Distribution of endocrine disrupting pollutants in the Long Island Sound

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Waste water treatment facilities (WWTFs) input fresh water, nutrients, and contaminants to the Long Island Sound (LIS). While nutrient loads and concentrations for some pollutants are regulated, there are many emerging contaminants that are not controlled as the full range of toxic effects are still to be determined, and the regulatory framework controlling their usage and allowable inputs into coastal waters has yet to be established. The water and sediment of the LIS are routinely sampled for concentration data of classical persistent organic pollutants, however there are numerous emerging contaminants, with known endocrine disrupting and bioaccumulating properties, that have yet to be detected in this economically important region. It is critically important to investigate the inputs of emerging contaminant throughout the LIS, in order to determine the potential impacts on this estuarine ecosystem. Eight waste water treatment facilities (WWTF) which discharge directly into the LIS were tested for several classes of known endocrine disrupting compounds (EDCs); perfluorinated compounds (PFCs), phthalates esters (PAEs), phenolic compounds (nonylphenol, octylphenol and bisphenol A) and steroidal estrogens. These EDCs have been detected in many water bodies and sediments, yet partitioning data between these compartments are limited. WWTFs are a known point source of these pollutants; research indicates that biodegradation during secondary treatment can increase concentrations of PFCs in wastewater effluent. The distribution of several of the target compounds between the suspended particulate and dissolved phases in the effluent waters was determined to be a function of the organic carbon content of both phases; organic carbon normalized data indicate that partitioning to dissolve organic matter (DOM) is important as a third phase for several target pollutants. The fate and distribution of target compounds entering the saline estuary from the effluent was further investigated; an important factor in distribution of these target compounds is the scavenging by particulates which increases as a function of ionic strength. The water-sediment distributions of target EDCs in different salinity and sediment quality regions of the LIS are presented. These results are an important first step in the investigation into the role of wastewater organic matter in the transport and distribution of target EDCs in an estuary.

MO 180

Assessment of intersex severity in grey mullets from three Portuguese estuaries - preliminary data

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All over the world, plentiful chemicals reach continuously the environment, and some of them are endocrine disrupters compounds (CDEs) which can cause diverse harmful effects in wild fish (also under low concentration) including intersex condition, namely ootestis (oocytes in the testis), but can also affect human health.

The presence of intersex condition in ichthyofauna has been reported in numerous countries, including (scarce information) in Portugal, but no data was published based on a large sampling and covering several estuaries, and usually no ranking system for severity assessment is done. For this aim, we have been studying adult grey mullets (*Mugil cephalus*) caught in the estuaries of the rivers Mondego (2006), Douro (2007) and Ave (2008). Collections took place twice a year, aiming 50 animals per survey, in spring/summer (SS) and autumn/winter (AW).

So far, testis histopathology showed intersex condition (ootestis) at the following rates (SS vs AW): 12 % vs 8 % in Mondego; 30 % vs 8 % in Douro, and 13 % vs 39 % in Ave. Despite the presence of ootestis in all estuaries, the density and oocyte development stage in testis varies greatly.

We used the Ootestis Severity Index (OSI) in this work (proposed by Bateman et al. 2004). OSI data obtain are (SS vs AW): 0,6 and 0,3 in Mondego mullets, 6,1 and 2,8 in Douro mullets, and 12,0 and 5,8 in Ave mullets. The OSI was low in Mondego fish, which agree with the less contaminated estuary studied (our reference estuary). In Douro, the fish present higher OSI, but Ave mullet show the highest value. No seasonal differences were detected in OSI values.

In some cases we observed also considerable differences in histopathology of the gonads (fibrosis, melanomacrophage centers, interstitial inflammation, as well as unspecific and parasitic granulomas). The estuaries presenting higher rate of OSI, correspond to the higher pollution levels, and show also higher rate of gonad lesions. These data are in correspondence with our published chemical data, as both Mondego and Douro have estrogens in the water (with a lesser load in Mondego). This work corroborate that mullet are waterborne exposed to estrogenic compounds and OSI should be used in this kind of studies.

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MO 181

Effects of antiandrogenic compounds on roach (*Rutilus rutilus*) steroidogenesis : an in-situ study based on environmental occurrence and relevant gene expression analysis in gonads

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Wildlife is exposed to an increasing pollution pressure which can disrupt homeostasis and ultimately fitness of the more sensitive species and may thus lead to biodiversity erosion. For more than a decade, efforts were made by the scientific community, to assess and understand the impact of endocrine disruptors, especially estrogenic compounds, on fish breeding cycle. Feminization and demasculinization of fish were demonstrated in the field and correlated not only to estrogenic contaminants but also to antiandrogenic compounds. Although less characterized, these latter might be of importance. They can interfere with the androgen pathways at the receptor level and/or by modulating transcriptional or enzymatic activities related to steroidogenesis. In this study, Roach was chosen with regard to its large repartition in freshwater systems and to its sensitivity to endocrine disruption. Four different sites of the Seine river catchment basin in the Seine presenting an overall contamination gradient were studied. Sediments and fish bile were sampled in order to determine the occurrence of androgen antagonists in the environment and in the exposed animals using the anti-yeast androgen screen (anti-YAS) assay. Liver and gonad of sexually mature individuals were dissected to carry out transcriptional analysis of key genes involved in steroidogenesis. This includes genes encoding for the steroidogenic acute regulatory protein (StAR) responsible of the import of cholesterol in mitochondria that represents the rate limiting step of steroidogenesis; the mitochondrial CYP 11A1 that converts cholesterol to pregnenolone; the CYP 17A1 (17 α -hydroxylase/17,20-lyase) that catalyzes oxidation and cleavage of intermediary steroids; the CYP 19A1, so-called aromatase that converts testosterone in estradiol 17- β . Furthermore, transcriptional level of a CYP 3A isoform catalyzing sexual steroid inactivation was assessed in order to appreciate whether environmental pollutants disrupt the biotransformation balance of endogenous steroids. To our knowledge, this is the first in situ study that underlines in the same time the occurrence of androgen receptor antagonists in sediments and antiandrogenic effects on gonadal mRNA levels.

MO 182

Analysis and occurrence of endocrine disruptors and related compounds in surface waters and sediments in rivers of the Iberian Peninsula

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The Mediterranean basin is one of the most vulnerable regions of the world to the global change due their climatic conditions characterized by summer drought and their characteristic topographical regions. Moreover, the high activity close to the Mediterranean rivers and the high population density in these zones, are the principals arguments to evaluate the water quality of these rivers.

The objective of this work was the determination of endocrine disruptors and related compounds belonging to different groups of chemical substances (10 estrogens, natural and synthetic, in free and conjugated form, 8 alkylphenols, 4 antiseptics, 2 antibacterials, 3 flame retardants, 2 anticorrosives and BPA) in four representative watersheds in Spain: Llobregat, Ebro, Júcar, and Guadalquivir.

In this study, a multiresidue analytical method for the analysis of water samples was applied, using the Thermo Scientific Equan LC-MS system, an automated online preconcentration method. For the analysis of sediment samples a pressurized liquid extraction (PLE) was carried out using a fully automated ASE 200 system (Dionex, Sunnyvale, CA, USA), the solvent extraction was methanol:acetone (1:1) mixture, for clean-up a solid phase extraction (SPE) was performed. A Thermo Scientific TSQ Vantage triple quadrupole mass spectrometer with an ESI source was used for the MS analysis.

The results show widespread occurrence of target compounds, although the level of concentrations of different compounds detected varied considerably depending on the sampling point. For example, in the case of surface waters the 3 chemical groups with higher contribution in terms of concentrations were alkylphenolic, corrosives and organophosphorous flame retardants with levels between ng/L to μ g/L. The natural and synthetic estrogens in free and conjugated forms were found in some points in levels lower than 10 ng/L.

In order to estimate the estrogenicity of samples, the EEQ were calculated using relative estrogenic potency (relative to 17 β -estradiol). As it was expected, the results showed that estrogens, especially estradiol, were the largest contributors to the estrogenic potential with values between nd-4.60 ng/L. However the contribution of other compounds such as alkylphenols, BPA and anticorrosives to the total estrogenicity was low and ranged between nd-0.09 ng/L.

MO 183

Specific accumulation of polychlorinated biphenyls and brominated flame retardants in human breast milk and scalp hair from the Philippines: levels, distribution and profiles

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Due to the lack of adequate treatment facilities, large amount of municipal wastes are directly dumped at dumping sites in the Philippines without proper management, probably causing several adverse environmental consequences and increased human health risk to local communities. Overall, the data on human exposure to organohalogen compounds in the Philippines is rather scarce and comprehensive studies have not been made. The present study was therefore carried out to determine the concentrations of three group of persistent organohalogen compounds such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) in human breast milk and scalp hair samples from mothers living in two locations in the Philippines, viz., Payatas, a waste dumpsite, and Malate, a non-dumpsite. In addition, the present study examined relationships between contaminant levels in milk/hair samples. Human breast milk and scalp hair samples (n=30) from primipara and multipara mothers living in two locations in the Philippines were obtained in 2008 and analyzed for three groups of organohalogen compounds (PCBs, PBDEs and HBCDs). PCBs were

the predominantly identified compounds in all the breast milk samples. The highest concentrations of PCBs in breast milk (115 ng/g lipid wt., sum of 12 congeners) were found in a sample from a 28-year old woman, living in the periphery of the dumpsite (Payatas) and nursing for the third time. Levels of PBDEs in human breast milk samples from the Philippines were similar to Europe and other Asian countries. Among PBDEs, BDE-47, -99, -100 and -153 were the major congeners. For HBCDs, -isomer was the predominant followed by - isomer in both locations. PBDE concentrations (including BDE -209) were significantly higher than those of PCBs and HBCDs in all the scalp hair samples. On a congener basis, the levels of PBDEs found in scalp hair were higher than those in Spain (children and adults) and China (e-waste workers). PBDE congeners in scalp hair were dominated by those found in Deca- and Penta-BDE mixtures. To our knowledge, this is the first comprehensive research on HBCDs in human scalp hair from the world. Human hair as a biological measure of exposure to persistent organic pollutants has some advantages over the more commonly used blood, milk and adipose tissue samples.

MO 184

Alkylphenolic substances, steroid estrogens and bisphenol A in water, sediment and fish from Dan-Shui River, Taiwan

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This study investigated the distribution of feminizing chemicals including 4-*tert*-octylphenol (OP), 4-nonylphenol (NP), nonylphenol monoethoxycarboxylate (NP,EC), nonylphenol monoethoxylate (NP,EO), nonylphenol diethoxylate (NP,EO), bisphenol A (BPA), estrone, 17 β -estradiol, estrinol and 17 α -ethinyl estradiol in river water, sediment and fish in the Dan-Shui River, Taipei, Taiwan. Sampling was conducted every one and a half months in nine months. The concentrations in fish organs were also determined to estimate the organ distribution and the bioaccumulation of these chemicals. Water, sediment and fish (*Oreochromis mossambicus* and *Oreochromis niloticus*) were taken from the Dan-Shui River. Water was pretreated with automated solid-phase extraction. Sediment and biota were extracted with matrix solid-phase dispersion. The quantification was done with ultra-high performance liquid chromatography/tandem mass spectrometry and isotope dilution techniques.

Alkylphenolic substances and BPA were detected in all of the water samples. BPA was the most abundant analyte in river water (547 $\pm$ 602 ng/L). In the tributary of the Dan-Shui River, the concentrations were the highest near a wastewater treatment plant (WWTP). The average concentration of BPA in the main stream of the Dan-Shui River (1,169 $\pm$ 906 ng/L) was four times higher than that in the tributary (313 $\pm$ 144 ng/L). The spatial variation of concentrations in water were associated with the emission from point sources. NP was the most abundant analyte in sediment (1,131 $\pm$ 839 ng/g w.w.) and in fish muscle (245 $\pm$ 167 ng/g w.w.). Only few ng/g w.w. of steroid estrogens were detected. Unlike the feminizing chemicals in water, the higher concentrations in the sediment were detected at the downstream of a WWTP.

Although BPA is easy to degrade, up to hundreds of ng/g w.w. were detected in fish muscle. Concentrations of feminizing chemicals in fish organs were found to be higher than that in muscle. NP was abundant in liver (3,036 $\pm$ 3,115 ng/g w.w.), gonad (9,597 $\pm$ 10,536 ng/g w.w.) and eggs (6,376 $\pm$ 5,142 ng/g w.w.). The bioconcentration factors (BCFs) of NP were also the highest among these chemicals (1.1 L/g in muscle, 15.8 L/g in liver and 45.8 L/g in gonad). There might be bioaccumulation of alkylphenolic substances in liver and gonad. Skewed sex ratio (female: male = 1.7) could relate to high concentrations in the organs.

MO 185

Determining outliers in fathead minnow (*Pimephales promelas*) plasma vitellogenin concentration and their correlation to other assay endpoints

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Plasma vitellogenin concentration is an important biomarker and is a data endpoint collected in the OECD 229, 230 and OPPTS 890.1350 test guidelines used to screen chemicals for potential endocrine activity in sexually mature male and spawning female fathead minnows (*Pimephales promelas*). Plasma vitellogenin concentration is also an important endpoint in the OECD 234 test guideline in maturing freshwater fish. Due to the inherent variability observed within a typical population of fish, examining historical control data and determining outliers is imperative. The objective of this presentation is to outline historical control ranges of vitellogenin concentrations in plasma and to provide recommendations for considering a sample result to be an outlier. The outlier samples from female fish are typically characterized by low vitellogenin recoveries while the outlier samples from male fish are typically uncharacteristically high vitellogenin recoveries. Samples that do not fall within the typical ranges are most often extreme outliers, often observed to be orders of magnitude different from the expected ranges found in historical control data. Inclusion of these outliers in statistical analysis can skew means and decrease sensitivity of the analysis causing some empirical or statistically significant differences to either be magnified or masked. Exploration of vitellogenin results and correlation to effects observed in the remaining endpoints (i.e. gonad histopathology, percent gonadosomatic index, percent of viable eggs, fecundity, etc.) is also considered when evaluating possible outliers. This correlation is important in deciding if plasma vitellogenin concentration is a stand-alone endpoint where an outlier can be individually excluded or if an outlier should be removed from other endpoints as well. Based on the historical control data base and the procedure for determining outliers within a vitellogenin dataset, there is no indication that the other endpoints are affected in either female or male fish and therefore would not warrant exclusion from further analysis.

MO 186

Application of ultra performance liquid chromatography-tandem mass spectrometry for the determination of steroid estrogens and non-ionic surfactants in wastewaters

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Separate ultra-performance liquid chromatography methods using a triple quadrupole mass spectrometer was developed and validated for the determination of steroid estrogens and alkylphenol ethoxylates in wastewater matrices. To date, analytical methods established in the literature for 17 α -ethinylestradiol have been unable to achieve the respective proposed predicted no effect concentration of 0.1 ng l⁻¹. The estrogen analytical method enables 17 α -ethinylestradiol to be determined below the PNEC with a method detection limit of 0.06 ng l⁻¹ which has been validated in real environmental matrices. During the validation process, a trickling filter wastewater treatment works was monitored to demonstrate the methods application. Estrogen removal across the filters demonstrated good removals of natural free estrogens (≥ 62.0 %) with lower removals of synthetic estrogen 17 α -ethinylestradiol (29.2 %) from wastewaters at 10 °C. The methods application illustrates the proposed methods capability to detect estrogens below PNEC values in real samples. Furthermore, a complete process mass balance for 17 α -ethinylestradiol is now attainable which has previously posed a challenge due to the low environmental concentrations typically exhibited, but more significantly as a result of the lower sensitivity inherent in previously reported analytical methods. The ultra performance liquid chromatography method developed for alkylphenol polyethoxylates enables these compounds to be determined in the ng l⁻¹ range which is crucial due to the reduced concentrations in crude sewage experienced over recent years.

MO 187

Occurrence and behavior of Dechlorane plus, Polybromodiphenyl ethers and Emerging BFRs in raptor eggs from Doñana National Park (south-western Spain)

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Dechloranes 602 (Dec 602), 603 (Dec 603) and 604 (Dec 604), syn- and anti-Dechlorane Plus (sDP and aDP), are halogenated flame retardants introduced as replacements of Mirex. Presence of these emerging pollutants has been observed in sediments and biological matrices such as fish and eggs, showing their bioaccumulation potential. Recent studies report the effects of syn- and anti-DP as endocrine disruptors. Dec 602, Dec 603, and Dec 604 are supposed to have similar toxicological properties than DP. The knowledge about the environmental occurrence, fate and behavior of dechloranes and related compounds is still limited. Moreover, most of the reported levels are near production facilities (China and USA), where the concentration levels of these contaminants is expected to be higher than in other places of the world. On the other hand, polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants for many years and concentration levels have been severally reported. Along with other harmful effects, PBDEs are endocrine and thyroid disruptors. Thus, production and use of commercial PBDE mixtures are banned in Europe. However, alternative non-BDE BFRs such as pentabromoethylbenzene (PBEB), hexabromobenzene (hexaBBz) and decabromodiphenylethane (deBDethane) are being developed and used.

The purpose of this study was to determine the occurrence of Dechloranes, PBDEs and emerging BFRs in different raptor eggs. Eggs of white storks (*Ciconia ciconia*) and black kites (*Milvus migrans*) were collected during the breeding season of 2011. Additionally, samples of sediments, mullets (*Mugil cephalus*), carps (*Cyprinus carpio*), barbel (*Luciobarbus sclateri*) and red swamp crawfishes (*Procambarus clarkii*) were also collected for bioaccumulation and bioconcentration studies. Sample preparation methods were developed and optimized for all the flame retardants included in the study, and for all the selected matrices. Instrumental determination was carried out by GC-NCI-MS for PBDEs and emerging BFRs, whereas Dechloranes were analyzed by GC-NCI-MS-MS method.

Both PBDEs and dechloranes (DP isomers, Dec 602 and Dec 603) were detected in egg samples, as well as in sediments and biota samples collected along the Doñana National Park. This is the first time that these emerging contaminant levels are reported in food webs from Spain. The study of Fanti ratio showed a decrease in the biota samples, suggesting that syn-DP has more bioaccumulation capacity than the anti-DP.

MO 188

Hexabromocyclododecane (HBCDD) a flame retardant with POP-properties used in polystyrene insulation products

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Hexabromocyclododecane (HBCDD or HBCD) is an emerging chemical used mainly as a flame retardant in polystyrene-based building insulation products.

There are 64 theoretical possible isomers, of which only 16 exist and 3 dominate. Commercial products contain mainly the -isomer (75-89%) and less of the - (10-13%) and -isomers (1-12%).

Of particular concern is the capacity of this lipophilic and persistent organic pollutant to accumulate in organisms and magnify in the food chain, leading to progressively increasing background levels in human tissues - including mothers' milk - and in wildlife, especially of the most stable -isomer. The extent of this accumulation correlates directly with its ever-more prevalent use.

Limited toxicological information is available to assess the long-term implications for health or the environment of the HBCDD contamination but HBCDD is an endocrine

disruptor in animals.

In 2008 The European Chemicals Agency identified HBCDD as 1 of 14 substances of “Very High Concern”, and in September 2010 HBCDD was added to REACH's Authorization List. In February 2011 HBCDD was selected to be phased out by EU REACH Regulation before 2015, if authorization is not granted.

In October 2010 and October 2011 POP Review Committee under the Stockholm Convention has assessed the risks from HBCDD, and it was concluded that HBCDD fulfils the criteria of a persistent organic pollutant (POP).

This presentation will provide a succinct up-to date overview of HBCDD's properties, uses, and regulation and discusses the risks associated with its prevalence in buildings, our homes and immediate environment.

MO 189

Statistical models for sex ratio data

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Studies following OECD Fish Sexual Development Test (FSDT) Test Guideline 234 call for 50% male and 50% female fish during the sex labile period to be placed in each replicate tank in the control and test concentrations at the start of the experiment, with the primary goal to determine whether the test substance causes sex reversal. The observed percent male has its maximum variance when the true percent is 50%. Thus, all analyses of sex-ratio must deal with high variability. This is quite different from typical survival analysis, where control survival is close to 100%.

This variability affects both ECx and NOEC analyses. For NOECs, there is reduced power to detect an effect. With regression, there are increased model uncertainty and width of confidence intervals for ECx. One idea to reduce variability is to “normalize” sex-ratio by dividing each replicate response by the control mean response. Normalized responses may still be highly variable, since they are ratios of two random variables. Also, such data are no longer independent, since they all have the same random variable denominator, thereby violating a basic requirement of regression and hypothesis testing models. Mathematical details show that the correlations among the normalized responses are not trivial and cannot be ignored.

The quality of regression models and resulting ECx estimates based on non-normalized responses were investigated using data from numerous studies done following TG234. Numerous well-accepted regression models for toxicity data were fit to the data, goodness-of-fit was assessed, and confidence intervals for ECx estimates for x=5, 10, 20, and 50 were obtained. Several hypothesis tests were used to obtain NOECs. These methods were compared to one another and to the regression results. Both regression and hypothesis test results were supplemented by computer simulations that showed the conclusions are true in general.

The results show that for fish sex-ratio data based on phenotypic sex, regression models often fail to provide meaningful estimates of ECx for x≤20 and sometimes for larger values of x. This is true regardless of any practical experimental design for the FSDT. The step-down Jonckheere-Terpstra test usually provides meaningful NOEC values that are useful for risk assessment. When genetic sex is known, so true sexual reversal can be measured, the situation is quite different and regression models are very useful.

MO 190

Progress of assessment under the Japanese program on endocrine disruption: EXTEND2010

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Ministry of the Environment, Japan implemented their third research program on endocrine disruption titled “EXTEND2010” (EXTEND: Extended Tasks on Endocrine Disruption) in July 2010. Target of the new five-year program is to accelerate hazard assessment on endocrine disruption of chemical substances, prioritizing organisms in the environment, and to consequently conduct risk assessment to see whether any regulatory risk management measures should be introduced.

Test protocols of fish, amphibian and invertebrates have been developed through bilateral or multilateral collaborations. *In vitro* assays using receptors of fish, amphibians and invertebrates are also being developed in the ministry's program. Two-tiered framework for assessing endocrine disrupting effects to organisms in the environment is being developed. This framework is designed to effectively identify potential candidates for endocrine disruptors using available information and test results. Reliability evaluation of available information that might be relevant to endocrine disruption is being conducted to select candidate chemicals subject to testing to assess their endocrine disrupting effects to aquatic organisms. The existing knowledge is re-evaluated to identify which *in vitro* assays should be conducted for prioritization to select candidate chemicals for *in vivo* tests.

After the two batches of reliability evaluation twenty-five chemicals were identified as candidates for testing. *In vitro* assays were conducted for eighteen chemicals in FY 2010 and 2011 and estrogenic and anti-estrogenic activities were detected in some of them. The way of prioritized selection of chemicals subject to tier 1 *in vivo* testing is being discussed based on the results.

Progress and updated status of the assessment of chemicals under the EXTEND2010 will be demonstrated.

MO 191

Impact of animal manure separation technologies on steroid hormone distribution - consequences for agricultural practices

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When steroid hormones are emitted into the environment, they may have harmful effects on the reproduction system of aquatic life. Until now, research has primarily focused on human excretion, demonstrating that steroid hormones reach the aquatic environment due to insufficient removal in waste water treatment processes. However more recently, it has been revealed that agricultural practices also may add to the environmental burden of steroid hormones. So far, research activities have mainly focused on steroid estrogens, but also androgens, progestagens and glucocorticoids, expressed in the vertebrate steroidogenesis, may occur at substantial levels in animal manure and should be addressed. In agricultural practices the animal manure can be applied to the soil as raw manure, but also as a solid or liquid manure fraction, since current livestock production facilities utilizes a recently developed technology, which separates raw animal manure into a solid and a liquid fraction. This technology offers an improved handling and refined distribution of the manure nutrients to the farmlands and the possibility to reduce the environmental impact of manure nutrients, especially avoiding the surplus load of phosphorous.

In the present work we investigated the distribution of 9 steroid hormones (pregnenolone, progesterone, dehydroepiandrosterone, androstenedione, testosterone, dihydrotestosterone, estrone, 17 α -estradiol and 17 β -estradiol) in raw manure and manure separates from 10 to 15 different pig farms in Denmark utilizing 4 different separation technologies. Furthermore, we investigated a possible relationship between the steroid hormone concentration and the different manure fractions and separation technologies. The chemical steroid hormone analysis was done by inverse and integrated clean-up pressurized liquid extraction, and further cleaned by a two step solid-phase extraction before derivatization and finally analyzed by GC-MS/MS.

It was found that the steroid hormones were predominant in the solid manure separate calling for manure management strategies to reduce the content of steroid hormones in separated manure solid fraction. This could potentially be achieved through composting or anaerobic digestion for biogas production of the solid fraction; however, the effects of these technologies on steroid hormones need to be verified.

MO 192

Criteria for endocrine disruptors: report from the Danish centre on Endocrine Disruptors (CEHOS)

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The aim of this session is to give a presentation of the report (both ENV and HH) on criteria carried out by the Danish Centre on Endocrine Disruptors (CEHOS) as a project contracted by the Danish Environmental Protection Agency. CEHOS is an interdisciplinary scientific network without walls and the main purpose of the Centre is to build and gather new knowledge on endocrine disruptors (EDs) with focus on information needed for the preventive work of the regulatory authorities. The aim of the report was to propose scientific criteria for the identification of ED substances of concern for human health and the environment. A number of issues relevant for the development of criteria for EDs were considered such as definition, potency, lead effects, specificity and relevance for humans and the environment.

The proposed criteria divide substances into three categories dependent on the available data: 1. Confirmed ED, 2a. Suspected ED and 2b. Indicated ED. The report describes the scientific evidence needed for fulfilling these criteria based on the OECD Conceptual Framework for endocrine testing and assessment. It considers non-test methods, test methods, epidemiology and field studies and gives examples of available ED data and relevant placement in groups. The overall purpose of the report is to provide scientific background for Danish input to the ongoing EU work within this field.

EP05P - Non-target analysis and identification of toxicologically significant emerging pollutants

MO 193

Innovative biodiagnosis meets chemical structure elucidation - New tools in effect directed analysis to support the identification and monitoring of emerging toxicants on a European scale (EDA-EMERGE)

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EDA-EMERGE aims to train a new generation of young scientists in the interdisciplinary techniques required to meet the major challenges in the monitoring, assessment and management of toxic pollution in European river basins considering the enormous complexity of contamination, effects and cause-effect relationships. By integrating innovative mode-of-action based biodiagnostic tools including in vitro tests, transgenic organisms and “omics” techniques with powerful fractionation and cutting edge analytical and computational structure elucidation tools, a new generation of effect directed analysis (EDA) approaches will be developed for the identification of toxicants in European surface and drinking waters. Innovative method development by young researchers at major universities, research centres and private companies will be closely interlinked with a joint European demonstration program and higher tier EDA and extensive training courses. EDA-EMERGE ESRs will learn to organise and run international and interdisciplinary sampling and monitoring campaigns and benefit from the expertise of one of the most experienced private companies in this field. Strong networking between academia, the private sector and leading regulators in the field of river basin management and pollution management ensures the relevance of the research for practice and excellent employment opportunities for EDA-EMERGE ESRs.

MO 194

Overview of available chemical analytical screening methods for identification of pollutants in European waters

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The need for collection and dissemination of information about the currently applied techniques for analytical screening of chemical pollutants in water has been recognized at European level. These techniques are identified as promising tools for the identification of the pollutants of concern at river basin or local level (river basin specific pollutants) under the Water Framework Directive WFD (2000/60/EC). They should allow a cost effective overview of the pollutants in the aquatic environment, in preparation of dedicated monitoring programmes.

The Italian Institute for Water Research IRSA, the EC Joint Research Centre and the NORMAN Association (Network of reference laboratories and research centres for emerging pollutants www.norman-network.net) for emerging pollutants have launched a survey for identification of currently available and used screening methods for the identification and quantification of chemical contaminants in water. A questionnaire was sent to the delegates of the chemical monitoring group under the Water Framework Directive Common Implementation Strategy (WFD - CIS) and through the NORMAN network. 20 compiled questionnaires were received. The response covered a wide geographical spread, including UK, Ireland, Portugal, Spain, France, Switzerland, Austria, Germany, The Netherlands, Denmark, Czech Republic and the Slovak Republic. The survey included questions about the scope of currently employed screening techniques, the instrumental approach and the validation scheme as well as method performance information and target compounds or compound classes. The reported methodologies include multiresidue methods with a defined target compound list, suspect screening techniques without reference standards (i.e. methods which are based on a mass spectra library with a large set of compounds or compound groups) and non-target screening approaches which include the identification of unknown compounds. A wide range and number of chemical pollutants can be covered by the use of liquid chromatography and gas chromatography coupled with mass spectrometer detectors. Methods are in most cases developed and validated in-house. Some of the methodologies are currently in use for monitoring programmes related to the implementation of the WFD. The survey shows that a common understanding of definitions and scopes is essential in exchanging information about most efficient approaches for target, suspect and non-target screening.

MO 195

Ionization pitfalls in nontarget screening by LC-high resolution mass spectrometry

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In nontarget screening approaches using high-resolution mass spectrometry, one important step is the determination of the molecular formula from accurate measured mass and isotope pattern. Similarly, suspect screening approaches derive the exact mass to be searched for from the molecular formula of the compounds of interest. In both cases the formation of protonated (positive ion mode) and deprotonated molecules (negative ion mode), is commonly anticipated when using electrospray (ESI) and atmospheric pressure chemical ionization (APCI). However, for a range of different compound classes, (de-)protonated molecules might be a minor species failing to reach selected intensity thresholds or even be completely absent. This results from (i) adduct formation with solvent or trace contaminant ions, (ii) gas-phase chemical reactions with solvents or trace contaminants, or (iii) electrochemically induced redox reactions at the electrospray needle. While available software tools can account for adducts with well-known species (Na^+ , NH_4^+ in positive ion mode; formic acid in negative ion mode) more complex substitution or redox reactions can hardly be addressed. We will present several case studies involving aromatic amines, nitroaromatic compounds or highly chlorinated weak gas-phase acids or bases. We will discuss approaches to allow for a correct assignment of their molecular formula in nontarget screening approaches and how the ions which are likely formed can be derived for different compound classes in suspect screening approaches.

MO 196

RISK-IDENT: assessment of previously unknown anthropogenic trace contaminants

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For the purposes of precautionary environmental protection, the project “RISK-IDENT” develops and applies an identification system for so far unknown anthropogenic trace contaminants in the aquatic system. The degradation of selected trace compounds is tested in lab-scale sewage treatment plants. Column and lysimeter studies are used to characterise mobility. Acute and chronic effect tests will contribute to the risk assessment process. Moreover an innovative elimination method as an additional sewage treatment step based on hydroxyl radicals will be proved.

The starting point of the project is the development of a database for relevant aquatic contaminants which allows an attribution to analytical data from the analysis of real water samples. This is done by

- Acquisition of substance data on the basis of REACH dossiers with particular reference to molecule-specific information (exact mass, log Pow, etc.) of these substances and their likely degradation products
- Observation and application of normalised retention time factors reflecting the molecular hydrophobicity as identification criterion and allowing independent interlaboratory HPLC methods
- Application of three different LC-MS and LC-MS/MS techniques using high-accuracy mass spectrometry for detection of contaminants via the exact mass and multiple-reaction-monitoring (MRM) for target- and nontarget-screening strategies with low- and high resolution tandem mass spectrometers.
- Optimisation of an procedure for identifying previously unknown trace compounds through the computer-aided comparison of analytical features with molecule-specific properties of potential water contaminants.

The poster presents the recently launched project “RISK-IDENT” and illustrates first results of the interlaboratory determination of normalised retention time factors and mass spectrometric information on the way to an independent data base applicable for all laboratories working on the water sector.

“RISK-IDENT” is supported by the German Federal Ministry of Education and Research (FKZ: 02WRS1273A).

MO 197

Detection and fate of Ionophores in the environment

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Anticoccidial agents or coccidiostats are the only anti-bacterial substances still authorised as feed additives within the European Union¹. Anticoccidial agents are used for the prevention and treatment of the disease coccidiosis, which is caused by a unicellular intestinal parasite. Coccidiosis is a major serious disease in poultry as well as in many other hosts.

Ionophores are the most heavily applied sub-group of the two sub-groups of anticoccidial agents, because they also have antibacterial properties. After the ban of antibiotic growth promoters ionophores are used extensively worldwide as prophylactic chemotherapeutics and growth promoters in livestock production. As an example, the yearly consumptions of active compounds are more than 10 tonnes in Denmark and for the Republic of Korea more than 800 tonnes²⁻³. In long term, this may cause problems with resistance development in the treatment of coccidiosis.

Several reports have revealed that ionophores are emerging environmental contaminants in agricultural run-off waters, surface waters, sediments, and ground waters, due to their continuously increased and constant application as feed additives in modern livestock production^{2,4-5}. Recent investigations have further reported that transformation

products of certain veterinary drugs such as antibacterial agents (i.e. tetracyclines) possess environmental effects on the soil-bacterial community at similar level as their parent compounds⁶. This has previously also been observed for antibacterial agents⁷.

The focus of this study is on general analytical methods for detection of ionophores and unknown transformation products in various matrices. The hyphenated method consists of an integrated clean-up with solid phase extraction followed by high-performance liquid chromatography tandem mass spectrometry (SPE-LC-MS-MS).

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MO 198

Prioritization of emerging pollutants on the basis of chemical structure

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The prioritization of hazardous chemicals is a useful procedure for the identification of critical substances and the optimization of experiments. This procedure became of particular relevance within the EU-REACH regulation, which encourages the minimization of animal testing also by the use of alternative in vitro and in silico methods.

Among these methods quantitative structure-activity relationships (QSARs) can predict missing data for the unknown activities and properties necessary to prioritize existing or not yet synthesized chemicals. The prioritization of four classes of emerging pollutants (brominated flame retardants, fragrances, perfluorinated compounds and (benzo)triazoles) is one of the topics of the FP7 European project CADASTER (Case studies on the Development and Application of in-Silico Techniques for Environmental hazard and Risk assessment). The final goal of the project is to exemplify the integration of information, models and strategies for carrying out hazard and risk assessments for large numbers of substances, organized in the four representative chemical classes.

The prioritization applied to CADASTER chemicals was crucial to focus the experimental design on critical substances on the basis of their chemical structure and potential ecotoxicological hazard.

The aim of this poster is to summarize the prioritizations performed within CADASTER project, also by applying “*ad hoc*” QSAR/QSPR models developed so far (WP3) for the four classes of compounds under investigation. Different prioritization procedures were applied to over 1000 chemicals by combining, through different approaches (similarity analysis, multivariate ranking methods, factorial design), the structural information, encoded in theoretical molecular descriptors, and the data (experimental or predicted) available for different toxicological and ecotoxicological endpoints. Chemicals belonging to the ECHA pre-registration list were also studied in the prioritizations. Priority compounds were suggested for focusing the experiments executed by other CADASTER partners (WP2).

MO 199

Studying nitrophenols as abiotic transformation products of phenolic microcontaminants in wastewater

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For many emerging contaminants in wastewater, biological treatment is incomplete. Rather than removal by mineralization, Sewage Treatment Plant (STP) processes lead to the formation of transformation products through the interaction of these compounds with the organisms in the sludge.¹ Recent publications have classified certain transformations as ‘biological’, i.e. involving direct interaction with living organisms, and others as ‘abiotic,’ implying they take place due to reactions with chemical species present in the milieu.^{2,3}

One form of abiotic transformation previously observed in STPs is the nitration of phenolic contaminants, forming their corresponding nitrophenol derivatives.^{2,3} Nitrophenols have toxic properties.⁴ We are investigating the nitration of phenols further to understand both the mechanism and its occurrence in STPs. Initially, we postulate that the nitration is due to the known reaction of a phenol with nitrogen dioxide (-NO₂), through a radical mechanism described by Beake et al.⁵

The source of -NO₂ is nitrite.⁵ Nitrite exists in equilibrium with its unionized form, HNO₂, which decomposes slowly leading to the formation, via -NO, of -NO₂. The nitration was first studied in batch experiments with phenol-containing compounds, including morphine. The time scale of the reaction warranted the development of a fast and direct sampling and analysis method for LC-MS/MS. Although the nitration is complete within a few hours, we are able to measure its progress. The evidence suggests that it is dependant on the concentration of nitrite, the pH, and involves radical intermediates.

Early experiments on morphine suggest that the formation of nitro-morphine by this reaction pathway would only occur in high-nitrite, low-pH conditions in the sludge.

It remains to be seen if such conditions could arise under regular STP operation. In order to determine if the results from the batch experiments correctly reflect a real STP, we will analyze effluent for the presence of nitrophenols. We have developed methods for this analysis based on solid-phase extraction. The goals are to elucidate the environmental significance of this abiotic transformation and gain evidence to support a proposed mechanism.

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MO 200

Linear solvation energy relationship models applied as classifiers in non-target analysis - a gas chromatographic approach

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The identification of unknown substances in complex environmental mixtures plays an important role in effect-directed analysis (EDA). Our approach for the identification of unknowns is based on the generation of possible structures followed by the progressive exclusion of structures that do not match experimental chromatographic and spectroscopic behaviour sufficiently. Linear Solvation Energy Relationships (LSERs) are applied as classifiers to predict the logarithmic retention factors log *k* in isocratic Gas Chromatographic (GC) measurements from the structures of candidate compounds. To demonstrate retention prediction and the application of the classifier model, twelve compounds with the molecular formula C₁₂H₁₀O₂ were selected, while experimental log *k* values were compared to the predicted values and exclusion of potential candidate compounds was performed. Predicted retention factors gained from calculated substance descriptors show poor quality. Prediction was enhanced by using experimental determined descriptors, also achieved by the GC measurements.

MO 201

Quantitative determination of different ether species in surface waters by Solid Phase Extraction and GC/MS

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Numerous ether species are of growing concern to human health as well as the environment. The production and use of ethers has been rising in many industrial sectors together with the exposure of these compounds to humans. There is a common lack of available information on the use, exposure and toxicity of these compounds. In this study method was developed for the extraction and enrichment of six compounds from the ether family: etbe, 1,4-dioxane, monoglyme, diglyme, triglyme and tetraglyme. These compounds are of concern because they easily dissolve in water and do not partition to soils, entering ground water systems and likely contaminating public water systems. They do not breakdown easily and might be difficult to remove from ground water sources.

Glymes are saturated polyethers which have found a widespread application in manufacturing of products such as printing inks, paints, coatings and batteries. They are also commonly used as reaction solvents in the area of pharmaceuticals or specialty chemicals production. Glymes have been found to be toxic to the reproductive and/or developmental systems causing infertility and harm to the unborn child. 1,4-dioxane has been widely used as a stabilizer for chlorinated solvents. It is also utilized in products such as paint strippers, dyes and greases. Based on the available data 1,4-dioxane is possibly carcinogenic to humans. The use of Etbe as an oxygenate gasoline additive in the production of gasoline is modest, but increasing. It has not been proven to pose any toxicity to humans.

In order to determine six ether compounds in the water samples, solid phase extraction procedure reflecting the method developed by the EPA for the determination of 1,4-dioxane in drinking water was utilized. 500 ml of a sample was passed through a charcoal containing cartridge and eluted with 10 ml of dichloromethane. All of the extracts were analyzed using a Gas Chromatography/Mass Spectrometry (GC/MS) in the selected ion monitoring (SIM) mode. To validate the method for all of the compounds a recovery study was performed, as well as a method detection limit was calculated for each ether. The same extraction procedure was used to determine the concentration of analytes of interest in the surface waters in Germany. Samples from six surface water bodies (Rhein, Main, Lippe, Wesel-Datteln Canal, Schwarzbach, Auesee) in twenty four different locations were collected, extracted and analyzed.

MO 202

UPLC/qTOF/MS and in silico screening for transformation products of pharmaceuticals in water/sediment tests

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Pharmaceuticals are widespread pollutants in the aquatic environment. Laboratory studies showed that the microbial community in river sediments is capable of transforming a variety of pharmaceuticals, and in some river systems this process is the dominating attenuation mechanism. In order to discriminate attenuation of pharmaceuticals along a river from dilution, suitable tools are needed. Among others, the determination of characteristic transformation products could be one such tool. The aim of this study was therefore to elucidate the transformation of several pharmaceuticals in river sediment and to evaluate this approach.

We carried out a series of incubations with sediment from three different rivers and synthetic river water. Tests were carried out at an initial concentration of 100-200 $\mu\text{g L}^{-1}$ for approx. 30 days; samples were filtered and subsequently analyzed by UPLC-qTOF-MS. The high-resolution mass spectrometric data were processed with a workflow combining the tools mzMine (<http://mzmine.sourceforge.net/>) and enviMass (<http://www.eawag.ch/forschung/uchem/software/enviMass1>). By comparing incubations containing pharmaceuticals to control samples without pharmaceuticals, this yields a list of peaks identified by exact mass and retention time for each time step of the incubation experiment. Using these time series data, peaks showing a temporal trend expected for transformation products (e.g., not present at the beginning of the incubation, increasing concentrations with time) can be located and processed further. Through application of this time series approach, the number of peaks to be processed further is substantially reduced.

The first results obtained by this approach are promising and provide a solid basis for the evaluation of the workflow, but also for scrutinizing its precision and robustness in detecting unknown peaks at low concentrations. This is necessary to increase confidence in the applied analytical and *in silico* methods for the specific aims of our study. We will present results of this evaluation, and we will discuss the tentatively identified transformation products with respect to their formation/elimination kinetics and their applicability as indicators for *in situ* micropollutant transformation.

MO 203

Evaluation of the contamination of aquatic micro-organisms by micro-QuEChERS-nano-LC-MS/MS

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It is currently accepted that the main route for pharmaceuticals and Endocrine Disruptors Chemicals (EDCs) to the aquatic environment is via sewage treatment plants receiving wastewater from households, industries and hospitals.

In a concern to follow their fates and their impacts in the environment, we developed analytical methods to quantify endogen and synthetic hormones, pharmaceuticals and chemicals in benthic species. We established a molecule list in accordance with prioritisation lists [1] and identification of contamination sources around the wastewater treatment plant (industries, hospital, villages): estrone, 17 α -ethinyl-estradiol, lorazepam, oxazepam, acetaminophen, carbamazepine, ibuprofen, 4-methylbenzylidene camphor, fluoroquinolones and sulfonamides for example.

We decided to evaluate the contamination of three different species which are well-known bio indicators of water quality. More precisely, invertebrates like gastropods (*Potamopyrgus antipodorum*), amphipods (*Gammarus*) and chironomidae larvae (bloodworms) are exposed to effluents from treatment plants..

In the interests of handling a few milligrams of biological matrices and of being accurate, we chose an adequate analytical technique: the innovative nano-LC coupled with tandem mass spectrometer. After cryo-grinding, we extracted samples with a salting-out assisted LLE followed by a purifying dSPE. The extract was evaporated then reconstituted in a proper mobile phase. Only a 1- μL injection is done on the pre-concentration cartridge and the capillary column. Thus, we can reach limits of detection of a few injected femtograms.

In conclusion, the comprehensive method was applied to the study of the fate and bio-accumulation of preoccupying micro-pollutants in aquatic micro-organisms.

MO 204

Occurrence and seasonal variations of preservatives and UV filters used in cosmetic products in Japanese rivers

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Preservatives and UV filters are widely used in many cosmetics. These compounds may enter the aquatic environment from showering, wash-off, and so forth via wastewater treatment plants or sewage treatment tank. Most of these preservatives are included in order to suppress microorganisms growing. UV filters are used to prevent skin damage from UV radiation, however some of them are hormonally active to aquatic organisms. Their ecological adverse effect on aquatic organisms should be concerned after discharge. But there is little published data of their occurrence in aquatic environment.

In this study, we determined twelve preservatives (2-Phenoxyethanol, Resorcinol, Chlroxylenol, 4-Isopropyl-3-methylphenol, Chlorphenesin, Triclosan, Methylparaben, Ethylparaben, Propylparaben, Isopropylparaben, Butylparaben, Isobutylparaben) and four benzophenone-type UV filters (BP-1, BP-2, BP-3, BP-6) using solid phase extraction and GC/MS. Surface water samples were collected monthly at six sites in four Japanese rivers from December 2010. 2-Phenoxyethanol, Resorcinol, 4-Isopropyl-3-methylphenol and Triclosan were found in most of the samples in all seasons. 2-Phenoxyethanol was detected at much higher concentration in winter. Isopropylparaben, Isobutylparaben, BP-2 and BP-6 were not detected at any sampling sites. The highest concentration in all samples was for 2-Phenoxyethanol at 1 $\mu\text{g/L}$ level. Further investigation is necessary to evaluate the ecological effect of high concentration preservatives including 2-Phenoxyethanol.

MO 205

Solid phase extraction and LC-MS/MS analysis of pharmaceuticals in the Irish aquatic environment

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Pharmaceutical and personal care products (PPCPs) are resisting water remediation techniques carried out currently in wastewater treatment plants and are thus being released into receiving waters. In previous studies PPCP presence has been detected in Irish effluent1 and soils2 in measurable quantities. In the work presented herein, 24 hour composite effluent samples were collected from two large Irish Wastewater Treatment Plants and analysed for a total of six pharmaceuticals from a range of therapeutic classes including non-steroidal anti-inflammatories, anti-biotics, lipid regulators and anti-epileptics. The complexity of these sample matrices requires a clean up and extraction step (Phenomenex Strata-X cartridges - 200mg, 6ml) before chemical analysis. Samples were eluted with 50:50 v/v ethyl acetate/acetone and reconstituted in 0.5mls of starting mobile phase. Qualitative and quantitative analysis was carried out using reversed phase LC separation (Waters Sunfire C18 3.5 μm 2.1 x 150 mm column with a 45 minute mobile phase gradient of 20 % acetonitrile with 13 mM ammonium acetate, pH6.2, to 80 % acetonitrile) with UV detection followed by MS/MS detection. Analyses were identified by comparing their retention times and precursor and product ions to those of a known standard.

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MO 206

Use of LC-Orbitrap accurate mass spectrometry in risk assessment after a chemical incident in the Netherlands

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Early 2011, a big incident took place in a chemical storage site in Moerdijk, the Netherlands. There was an extensive fire up to 500 meters high, with formation of soot. The column of smoke went in the northeastern direction within a large area. On the chemical storage site, many different inorganic, organic solid and liquid chemicals were stored. The fire extinguishing caused a vast volume of contaminated water.

Several Dutch water utilities have surface-water related raw drinking water in large storage reservoirs in the area under the plume. After storage, the raw water is treated by different treatment processes on the drinking water production location. These reservoirs were possibly influenced by the Moerdijk incident. For precautionary reasons, the reservoirs relatively close to Moerdijk were directly taken out of production and intake of possibly contaminated surface water was stopped. The quality of the drinking water produced by the utilities in the period during and after the incident has never come into play. Despite these precautionary measures, it was relevant to know if the storage reservoirs were negatively influenced by this Moerdijk incident. Therefore, several drinking-water related surface waters were sampled and analyzed using broad-screening analytical-chemical GC-MS and LC-MS accurate mass screening. The high sensitivity, the high resolving power and accurate mass measurement make this a valuable technique for the screening of trace levels of organic contaminants. In contrast to most current environmental monitoring campaigns, this approach does not target a predetermined set of (known) chemicals. Also, the developed approach does not aim at the detection of all organic chemical compounds present in a sample as in general unknown screening approaches. Instead the data was screened for the presence of 249 known chemicals and 386 unidentified chemicals. The list of 386 unidentified compounds was the result of broad screening campaigns in our laboratory in several Dutch water samples for the last 5 years.

In most of the storage reservoirs, no different compounds were found than normal. An exemption was a storage reservoir that was located 15 km from the incident.

MO 207

Polybrominated diphenyl ethers, alternate brominated flame retardants and Dechloranes in sediments from German Bight and Laizhou Bay, China

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Brominated flame retardants (BFRs) have been commercially used since the 1970s.

Since then more and more polymeric materials have been introduced for textiles, electronic equipment, upholstery, automobiles and building materials. Equally the production and usage of chemical additives for fire resistance of the mostly easy inflammable products have been increasing as well.

Due to their physicochemical properties i.e. low volatility, low water solubility and high Kow-values the BFRs tend to adsorb on particles. Therefore they were detected preferably in sediment samples. Benthic organisms and other contaminated sediment consuming animals can be the first step for bioaccumulation in the marine food web.

In this study 73 surface sediment samples were collected in Germany and China to investigate the levels, distribution, possible sources and different profile patterns of flame retardants (FRs) in the two countries.

25 German surface sediment samples were taken in the German Wadden Sea, the rivers Elbe and Weser and the German Bight in 2011. 48 Chinese samples were collected in Laizhou Bay and from discharging rivers in 2009.

The samples were Soxhlet extracted with dichloromethane and then purified by 10% water deactivated silica before measured by GC-ECN-MS. Quantification of 27 different FRs was obtained using 5 mass labelled internal standards ([13C]HBB, [13C]BDE-77, [13C]BDE-138, [13C]syn-DP, [13C]BDE-209).

The dominating compound in Laizhou Bay was Decabromodiphenyl ethane (DBDPE) and BDE-209 in German Bight, respectively. The alternate BFR DBDPE showed higher concentrations than BDE-209 in most Laizhou Bay samples and slightly lower concentrations in German Bight. Decachlorane Plus (DP) was detected in 71 out of 73 samples. DP concentrations were higher in German Bight except some Chinese river samples. Dibromopropyltribromophenyl ether (DPTE), Decachlorane (Dec) 602 and 603 were found in German samples only. Generally, the concentrations of FRs in German Bight and discharging rivers were quite comparable, while the Chinese samples showed big differences between marine and riverine sediment contamination.

MO 208

Water quality in the Three-Gorges Reservoir (China) - First results from the Yangtze Project WATERUSE

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To provide the public with high quality drinking water is a technological challenge, particularly in emerging countries. In the catchment area of the Three-Gorges Reservoir (TGR, China) productive and protected groundwater resources are very rare. Surface water is often used as a raw water source, but bank filtration as a first purification step is not feasible at TGR area. Thus, water works usually produce purified tap water directly from surface water, using standard filtration and disinfection techniques. The presented study focuses on the quality of surface water from Yangtze river and its tributaries in order to assess its suitability as drinking and irrigation water.

Hydrogeology and water chemistry of the 630 km long TGR are strongly influenced by the geology (red sandstones and clay stones, limestones) and a subtropical climate (mean temperature 19°C, precipitation ca. 1.138 mm/a). Soil erosion is leading to a high particulate matter load in the relevant rivers. Anthropogenic input of pollutants originates mainly from sewage, industrial discharge, garbage dumping and from agricultural activities.

Surface water, suspended solids and river bed sediments from TGR area were analysed for in-organic (anions, cations, heavy metals, contrast agents) using IC and ICP-MS and organic emerging pollutants (industrial chemicals, pesticides, pharmaceuticals, x-ray contrast media). Additionally, acute toxicity and Ah receptor mediated toxicity are assessed using the Neutral red and the EROD assays with the cell line RTL W1 obtained from rainbow trout.

First results of the inorganic river water analyses reflect the geogenic background. Concentrations of heavy metals and organic trace substances were usually well below international river water regulations. Particularly, pharmaceutical substances, which showed increased water concentrations in Germany in recent years, are almost absent in TGR water samples. Summer monsoons are controlling the highly varying river discharge. Thus, pollutant loads show a high seasonal variability with eco-toxicological impact.

Further sampling campaigns will focus on varying hydrological, geographical, and anthropogenic settings (e.g. high/low river water level) and the anthropogenic impact on the TGR chemistry.

MO 209

EDA of extracts from two different passive sampling devices in a polluted Norwegian river

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The Alna river running through Oslo has historically received pollution from a wide range of different industrial sources located nearby. Earlier investigations of the occurrence of contaminant in the river has shown high levels of PAHs, brominated flame retardants and PCBs in the water and sediments from the river. The river represents a significant contribution to the overall input of contaminants in the receiving Oslofjord. We wanted to investigate whether compounds other than those previously investigated contribute to the environmental problems in the river. We achieved this by using a combination of passive sampling devices exposed throughout the length of the river in combination with EDA (effects-directed analysis) and target chemical analyses to elaborate on the pollution status.

Six different stations downstream the river were investigated using two different passive samplers (silicone and LDPE - low density polyethylene) deployed for 6 weeks during summer 2011. Extracts from the passive samplers were analysed for environmental effects by using DR-CALUX assay, and were also subjected to both target chemical analyses (PAH, PCB, PBDE) and screening for non-target compounds by GC/MS-HR ToF.

The environmental effect of the extracts measured as DR-CALUX increased considerably (x100) downstream of the river source. The highest effects were measured in a tributary receiving run-off from a waste site upstream. GC/MS HR-ToF analyses revealed a difference in pattern of absorbed compounds downstream the river, and between the two different samplers. Initial results indicate that silicone seem to sample more of each compound, and a broader range of compounds, both in the relatively non-polluted upstream station, and the in the tributary.

These results will be presented in more detail, along with a comparison of the pattern of target chemical analyses downstream the river and between the two samplers, and with sampling rate estimates from performance reference compounds used with both sampler types. The results will be discussed in light of the different properties of the two passive samplers. Examples of identified compound and their occurrence downstream the river will also be discussed.

MO 210

Target and non-target screening of lake sediments using HR-MS/MS

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Sediments are excellent archives of environmental contamination if the chemicals persist over time under mostly anaerobic conditions. After a suitable extraction of sediment samples, the new technology LC combined with high resolution mass spectrometry (HR-MS) enables the performance of target screening of compounds which are known to be emitted in the aquatic environment in large amounts, suspect screening of compounds which are expected to be emitted, and non-target screening that up to now are unknown or unexpected aquatic contaminants. Besides the complexity in the ionization and detection of organic compounds, there are additional challenges to be addressed such as the lack of reference standards available for confirmation of many potential contaminants or their transformation products.

Results of target screening analysis show the detection of musk fragrances, personal care products (PCP), pesticides and biocides. The temporal resolution of selected organic pollutants in Lake Greifen were obtained with concentrations ranging from pg/gdw to ng/gdw. HR-MS/MS was also used to demonstrate the suitability of the method for the identification of compounds of interest which are expected to be present in the sediments based on consumption, discharge to the environment, and physical-chemical properties. Due to their high production volume and chemical characteristics, quaternary ammonium surfactants (QAs) might sorb strongly to soils and sediments and therefore deserve more attention. Several QA congeners were tentatively identified based on their retention times in accordance with their log Kow values and accurate masses. The identification of the QAs was confirmed by comparing the findings with purchased reference standards.

Non-Target screening is being explored by subtracting chromatograms of extracts of different layers from a sediment core. The top 5 cm of the core, which represent the condition of the lake from the past 10 years, was subtracted with a "blank sample" which corresponds to a sediment core layer from 100 years ago. The finding shows that around 41% of the detected peaks are unknown. The most abundant non-targets in the sediment correlate with the total phosphorus concentration in the lake. Automated exact mass filtering and peak detection in combination with in silico fragmentation for computer assisted identification of mass spectra is being used to identified possible non-target contaminants in the lake sediment.

MO 211

Characterization of surface water by multi-target screening of organic micropollutants combined with toxicological assessment

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In the past decades, several environmental and toxicological studies focused on persistent organic pollutants in domestic and industrial waste water. Due to incomplete retention by waste water treatment plants (WWTPs), tons of chemicals are yearly discharged to surface water. Although occurring in low concentration, these micropollutants might harm aquatic organisms. Especially mutagenic and endocrine substances have the potential to damage ecosystems. The aim of this study was to link the complex chemical composition of surface water samples with their multifactorial effects on organisms. Therefore, surface waters downstream of WWTP outlets associated to different sources including big cities, health resorts and chemical industry were examined. Water extracts were analysed by liquid chromatography-high resolution mass spectrometry for almost 300 environmental relevant substances, such as pharmaceuticals, pesticides, industrial chemicals or ingredients of personal care products. For biological characterization, the samples were tested for mutagenic and estrogenic effects by an Ames-test II and a zebrafish egg test, respectively. Obtained data were evaluated by chemometric tools such as partial least-squares regression. Chemical fingerprints and toxicological endpoints were correlated by a multivariate regression model. Groups of chemicals causing similar effects on organisms, as well as possible marker substances for waste water sources will be presented.

MO 212

Known unknowns: strategies for identification of toxicants in Effect Directed Analysis

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Technological innovations in the field of analytical chemistry have contributed significantly to the potential to find, identify and quantify unknown compounds present in the environment. Potentially, the application of accurate mass spectrometric techniques (e.g. time-of-flight, Orbitrap, FTMS) in environmental analysis can facilitate the

identification of unknown toxicants. One of the research areas that would benefit enormously from improvements regarding the identification of compounds is the field of Effect Directed Analysis (EDA). Our current work is dedicated to the development of a generic workflow aimed at the identification of compounds in extracts that are responsive in a toxicological/biological assay. In this presentation, we demonstrate our approach using a biotic sample that showed elevated thyroid hormone like activity. Using accurate mass LC-ToF-MS in the electrospray ionization mode, we were able to confirm the presence of a number of compounds that had been quantified in the samples using target analysis and that had very typical mass spectra: hydroxylated metabolites of PCBs. For compounds that have one or even a few halogens in their molecular structure, the typical isotopic patterns greatly enhance compound identification/confirmation. Using compound lists (e.g. list of pharmaceuticals, bioaccumulating compounds) target screening was performed on LC-ToF-MS data with Bruker Compass TargetAnalysis 1.2 software. Identities were established based on accurate mass, mSigma value (rate for the agreement of the theoretical and measured isotopic pattern of the mass peak of interest), molecular formula and elemental composition. In order to obtain significant progress regarding the identification of biologically active compounds in the framework of EDA, further development of mass spectral libraries are of major importance. Initiatives such as the NORMAN Mass Bank for sharing mass spectrometric data are expected to contribute to the advancement of identification of unknown compounds. In future work, more attention needs to be paid to the use of already available toxicological data, or software to derive theoretical compound characteristics.

MO 213

Silicone-based agricultural adjuvants: trace analysis in aqueous environment

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Pesticides fate and ecotoxicology have been an issue for the last decades but agricultural adjuvants are much less studied yet. Among adjuvants, trisiloxane surfactants are increasing in popularity because of their high efficiency compared to traditional carbon-based surfactants. They are often referred as superspreaders or superwetters because of their ability to promote a rapid spreading on the hydrophobic surface of leaves. This effect makes the silicone-based surfactants toxic for terrestrial insects. Trisiloxane surfactants may reach surface or ground waters after their application in agriculture but no data are available on their environmental fate. A method for trace analysis of trisiloxane surfactants in aqueous environment was therefore developed and validated. It allows the quantification of two important trisiloxane surfactants in different aqueous matrices with a limit of quantification in the ng/L range. The strength of the method is to consider every homologue as a single molecule and to obtain information on the oligomeric distribution of the surfactant.

MO 214

Tentative risk assessment of the UV filters

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In the last years, the impact of personal care products (PCP) and pharmaceutical on the environment has gained considerable attention. Still, little is known on specific classes such as the UV absorbing chemicals (UV filters).

UV filters are hydrophobic, bioaccumulative chemicals; few of them have been shown to have endocrine disrupting properties. UV filters are widely used and enter in the composition of sunscreens, cosmetics and in the protection of goods. Currently about 30 products are allowed by FDA and are present in the European cosmetic directive.

UV filters can enter in the environment directly as the result of recreational activities and through the WWTP. Although their use is widely spread, the information on their fate and ecotoxicity is scarce and sparse. After last REACH registration deadline, the amount of information has not increased as expected. About half of the allowed substances should have been registered in 2010 and the data of only 5 chemicals is available on ECHA website.

To understand their potential impact in the environment, we performed a risk assessment; unfortunately due to their properties, it is highly probable that the classical risk assessment underestimates the risk.

Whenever possible, the hazard was evaluated based on experimental data, with priority for information on REACH dossiers. We also used structure activity relationship methods to estimate missing ecotoxicity values.

To estimate the exposure, priority was given to measured quantities. When no data were available, the REACH deadline was used as an estimation of the European tonnage. A reliability score was given to the risk characterisation ratio (RCR); RCR calculated using experimental data obtained higher reliability score.

At the current usage, some of the UV filters have and RCR above one thus being potentially dangerous for the environment. In many cases, a refinement of the data, both hazard and exposure, is necessary and will improve our understanding of the potential danger of these chemical. In a few cases either it was not possible to calculate the RCR due to the absence of data, or it cannot be considered as reliable due to the high number of estimated data.

MO 215

Potential toxicity of sucralose

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Sucralose is an intensively sweet food additive derived from sucrose in a process where three hydroxyl groups are substituted for three chlorine atoms, producing a very stable substance and has become a popular substitute for sugar. Its exceptional stability in combination with high water solubility and popularity has resulted in measurable concentrations not only in recipient waters but also further out in the oceans. Animal and human studies have concluded that sucralose is safe for human use and earlier studies in aquatic organisms indicate low bioaccumulation potential and negligible acute/chronic toxicity. The close structural resemblance with sucrose in combination with the importance of sugar in nature implies that other assessment techniques than traditional methods might be of interest. The aim of this investigation was therefore to combine traditional methods with behavioral studies in crustaceans and algae. Our results show that no effects were obtained by traditional toxicological tests, but both physiology and locomotive behaviour were affected by exposure to sucralose. In *Daphnia magna*, the behavioural response was manifested as altered swimming height and increased swimming speed. In gammarids, effects on the time to reach food and shelter as well as activity responses measured by a Multispecies Freshwater Biomonitor[TRADEMARK] were observed. It is unclear whether these behavioural responses were initiated via traditional toxic mechanisms or stimulatory effects, but we imply that they still should be considered as a warning, since divergence from normal behaviour, may ultimately have ecological consequences.

MO 216

Antibiotic activity as a tool to identify and prioritize relevant point sources

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The research effort on pharmaceuticals in the last two decades has raised concern that pharmaceuticals, including antibiotics may enter the environment still as active substances. The route of entry of pharmaceuticals to the environment is largely via sewage treatment works. More and more is known that these systems are not always capable of removing these compounds to full extent. Next, too little is known on the effects of the substances and last, less is known on the effects of the mixture of compounds. In our research, we have calculated both emission data on loads to identify these mixtures and used available chemical analytical methods to identify known substances. However, the capacity to measure these emerging compounds is yet limited. For that reason, we have adopted a new bioassay that can be included as screening tools or in WET (Whole Effluent Test) procedures. The bioassay is capable of identifying the activity of 5 types of antibiotics. We present the latest results using this bioassay and discuss the potential as an initial screening step to prioritize effluents, one of the research needs as identified in the European Water Framework Directive.

MO 217

Chronic aquatic toxicity of sucralose to aquatic invertebrates and fish

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The intense artificial sweetener sucralose has been detected in municipal wastewater effluent and surface waters at concentrations ranging from ng/L to low ug/L. Few chronic ecotoxicological data are available in the peer reviewed literature with respect to sucralose. To address this data gap, 21 d *Daphnia magna*, 28 d *Americanysis bahia* (mysid shrimp) studies and a fish early-life stage study (ELS) were conducted to assess the effects of sucralose on the survival, growth and/or reproduction of these organisms. Concentrations ≤ 1800 mg/L resulted in no statistically significant reduction in *D. magna* survival or reproduction. Survival, growth and reproduction of mysid shrimp were unaffected by ≤ 93 mg/L sucralose. Collectively, these data suggest that the concentrations of sucralose detected in the environment are well below those required to elicit chronic effects in freshwater or marine invertebrates. Data will also be presented with respect to findings in the fish ELS study.

MO 218

Rapid acute toxicity screening of commercial and potential green plasticizers using bioluminescent bacteria

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The *Vibrio fischeri* bioluminescence inhibition assay (Microtox®) using an in-house diluent containing 2% DMSO was used to perform a rapid toxicity screening of 24 commercial and potential green plasticizers. This bioassay allowed us to better understand the impact of a key structural feature, alkyl chain size, on the toxicity of the target plasticizers. Results indicated that dibenzoate compounds with lower number of C atoms in their alkyl chains, such as 1,3 propanediol dibenzoate (PrDDB) and 1,4-butanediol dibenzoate (BDDB), were more toxic ($EC_{50} < 2$ μ M) than commercial glycol dibenzoates such as DEGDB and DPGDB ($EC_{50} = 14-31$ μ M); however dibenzoates with higher number of C atoms in their alkyl chains such as 1,5-pentanediol dibenzoate (PDDDB) and 1,6-hexanediol dibenzoate (HDDDB) had similar toxicities to those commercial plasticizers. Dibutyl and dihexyl compounds of the dialkyl esters (fumarates, maleates and succinates) were more toxic ($EC_{50} = 2-6$ μ M) than the commercial plasticizers di(2-ethylhexyl) phthalate (DEHP), 1,2-cyclohexane dicarboxylic acid diisononyl ester (DINCH) and di(2-ethyl hexyl) adipate (DEHA), which did not inhibit the bacterial luminescence. Nevertheless diethyl, dioctyl and di(2-ethyl) hexyl compounds are weakly toxic ($EC_{50} = 130-1292$ μ M) or non toxic at all to the luminescent bacteria. These data and previous studies on biodegradation and plasticizing properties indicate that 1,5-pentanediol dibenzoate (PDDDB), 1,6-hexanediol dibenzoate (HDDDB), dioctyl fumarate (DOF), dioctyl maleate (DOM) and dioctyl succinate (DOS) might be good candidates to replace current commercial plasticizers.

Contribution of PPCPs and surfactants on the sub-chronic toxicity of effluent-dominated urban creeks to aquatic organisms

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Japan Ministry of Environment started research on possible introduction of the bio-assay-based management system of wastewater and receiving water similar to USEPA's WET to Japan but information had been limited in Japan. Thus, our research group has examined the aquatic toxicity of some industrial and municipal wastewaters in Tokushima, Japan using the short-term chronic test mainly used in USEPA's WET Test Methods with slight modification and found significant adverse effects on the reproduction of *Ceriodaphnia dubia* and hatching/survival of eggs of Japanese medaka or zebrafish for several samples. On the other hand, we have been investigated on ecological risk of pharmaceuticals and personal care products and found some PPCPs including some antimicrobial agents and UV filters might be a possible threat for the aquatic organisms.

In the present study, the growth inhibition test of green algae (*Pseudokirchneriella subcapitata*), the *Ceriodaphnia dubia* reproduction test, and the zebrafish (*Danio rerio*) embryo/sac fly stage test was conducted to evaluate the sub-chronic toxicity of waters sampled in 12 urban streams of Tokushima, Kyoto, and Saitama, Japan, contaminated by treated or untreated domestic wastewater. The reproduction and survival of *Ceriodaphnia dubia* was adversely affected in the waters sampled in most of the sites at least once. The growth of green algae was significantly inhibited in those sampled mostly in summer season in at least three sites. Some potential toxicants, mainly PPCPs and surfactants, were measured for the water sampled using GC-MS or LC/MS/MS for the water samples in addition to the toxicity tests for approximately twenty individual PPCPs and surfactants. Toxicity unit (TU) for each individual PPCP was calculated based on NOEC values and compared with the TU of the whole river waters. Relatively higher concentrations of most of PPCPs were found in Tokushima compared to Kyoto and Saitama. Surfactants, triclosan and other antifungal agents slightly or moderately contribute the whole toxicity of the water samples for algae but the factors other than PPCPs may play important role for *Ceriodaphnia* and zebrafish.

ET08P - Extrapolation within wildlife toxicology**Variability in the outcome of outdoor mesocosms with three-spined Stickleback (*Gasterosteus aculeatus*) populations in control conditions: data from 20 replicates in two annual experiments**

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Ecotoxicology aims at protecting ecosystems and populations. Population level is rarely studied in ecotoxicity tests. However, the population level can be monitored with high realism relative to ecosystems through experiments in outdoor mesocosms. At Ineris, we focus on the three-spined Stickleback, *Gasterosteus aculeatus*, because it is a well-studied species, autochthonous in French rivers, and a promising species to assess the impact of contaminant.

All studies already performed in mesocosms suffer from an important variability between replicates for a same treatment (more than 50% of variation is generally observed on biological variables). Temporal replicability of mesocosms' outcomes is also an issue. In our study, we aimed at identifying the major causes of inter and intra annual variability in control conditions.

Thus 10 populations per year, for two years experiments were monitored between March and October 2010 and 2011. At the beginning of experiments, each compartment of the ecosystem (weight of macrophytes, number and mass of invertebrates, number, length and sex ratio of fish[3DOTS]) were introduced in standardized conditions. At the end of the experiments, all populations were defined by characteristics such as total number of fish, sex ratio, and percentage of mature organisms.

We found that, in 2010, the proportion of males and females fish introduced in the system was the most influent parameter to account for the population characteristics (total number of fish and structure of the population) at the end of the experiment. Inter-annual variability was mainly due to differences in water temperature profiles.

Intra-annual variability can be reduced through a better sex determination of the fish introduced at the beginning of the experiment. We developed an original methodology to achieve that (See another poster of de Kermoyan et al.: A non-invasive method based on head morphology to sex mature three-spined Stickleback (*Gasterosteus aculeatus*) in rearing conditions). Inter-annual variability, in link with temperature, could be quantified provided the influence of temperature on the main components of *G. aculeatus* population dynamics are understood and modelled.

Maternal transfer of lead in mallards under field conditions: biochemical and immunological effects on ducklingsM.E. Ortiz-Santaliestra¹, A. L  pez-Ant  a¹, M. Mart  nez-Haro¹, S. Esp  n², D. Vidal¹, R. Mateo¹¹IREC (CSIC-UCLM-JCCM), Ciudad real, Spain²University of Murcia, Murcia, Spain

The ingestion of Pb shot used for hunting constitutes the main cause of lead poisoning in waterfowl. Females may transfer some of the accumulated Pb to their eggs and, although most Pb is expected to accumulate in the eggshell, it can ultimately affect embryonic and hatchling health and development. We collected eggs of mallard ducks from the Ebro delta (NE Spain), an area with high Pb shot prevalences in waterfowl, and incubated them artificially. Blood samples were collected at days 3 and 28 post hatching to measure Pb levels, δ -ALAD activity, oxidative stress indicators, biochemical parameters, vitamins and carotenoids. Cellular immune function was quantified at day 14 as the intensity of swelling produced in response to an intradermal injection of phytohemagglutinin (PHA). Humoral immune function was measured at day 28 as the amount of antibodies synthesized against an intraperitoneal injection of sheep erythrocytes. Ducklings with blood Pb levels above 180 ng/ml died during the first 7 days post hatching. Eggshell Pb levels were significantly correlated with blood Pb levels in ducklings at day 3 ($r=0.549$, $p=0.002$), but not at day 28. δ -ALAD activity and cellular immune function were negatively correlated with Pb levels in eggshell (δ -ALAD: $r=-0.383$, $p=0.040$; PHA reaction: $r=-0.471$, $p=0.048$) and blood of mallard ducklings (δ -ALAD: $r=-0.468$, $p=0.009$; PHA reaction: $r=-0.639$, $p=0.003$). Neither the humoral immune function nor the rest of blood parameters were affected by blood or eggshell Pb concentrations. δ -ALAD activity decreased linearly at blood Pb concentrations above 60 ng/ml, as it has been described for adult mallards [6]. The results suggest that some of the Pb present in eggshells was accumulated in target tissues (i.e. liver, spleen, bone), potentially contributing to the observed immunotoxic. Because range of Pb levels is wider in eggshells than in blood samples, eggshell contents may be used to estimate in ovo Pb exposure of the ducklings, being a more representative indicator than blood levels to estimate the effects of Pb intoxications.

Blood concentrations of heavy metals (Ba, Cd, Hg and Pb) and Metalloid (As) in raptor species (*Buteo buteo*, *Milvus migrans* and *Gyps fulvus*) from PortugalM.A. Carneiro¹, B.J. Cola  o¹, R. Brand  o³, C. Ferreira³, N. Santos⁴, V. Soeiro⁵, A. Cola  o¹, M.J. Pires¹, P. Oliveira¹, S. Lavin  ¹Universidade de Tr  s-os-Montes e Alto Douro, Vila real, Portugal²Centro de Ecologia, Recupera  o e Vigil  ncia de Animais Selvagens, Gouveia, Portugal³Centro de Recupera  o e Investiga  o de Animais Selvagens da Ria Formosa, Olh  o, Portugal⁴Centro de Recupera  o de Fauna Selvagem do Parque Nacional da Peneda do Ger  s, Braga, Portugal⁵Centro de Recupera  o do Parque Biol  gico de Gaia, Gaia, Portugal⁶Servicio de Ecopatologia de Fauna Salvaje. Universidad Aut  noma de Barcelona, Barcelona, Spain

Heavy metals are ubiquitous elements present in the environment and they can easily enter the food chain and cause possible toxic effects on humans and animals, being a serious threat for the stability of ecosystems and living organisms. Birds of prey can be useful as sentinel species to monitor the quality of the environment, once they forage over a large geographical area and as top consumers in food chain it would be expected that they accumulate environmental contaminants, like heavy metals.

The purpose of this study was to evaluate the exposure to arsenic (As), barium (Ba), cadmium (Cd), mercury (Hg) and lead (Pb) in three raptor species from different areas of Portugal. Birds of this study were brought alive, but injured or debilitated for various reasons, to four Portuguese Wildlife Rehabilitation Centers. Blood samples were collected from 72 common buzzards (*Buteo buteo*), 22 black kites (*Milvus migrans*) and 33 griffon vultures (*Gyps fulvus*) and concentrations of toxic elements were measured by inductively coupled plasma mass spectrometer (ICP-MS), after blood wet digestion.

Results obtained showed the presence of various toxic elements in all samples analyzed. Cd means concentrations were the lowest in the three species and no statistically significant differences were found between species ($p=0.758$). However, statistically significant differences were observed between species for As ($p<0.001$), Hg ($p<0.001$) and Pb ($p<0.05$), showing griffon vulture and black kite the highest blood Pb concentrations (respectively 21.278 ± 13.588 and 20.536 ± 32.662 $\mu\text{g/dl w.w.}$), common buzzard the highest Hg concentrations (17.156 ± 22.439 $\mu\text{g/dl w.w.}$) and results concerning As, black kite presents the highest values (4.607 ± 5.090 $\mu\text{g/dl w.w.}$). The area of origin seems to influence blood Hg concentrations in common buzzard, once were found statistically differences between Braga and other five districts ($p<0.05$), presenting the animals of Braga the highest levels of Hg.

Results concerning Hg and obtained in this study suggest that common buzzards are exposed to high levels of this toxic metal, when compared with other elements. Black kite and griffon vulture are exposed to high levels of Pb, possible to cause subclinical or clinical toxicity. Although concentrations could not be directly related to lethal effect, they might constitute a serious environmental factor affecting the survival, and special attention should be given at species in decline.

A multi-species approach for assessing lead bioavailability in the ecological risk assessment of a shooting range

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Ecological risk assessment is an important tool for evaluating potentially hazardous contaminants on a site-specific basis. Few methods exist for evaluating population and community level effects of contaminants on terrestrial invertebrates, though such assessments may aid in site evaluation and subsequent decision-making with regard to site remediation. In the current study, we investigated the utility of ground beetles (Carabidae) as indicators of elevated metal concentrations originating from lead-based shot at a shooting range. Ground beetles were collected in 2008 and 2009 using pitfall traps in shotfall and reference areas. Forty-five species were collected and overall species richness did not differ between sites. Abundance of the most common species was not significantly different between sites or was more abundant at the reference site. Collection of ground beetles is on-going and additional years of data may reveal different trends in ground beetle distribution and abundance. In addition to ground beetle data, bioassays using earthworms (*E. fetida*) were used to estimate bioaccumulation and sublethal effects of the metals in shooting range soil. Twenty-eight day bioaccumulation

and reproduction tests were conducted with earthworms. Cocoon production was lower in shotfall soil compared to reference soil and Webster (laboratory standard soil). No mortality was observed and Pb bioaccumulation increased with increasing Pb concentration in soil. Pb bioavailability will also be estimated using a modified in vitro gastrointestinal method (mimicking the short-tailed shrew, *Blarina brevicauda*). Field soils and earthworms exposed to field soils will be analyzed for bioaccessible Pb. In the future, we hope to collect earthworms and shrews from the shooting range for the determination of Pb concentrations in their tissues. If a correlation is established between field Pb levels in shrews and the relative bioaccessibility, then these modified methods may be a useful tool for Pb exposure estimates in ecological risk assessment.

MO 245

Effects of lead shot ingestion on constitutive immune function of red-legged partridges

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The effects of toxic levels of Pb are diverse and represent several functional systemic alterations. Although the effects of exposure to Pb on the immune system of mammals, and especially in humans, have been extensively studied, little is known about how this heavy metal affects the constitutive immune function in birds. Pb intoxication may be a major problem for birds as they accidentally feed on Pb shots that are confounded with grit particles. We present a preliminary study based on different tests to assess changes in innate immune response of red-legged partridges (*Alectoris rufa*) subjected to Pb shots. We used four experimental groups (0-9 Pb shots) with six replicates each. A marginally significant lethal effect was detected for the highest Pb treatment (60% mortality; $p=0.057$). Among survivors, decrease in body mass after Pb exposure affected especially to females. Blood samples were obtained from the survivors 25 days after exposure. We compared blood Pb levels, analyzed by Atomic Absorption Spectrophotometry with graphite furnace, with different parameters indicative of the innate immune response. The ratio between lymphocytes expressing CD4 and CD8 membrane receptors, analyzed by flow cytometry, was affected by the interaction between treatment and sex in a less than significant manner ($p=0.060$). Haematocrit, serum lysozyme concentrations, bactericidal activity of the whole fresh blood and the ratio heterophils/lymphocytes were unaffected by the exposure to Pb. Our results highlight the necessity to analyze several parameters to assess the immunotoxicity of a substance. Nonetheless, these and other tests should be repeated with a larger sample size to obtain meaningful results. Analyzing constitutive immune function is important in wild populations as it represents the organism's ability to display a quick response against opportunistic pathogens.

MO 246

Assessment of fungicide- and insecticide-coated seed risks for red-legged partridges

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The use of agrochemicals is suspected to be a major cause of population declines of farmland birds from Western Europe. Apart from indirect effects like reduction of available food and optimal habitat, ingestion of pesticide-coated seeds constitute a potential way of direct intoxication. Although the most toxic chemicals are being legally restricted, some of the pesticides currently used for seed coating can cause adverse effects when consumed by birds. The aim of the present project is to test the effects of coated seed ingestion on red-legged partridge (*Alectoris rufa*) physiology, fitness and reproduction. We tested two insecticides (natural pyrethrins and imidacloprid) and two fungicides (maneb and thiram). Each experiment consisted in two groups of 16 pairs of partridges exposed to two doses: a high dose corresponding to recommended for seed coating and a low dose being the 20% of the recommended one. Exposure was split in two periods, corresponding to the two seasons of cereal sowing; 25 days in autumn and 10 days in late winter. Imidacloprid was the most toxic compound, killing all partridges at the recommended dose in 21 days. All pesticides affected hematocrit (controls: 42.3%; treated: 38.3-41.0%), whereas maneb at high dose reduced body condition after the first exposure period (10.6% lower than controls). Reproduction was affected especially by the fungicides, and at low doses rather than at high ones. The two fungicides reduced the fecundation rate (34-54% vs. 62% in controls) and thiram caused a significant decrease in chick body condition 24 days after hatching (18% lower than controls) that ultimately caused a marginally significant mortality ($p=0.07$). At high dose, this fungicide also caused an immunosuppressive effect, as estimated by the PHA test to quantify the cellular immune response. The use of coated seeds is a widespread practice, and cereal seeds constitute a major portion of partridge diet during autumn and winter, when alternative food sources are scarce. On the other hand, colour, taste or texture of the coat can make the seed unpalatable for birds, reducing the risk of ingestion. Upcoming analyses will include an assessment of the environmental exposure of partridges to coated seeds that will be achieved by the study of crop contents of field collected partridges provided by hunters.

MO 247

Long-term effects of Pb pollution on mineralization and biomechanical properties of bone in wild ungulates: relationship with vitamins A, D and E

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Long-term effects of environmental lead (Pb) exposure in bone could play an unknown role threatening some populations of wildlife. Certain physiologic disorders attributed to Pb exposure are related to the generation of oxidative stress, which may be involved on the depletion of antioxidant vitamins. The aim of this work was to study the occurrence of abnormalities on bone tissue composition and turnover mechanisms through the Pb-mediated disruption of vitamins (A, E and D) homeostasis in wild ungulates living in a Pb-polluted mining area. Red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*) from the mining area had significantly higher liver and bone Pb levels than in the control area, which were associated with the depletion of liver retinyl esters and the corresponding increase of free retinol levels both in deer and boar from the mining area. The most characteristic trait in bone mineralization of Pb-exposed adult deer was a lower carbonate content in bone mineral that was associated with the increased free retinol percentage, which in turn had consequences on bone biomechanics. Although Pb effects on wild boar have been less significant, the degree of bone mineralization was also positively associated with higher burdens of retinyl esters. In conclusion, we suggest that changes in antioxidant vitamins found in deer may be involved in the toxic effects of Pb on bone composition.

ET09P - Global climate change: implications for environmental toxicology and chemistry

MO 249

The influence of global climate change on the scientific foundations and applications of environmental toxicology and chemistry: summary from a SETAC international workshop (July 2011)

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This is an introduction to and summary from a SETAC international 'Pellston' workshop titled "The Influence of Global Climate Change on the Scientific Foundations and Applications of Environmental Toxicology and Chemistry". Held July 16-21, 2011 it involved some 36 scientists from 11 countries. The central question addressed was: *How will global climate change influence the environmental impacts of chemicals and other stressors; and, the way we assess and manage chemical contamination and other stressors in the environment?* While more detail is found in the complete series of papers, some key consensus points are: 1. human actions (adaptation, mitigation, etc.) may have as much or more influence on the fate and effects of chemical contaminants as does GCC, and modeled predictions should be interpreted cautiously; 2. effects of GCC may be slow, variable and difficult to detect though some species and populations of high vulnerability (polar, coral reefs) may exhibit responses sooner and more dramatically than others; 3. future approaches to human and ecological risk assessments will need to incorporate multiple stressors, cumulative risks (positive and negative) considering the wide spectrum of potential impacts stemming from GCC; 4. baseline / reference conditions for estimating resource injury and restoration / rehabilitation will continually shift due to GCC and represent significant challenges to practitioners.

MO 250

Consequences of metal adaptation on sensitivity to future climate change stressors in *Daphnia pulex* populations

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Genetic variability may allow for natural populations to adapt to a stressor, which is manifested by an increased tolerance to the stressor. As a consequence of this genetic adaptation, populations may also become more (co-)tolerant or less tolerant (cost-of-tolerance) to other stressors. In this research we investigated the cost-of-tolerance hypothesis in the context of climate change, i.e. that populations of the waterflea *Daphnia pulex* that adapted to cadmium (i.e. more tolerant to sublethal cadmium concentrations) are less tolerant to exposure to two climate change stressors, cyanobacteria and hypoxia. Cyanobacteria are a common stressor to most zooplankton species and are predicted to play an increasingly prominent role in aquatic ecosystems as their occurrence in harmful algal blooms is predicted to increase due to climate change. Increasing deposition of atmospheric nitrogenous gases and phosphorus loading from agricultural fertilization and fossil-fuel emissions into the world's surface waters increases eutrophication of these waters, eventually resulting in an increased amount of sinking organic matter. Hypoxia is caused by an increase in microbial, oxygen requiring processes which cope with digestion of this organic matter. Furthermore, higher water temperatures tend to increase the duration and strength of thermal stratification by reducing the mixing of oxygen rich water layers with oxygen poor layers, resulting in a prolonged hypoxia zone. To test the cost-of-tolerance hypothesis, animals were exposed to either a diet containing 50% *Microcystis aeruginosa*, 2 mg·L⁻¹ O₂, or 0.5 µg Cd·L⁻¹ to assess cyanobacterium, hypoxia, and cadmium tolerance, respectively. Control animals were maintained at an oxygen level of 9 mg·L⁻¹ O₂ and were fed 100% *Ankistrodesmus falcatus*. Tolerance was assessed as control normalized

reproduction and growth. Contrary to the cost-of-tolerance hypothesis, we found significant correlations between cadmium and both *M. aeruginosa* and hypoxia tolerance, suggesting that co-tolerance rather than a cost-of-tolerance occurred. These findings suggest that cadmium adapted *Daphnia* populations are better suited to face some future climate change stressors than non-cadmium adapted populations.

MO 251

Toxicity interaction of salinity and copper on the development of early life stages of Zebrafish

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Climate changes are expected to modify environmental parameters in several aquatic ecosystems. Within these scenarios, an increase in salinity, due to the sea level rise, is predicted to occur in coastal freshwater ecosystems. According with this scenario, a set of works has already addressed the sensitivity of several freshwater species to increased salinity in order to evaluate the potential risks associated with the salinization of such coastal ecosystems. However, as many of these coastal freshwater lagoons are already impacted with metal contamination, it is pertinent and important to assess and understand the combined effects of metals and increased salinity to freshwater species. Accordingly, the present work intended to evaluate the possible interactions of copper and NaCl (as a measure of increasing salinity) on the development of zebrafish. To achieve this goal, larvae (96hpf) were exposed to several concentrations of copper (0.44 - 7.38 µM) and NaCl (5 - 7.93 g/L), both individually and combined (using a full factorial design), during 96h. During exposure several lethal and sublethal endpoints (mortality, swim-bladder size, edemas, malformations, and activity) were monitored. Preliminary results showed that in the lowest copper concentrations (without NaCl), mortality was low or null, but, when combined with NaCl, mortality increased during the four days of the experiment. Furthermore, the highest copper concentrations combined with NaCl caused higher mortality rates, but only after 48h of exposure. These data suggest a possible synergism between copper and NaCl, which may lead to higher rates of mortality than those expected from their individual toxicity. Additionally, in treatments with NaCl alone, no significant rates of malformations or edemas were observed and when the same concentrations were combined with copper there was an increased incidence of these two parameters. This same scenario was observed with copper alone. It is then expected that this synergistic effect between compounds may affect as well other significant sublethal endpoints. These preliminary results suggest that attention must be paid to the effects of salinization on aquatic ecosystems impacted with metals as interactions are likely to occur, and already chemical-impacted populations may be at a higher risk comparatively with non-exposed ones.

MO 252

In vitro testing of biofuels - Verifying the environmental friendliness of biofuels in comparison with fossil fuels

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In the attempt to reduce the emission of greenhouse gases and simultaneously decrease the amount of fossil fuels that is used in the transport sector, fuels produced from biomass, so called 'biofuels', are becoming more and more popular. In this context, the amount of biofuels in the transport sector will be increased during the next decades. In 2022, 10% of the total fuel consumption in the European Union should be obtained from biomass. The United States of America, on the other hand, plan to increase the amount of biofuels up to 30% until 2030. While the negative effect on the global climate due to decreased carbon dioxide emission from fossil fuels will be reduced, significant effects on the environment can be expected! However, there is nearly no data available on (eco)toxic effects these biofuels could have on the environment or the human health. In this study, we want to investigate the use of in vitro biotests for the (eco)toxicological investigation of biofuels on the basis of the three potential biofuels ethyl levulinate, 2-methyltetrahydrofuran and 2-methylfuran. These potential biofuels are currently investigated by the excellence cluster "Tailor-made fuels from biomass" of the German excellence initiative. We want to identify relevant endpoints and test organisms that could be used for a comprehensive hazard assessment of biofuels. There are, however, some points that need to be addressed due to the nature of the test substances. The particular physical-chemical properties of these potential biofuels and the also investigated fossil fuel reference substances require some adaptations of the test designs.

We investigated three in vitro biotests and ecotoxicological endpoints. Acute Cytotoxicity was determined by means of the Neutral Red Retention assay and ah-receptor mediated activity, so called dioxin-like activity, was detected using the mechanism-specific 7-Ethoxy-o-resorufindeethylase (EROD) assay, both using cells from the permanent cell line RTL-W1 (Onchorynchus mykiss) as a test organism. Furthermore, genotoxicity was determined by means of the micronucleus assay using the cell line V79 (Chinese hamster) (ISO21427-2). Besides the direct dosing approach, which is more or less a standard method, a passive dosing approach using Polydimethylsiloxan-Rings was also applied, in order to investigate the suitability of this method for these substances.

MO 253

How important is chemical toxicity for biodiversity conservation?

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In the early 1990's, biodiversity was recognized as an important factor determining ecosystem functions. Ecotoxicology approaches biodiversity conservation from a chemical toxicity perspective using time scales that match those of the exposed organisms' life cycles (hours to months). However, the contribution of chemical toxicity to centennial trends in biodiversity remains unknown. Here, we propose a combination of paleo-ecological approaches with statistical modelling to increase our insight into the quantitative importance of chemical toxicity to biodiversity changes over long time periods. Specifically, we analysed if and to what extent fluctuations of two biodiversity indicators (richness and evenness) during the last 2000 years in a copper mining region in central Sweden were steered by copper toxicity, relative to land-cover changes and continent-wide temperature anomalies. Historical trends of copper, biodiversity and land-cover were reconstructed from copper concentrations, counts of fossil diatoms, and counts of terrestrial pollen - all measured in sediment cores - respectively. Temperature anomalies were obtained from climate models. Using additive modelling, we found that 76% and 50% of the variability in evenness and richness can be explained by lake identity and the degree of land disturbance. Addition of more predictors (including Cu concentrations) did not improve model fit. However, the fitted lake-specific effects indicated that the two lakes located next to metal smelters (Cu up to 3000 µg/g) had communities that were less rich (30 to 40 taxa less) and had a lower evenness (0.1 lower than lakes located further away from mining activity). The absence of significant effects of Cu concentrations between 10 and 100 mg/kg on biodiversity could be explained by the replacement of sensitive taxa by tolerant taxa. The beneficial effect of land disturbance on biodiversity was explained by increasing abundances of r-strategist species with increasing disturbance. Our findings illustrate that - even in a metal polluted area - land-cover was the major driver for biodiversity fluctuations.

MO 254

Temperature-dependent effects of the fungicide pyrimethanil on the reproduction of Physella acuta

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The environmental impact of climate change is meanwhile not only discussed in the scientific community but also in the general public. However, little is known about the relationship between pesticides and climate change. A combination of multiple stressors may lead to severe alterations for organisms such as changes in time of reproduction, reproductive success and growth performances, mortality and geographic distribution. Recent research revealed that aquatic model organisms tend to react more sensitive towards contaminants under climate change conditions.

To understand the sublethal impact of contaminants and how temperature influences sensitivity of molluscs as non-model organisms we assessed an embryo toxicity test as well as a whole life-cycle test (F1 and F0-generation) with the snail *Physella acuta*. Therefore embryos and adult snails were exposed towards the fungicide pyrimethanil and three different temperatures (15, 20 and 25°C).

Independent of pyrimethanil concentrations, more embryos hatched the higher the temperature was. Furthermore pyrimethanil caused high mortality at the highest concentration of 1.0 mg L⁻¹ (chronic EC10 for *Daphnia magna* = 0.95 mg L⁻¹) at all temperatures. In the life-cycle test more egg masses were produced the higher the temperature was in the F0-generations and the snails reacted more sensitive towards pyrimethanil the lower the temperature was. Due to high mortality no snails were available for the F1-generation at the highest concentrations (0.5 and 1.0 mg L⁻¹). Compared to the F0-generation, overall more egg masses were produced in the F1, while all egg masses were fertile and no mortality occurred. For the F1-generation the strongest pyrimethanil effects were detected at 15°C. A comparison of effect concentrations between both generations showed that the F1-generation is more sensitive than the F0-generation.

These results indicate that an exposure over more than one generation may give a better overview of the impact of xenobiotics. With the establishment of an embryo and reproduction test under different temperatures and various concentrations of pyrimethanil with *P. acuta* we could successfully show that molluscs can respond more sensitive than model organisms and that both, chemical and thermal stressor strongly influence the behaviour of the snails.

MO 255

Health condition of Minho and Lima Corbicula fluminea populations in relation to summer mass mortality events

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The Asian clam *Corbicula fluminea* is one of the most important non-indigenous invasive species (NIS) in aquatic ecosystems that has been causing considerable negative ecological and economic impacts, especially in Europe and North America. In the last decade, massive mortalities of *C. fluminea* have been recorded periodically at the end of the summer in the Minho River (NW Iberian Peninsula) which has been related with extreme events (draught conditions) and variations in water quality possibly including chemical stress effects. To go further on the causes contributing to such die-offs, a monitoring study investigating the health status of the population in relation to water quality and other abiotic variables has been carried out.

Several biomarkers and other parameters indicative of the health condition of the animals were investigated monthly between July and October. Results indicate changes in the health condition of animals (e.g. oxidative stress) in relation to the variation of abiotic conditions, and the multivariate analysis of data associates a decrease of health condition with individual abiotic factors. These findings are discussed in relation to the reduction of the diversity of the molluscan community of the Minho River estuary and ecosystem functioning.

This study was carried out in the scope of the project “NISTRACKS - Processes influencing the invasive behaviour of the non indigenous species *Corbicula fluminea* (Mollusca: Bivalvia) in estuaries - identification of genetic and environmental key factors” funded by the Portuguese Foundation for the Science and the Technology (FCT) (PTDC/AAC-AMB/102121/2008) and FEDER COMPETE funds (FCOMP-01-0124-FEDER-008556). C. Oliveira had a PhD grant from FCT (SFRH/BD/68423/2010) funded by national funds of the MCTES and FEDER European funds (POPH-QREN-Tipology 4.2.).

MO 256

Differential proteomic responses of larvae of the marine medaka *Oryzias melastigma* upon cadmium exposure at thermal extremes

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Upon facing unpredictable extreme temperature events under the human-driven climate change, marine ectotherms would generally be more susceptible to additional stressors like toxicants. This study aimed to reveal the stress responses in marine medaka larvae *Oryzias melastigma* upon exposure to cadmium (Cd) under different thermal conditions including extreme temperatures. Larval fish were thermally treated by elevating or decreasing temperatures at a rate of 1°C per hour followed by 7 days of acclimation to the test temperatures, before commencement of 4 days of Cd treatments. The associated toxic stress responses were elucidated using two-dimensional gel electrophoresis (2-DE) coupled with multivariate statistical analyses. Proteins of fish larvae from temperature treatment groups (i.e., 10°C, 25°C and 32°C) were successfully separated using IEF strip with pH 3-10 and gradient SDS-PAGE with gel percentage of 4-12.5%. The total- and phospho-proteins were visualized using Sypro Ruby and Pro-Q Diamond stains, respectively. Similar numbers of total protein spots (from 206 to 225) were detected in the three temperature treatments. Higher percentage number of phosphoprotein spots was however detected at 10°C (~25%) than at 25°C (~12%) and 32°C (~12%). This observation indicated that alteration of physiological pathways can take place at the low extreme temperature, i.e., 10°C. In view of the progressive decrease in whole-organism aerobic scope and shift towards anaerobic metabolism under the extreme temperatures, and needs for extra energy for detoxification and cellular repairing processes under chemical exposure, adverse toxic responses could be expected under the extreme temperatures. Proteomic response of the fish larvae under combined Cd and thermal stresses is under investigation. The results of this study could provide certain essential and fundamental information to further advance our understanding on specific toxic effects of trace metals under temperature extremes.

MO 257

The influence of fluctuating temperature on the effect of copper toxicity in *Caenorhabditis elegans*

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Climatic variables like humidity and temperature have been shown to affect the toxic effect of some chemicals. Temperature especially has a direct effect on the toxicity of metals and pesticides, generally causing toxicity to rise with increasing temperature, in particular when approaching the temperature tolerance limit of the test organism. Studies on the effect of temperature differences on toxicity have so far been done at static temperature regimes, which ignore any additional stress which may be caused by fluctuating temperature. Since daily temperature changes in the terrestrial environment can fluctuate with more than 10 degrees, it is questionable whether information from static conditions can be extrapolated to describing the effect of temperature on toxicity in the field. We hypothesize that fluctuating temperatures will prove to be a stress factor in itself, which may increase the effect of a toxicant, as compared to the equivalent static scenario at the average temperature. In the present study we expose the nematode *Caenorhabditis elegans* to a range of copper concentrations at three static temperatures (12, 16 and 20°C), as well as in a non-static temperature scenario with a daily temperature fluctuation from 12 to 20°C and an average daily temperature of 16°C. The temperature exposures were chosen to be within the approximate optimal temperature range of *C. elegans*. The study use full life cycle exposures, with daily measurements of reproduction and survival during the entire lifespan. To our knowledge this is the first study to compare the effect of static and fluctuating temperature on toxicity of copper.

MO 258

Effect of temperature on embryotoxicity of the antifouling biocide zinc pyrithione to *Sphaerechinus granularis*

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The present study investigated combined effects of the antifouling biocide zinc pyrithione and different temperature (17, 23 and 26 °C, control at 20 °C) on embryonic development of sea urchin, *Sphaerechinus granularis*. *S. granularis* embryos were reared in zinc pyrithione contaminated seawater at concentrations ranging from 10⁻⁸ to 10⁻⁶ M for 48, 72 or 96 h, respectively depending on the temperature. Embryological analysis was carried out to determine percent developmental anomalies and/or embryonic mortality. The median effective concentrations (EC50) were 65,9±8,3nM at 17°C, 83,1±15,2nM at 20°C, 81,5±10,8nM at 23°C and 55,9±15,9nM at 26°C for *S. granularis* embryos. Within the tested temperature range, increased developmental anomalies and/or embryonic mortality occurred particularly at concentrations of 50.0 and 500 nM zinc pyrithione. Our results verified that toxicities of the zinc pyrithione, were highly temperature-dependent. The EC50 values of *S. granularis* exposed to zinc pyrithione decreased with increasing temperature between 20-26°C, whereas the EC50 values at 17°C were significantly lower than 20°C. To conclude, higher temperatures increased the sensitivity of *S. granularis* embryos to zinc pyrithione stress.

MO 259

Temperature-dependent toxicity of DDT to larvae of the marine medaka *Oryzias melastigma*: pollutant responses at thermal extremes

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In light of recent predictions of an increase in average sea surface temperature (e.g., a rise of 3°C by the end of this century) and incidences of temperature extremes involved in global climate change, it is important to study the temperature-dependent chemical toxicities on marine organisms and elucidate toxic mechanisms associated with the interacting effect of thermal stress and pollutant exposure. The vast majority (>95%) of aquatic species are ectothermic, making temperature a key environmental factor in controlling their fundamental physiological processes. This study aimed to examine the combined effects of temperature and dichlorophenyltrichloroethane (DDT) pollution to larvae of the marine medaka fish *Oryzias melastigma* with a focus on their molecular and physiological responses. A stepwise acclimation was applied for the fish larvae at a rate of 1°C/h from 25°C to 13, 15, 20, 28 and 32°C, followed by 7 days of acclimation and another 4 days of exposure to DDT or the control. Cumulative mortality was under 50% for all selected thermal points at the end of acclimation period. Growth performances, in terms of length and weight, varied across different temperature treatments, having the lowest growth at 13 and 15°C. Active metabolic rate in terms of oxygen consumption was significantly higher at 32°C but was the lowest at 13 and 15°C. After 96h exposure to DDT, thermal treatment of 32°C did exert a significant influence on cumulative mortality and LC50. Further studies on enzyme activities (e.g., lactate, malate and succinate dehydrogenases) and expression of proteins (e.g. heat shock proteins) through advanced proteomic analysis are ongoing and the results may yield a mechanistic explanation on the observed synergistic effect of temperature and DDT.

ET12P - Soil ecotoxicology

MO 261

Are natural biocides used in agriculture safe for soil ecosystem? Case study of non-target bioactive compounds produced by rhizosphere soil *Streptomyces sioyaensis* TM 822

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Streptomyces sioyaensis TM 822 was isolated from turmeric rhizosphere soil at Chiang Mai, Thailand. Crude bioactive compounds produced by strain TM 822 showed antagonistic activity against Gram-positive bacteria, filamentous fungi and yeast, moreover, they were used directly for biological control of white root disease in rubber tree causing by *Rigidoporus* sp. This work, we aim to assess the ecological risk of emerging natural biocides used in agriculture. Impact in ecotoxicology of the crude bioactive compounds derived from the strain was tested with both eukaryotic and prokaryotic organisms. Lethal effect of the crude bioactive compounds against normal cell line; mouse fibroblast (3T3 cells) revealed less in cytotoxicity with a mean lethal dose (LD50) of 2.5 µg mL⁻¹ within 48 h of incubation, however, no lethal effect was observed in all concentrations tested during incubation for 12 h. The crude bioactive compounds exhibited stronger antifungal with a median effective dose (ED50) of 5.7% (v/v) against mycelia growth of *Rigidoporus* sp., compared to positive control (metalaxyl). Microbial numbers in agricultural soils that applied with the crude bioactive compounds varied according to the concentrations of the crude used. We suggest that suitable dose of either natural or synthetic biocides should be evaluated before use for agricultural purposes with avoiding side effect to biological components in soil ecosystem.

MO 262

Copper toxicity in soils amended with copper containing fungicides

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The long-term application of copper containing pesticides can cause a significant accumulation of Cu in soils. In order to assess the potential toxic effects of these increased Cu concentrations, a comprehensive comparison of Cu toxicity in a set of soils with high Cu concentrations due to the application of Cu pesticides and in corresponding reference soils was performed. Soils with a long history of application of Cu containing fungicides were sampled at 11 vineyards across Europe including an uncontaminated control soil outside each vineyard. Standard ecotoxicity tests for plant growth, root elongation, nitrification and invertebrates (*Enchytraeus albidus*) reproduction were performed on each soil of both the vineyard gradient and the corresponding control soil spiked with CuCl₂. For all vineyard gradients, the increased Cu concentration (maximum 349 - 689 mg Cu/kg) did not negatively affect the response of any test compared to the corresponding control soils. Spiking these control soils with CuCl₂ however resulted in clear dose-response curves and toxicity data for these freshly spiked control soils are within the range of toxicity data for these endpoints reported in the European Voluntary Risk Assessment Report (VRAR) on Cu and its compounds. Soil limits (Predicted No Effect Concentration, PNEC) calculated according to this VRAR are a factor 2.2 to 5.7 below the Cu concentrations observed in the sampled vineyard soils and therefore seem to be over-protective for soils contaminated with Cu due to

the long term use of Cu containing pesticides. It is assumed that long-term accumulation of Cu in soils due to the annual application of limited amounts of Cu, as is the case for Cu containing plant protection products, may result in stronger attenuation of Cu availability with time and hence a lower toxicity of Cu in soils compared to the default assumptions in the VRAR on the effect of ageing on toxicity. The effect of ageing is commonly taken into account in the effect assessment through the application of a so-called leaching-ageing factor. Therefore, a larger scenario-specific leaching-ageing factor is proposed for the effects assessment of Cu in soils affected by long-term application of Cu containing pesticides.

MO 263

Toxicity of Tributyltin (TBT) on terrestrial invertebrates and plants

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Tributyltin compounds are highly toxic organotins that were deliberately introduced into the marine environment by antifouling paints since 1960s. Their slow degradation rate and high adsorption to sediments and suspended matter contributed to the high levels of TBT found in sediments. Their disposal on land as well as the disposal of contaminated sewage sludge and application of TBT-based biocide products on agricultural fields constitute an important source of TBT contamination in soils. Due to their adverse effects to non-target organisms at very low concentrations (ng.l⁻¹) and bioaccumulative properties, TBTs are a hazard to soil flora and fauna.

This work aimed to assess the effects of TBT on invertebrates and plants species. An agriculture soil collected from Portugal was used for the TBT exposure.

For this evaluation several bioassays were performed: two feeding inhibition assays (with contaminated soil and with contaminated food) and one avoidance behaviour test were carried out with the isopod *Porcellionides pruinosus*. Likewise, a reproduction test with the collembolan *Folsomia candida* and seed germination and plant growth test with the rapid cycle turnip *Brassica rapa* and common wheat *Triticum aestivum* were also performed.

Feeding parameters (consumption, assimilation and egestion ratios) and isopod survival decreased when exposed to TBT. *P. pruinosus* was able to detect very low concentrations of TBT in soil and thus could possibly escape from the contaminated sites. A dose related response between the decrease in the number of juvenile springtails and adult survival with the increase of TBT exposure was also observed for the springtail exposure.

For the plants' exposures a dose-response relationship was observed, showing a delay and decrease on seed germination in both plant species *B. rapa* and *T. aestivum*. With increasing tributyltin concentrations, a decrease on growth parameters, including biomass production and plants' length, was also observed.

MO 264

Effects of zinc oxide nanoparticles on developmental stages of soybean in standard soil

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Long-term effects of zinc oxide nanoparticles to soybean (*Glycine max* (L.) Merrill) growths were evaluated in the OECD standard soil. The OECD standard soil was amended as 0, 50, and 500 mg/kg soil with ZnO nanoparticles (< 50 nm). Plant experiments were conducted in a greenhouse. The growth and developmental stages of soybean were measured during exposure period of 8 to 9 weeks. The results showed that development of soybean was significantly affected at 500 mg/kg dry soil. This developmental delay led to a failure in the formation of seeds. The results demonstrated that ZnO nanoparticles affected negatively the life cycle of soybean.

MO 265

Soil risk assessment with terrestrial model ecosystems (TME): Population dynamics of microarthropods under field (grassland) and semi-field (TME) conditions

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Since the PERAS workshop in 2007 Terrestrial Model Ecosystems (TME) are discussed as a higher tier option in soil Risk Assessment. In comparison with standard laboratory tests they maintain an intact and complex soil community with a lot of potential faunistic endpoints from e.g. the taxa Collembola or Oribatida under natural climatic conditions.

Until now several experiments have been conducted to study the TME community under different management [Theißen et al. 2010, Toschki et al. 2011] or pesticide application regimes [e.g. Scholz-Starke et al. 2011]. The results did either show no detrimental effect (management) or just short term effects (pesticide application) indicating, that the system itself can be resilient due to external impacts. Nevertheless these studies were conducted under artificial conditions testing a TME community potentially being under disturbance in the new test facility situation.

There is no doubt that TME entail some limits inherent to the system. In contrast to the field the horizontal mass flow, the growth of plant roots as well as the emigration/immigration of animals is inhibited. Thus life within TME is constrained to a small scale, potentially selecting organisms e.g. with restricted spatial requirements.

In our study we wanted to examine the resilience of the former meadow community after its transfer into TME steel cylinders. We present data on the population dynamics of springtails and mites in TME and the meadow, where the TME we cored from. Results shall contribute to a discussion to what extent TME can reflect natural conditions.

Theißen, B., Scholz-Starke, B., Hammers-Wirtz, M., Schäffer A., Kölzer, U., Leicher T., Roß-Nickoll M. (2010): Does "sequentially sampling" influence population dynamics of soil mesofauna in TME? Poster, SETAC Europe 20th Annual meeting 23. - 27. May 2010, Sevilla, Spain.

Toschki, A., Hammers-Wirtz, M., Hoelscher, C., Ross-Nickoll, M., Theissen, B. (2010): Requirements for Terrestrial Model Ecosystems (TME) in Environmental Risk

Assessment - influence of coring sites and management on microarthropod communities. Poster SETAC Europe 21st Annual meeting 15- 19. May 2011, Milano, Italy.

Scholz-Starke, B., Nikolakis, A., Leicher, T., Lechelt-Kunze, C., Heimbach, F., Theißen, B., Toschki, A., Ratte, H. T., Schäffer, A., Roß-Nickoll, M. (2011): Outdoor Terrestrial Model Ecosystems are suitable to detect pesticide effects on soil fauna: design and method development. *Ecotoxicology*, 20(8):1932-1948.

MO 266

Phosphogypsum as a soil fertilizer: evaluation of the impacts on soil habitat and retention functions

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Phosphogypsum (PG) is a metal and radionuclides-rich by-product of phosphoric acid production. For every tonne of phosphoric acid made, from the reaction of phosphate rock with acid, commonly sulfuric acid, about 3t of PG are created. There are three options for managing PG: (i) disposal or dumping (ii) stacking and (iii) re-use in agriculture, construction, or landfill. Despite some authors have suggested the use of PG as a fertilizer, to the best of our knowledge, till now any evaluation was made about the real impacts of soil amendment with PG, on soil habitat and retention functions. In this work reproduction assays with earthworms (*Eisenia andrei*), collembolans (*Folsomia candida*), enchytraeids (*Enchytraeus crypticus*) and mites (*Hypoaspis aculeifer*) and seed germination and growth assays with plants (*Zea mays* and *Lactuca sativa*) were performed to assess the effect of soil amendment with 2.5, 16.6, 11.1, 7.4, 4.9% of PG, following standard protocols. The standard OECD soil was used as test substrate for these amendments. Further soil elutriates were obtained from OECD soil amended with 6.25, 12.5 and 25% of PG, preparing soil suspensions with water in a proportion of 1:4 (m/v). Assays with the bacteria *Vibrio fischeri*, the cladoceran *Daphnia magna*, the green algae *Pseudokirchneriella subcapitata* and the aquatic plant *Lemna minor* were performed to assess the toxicity of elutriates, testing a range of dilutions as suggested by standard guidelines. As far as assays with soil invertebrates are considered a similar hormetic pattern in reproduction inhibition was observed for all the species. The number of juveniles produced was significantly lower in soils with the lowest and highest concentrations of PG. No toxic effects were recorded for plants. Results obtained for the aquatic assays, showed no toxicity of all elutriates for *L. minor*, as well as only a slight inhibition in the growth of *P. subcapitata*, *V. fischeri* and *D. magna* were more sensitive. Only the elutriate obtained from soil amended with 6.25% of PG was not toxic to the bacteria. However, all the elutriates were acutely toxic to *D. magna*, despite an EC50 close to 100% has been obtained. The results gathered in this study raise concerns about the potential contamination of freshwater resources caused by amendment of soil with PG for agricultural purposes. However, it stills precocious to advice the use of PG for such purpose before assessing cumulative effects of amendment within other aspects.

MO 267

Modeling effects of NaCl and of its interactions with organic matter and pesticides towards soil fauna

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Soil salinization consists in the accumulation of salts (mainly NaCl) in the upper layers of the soil by natural processes and human activities, which can cause various problems, including soil desertification. This threat to the soil system become a growing concern in the recent years, mainly in the Pannonian and Mediterranean regions.

To complement the published information on the effect of salt as a stressor for soil fauna, avoidance and/or reproduction tests were performed with three soil organisms: *Folsomia candida*, *Eisenia andrei* (avoidance tests only) and *Enchytraeus crypticus* (reproduction tests only). The effect of NaCl alone (1, 2 and 4g/Kg soil), as well as the interaction between salt and soil organic matter content (1%, 2.5% and 5%), was tested in these endpoints. Furthermore, the interaction of NaCl with a commonly used agricultural pesticide (Lambda-cyhalothrin) was also tested using reproduction tests with *F. candida*.

Generalized Linear Modeling (GLM) techniques were used to predicted avoidance and reproductive responses due to NaCl, its interaction with soil organic matter and also to evaluate the shift in toxicity of the Lambda-Cyhalothrin when tested on saline soils.

Results showed an increased avoidance response with increasing salinity on both test organisms, with earthworms showing a much higher sensitivity than collembolans.

Reproduction was impaired starting at medium salinity (2g NaCl/Kg soil) in *F. candida* and at low salinity (1g NaCl/Kg soil) in *E. crypticus*, and ceased completely at high salinity (4g NaCl/Kg soil). Models for the avoidance response were able to explain only 45% of the variation on both test organisms, and were able only to predict the response up to a certain level of salinity. In opposition, models on reproduction data explained over 70% of the variance in reproduction data. Although NaCl was the major driver of the responses obtained, all models showed a significant response of the OM content and of the interaction between these two factors. Lambda-Cyhalothrin caused a dose-response decrease in the reproductive output of *F. candida* in all NaCl treatments tested but the models showed the positive interaction with salt, with EC50 values increasing from low to medium salinities.

MO 268

Using the comet assay to determine species sensitivity of earthworms to copper

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The endpoints normally used to construct Species Sensitivity Distributions (SSDs), comparing the various sensitivities of species for toxicants, are predominately whole body or life-cycle characteristics. Biomarkers are easy and rapid tests. This study investigated whether a biomarker, the comet assay, could be used to compare species sensitivity between earthworms. This assay, measuring DNA damage in the form of single strand breaks, gives an indication of the genotoxicity of a toxic substance for an exposed organism. Four earthworm species from three different families were experimentally exposed to copper in the form of the fungicide, copper oxychloride. The species used were a lumbricid species, *Eisenia andrei*, two megascolecid species *Perionyx excavatus* and *Amyntas diffringens* and *Chilota* spp., an endemic South African species of the family Acanthodrilidae.

At toxic concentrations, Cu can be damaging on various levels and, as shown by the results of the present study, also acts as a genotoxic agent. Adult specimens were exposed to the fungicide in artificial OECD soil, with benomyl as positive control. An exposure concentration series of 0, 20, 80, 160, 320 and 640 mg/kg Cu was used. Exposures were done in four replicates for a duration of 14 days. After the exposure period coelomocytes were harvested from the organisms and the cells prepared for the comet assay according to the method of Singh et al (1988) as adapted by Reinecke & Reinecke (2004).

The Tail DNA % parameter was used to determine the comet values. The highest mean damage to the DNA at all exposure concentrations was observed in *A. diffringens* and the lowest in *P. excavatus*. EC10, LOECs and NOECs were determined and SSDs constructed using EC10 and LOEC values. These showed that *A. diffringens* was the most sensitive and *P. excavatus* the least sensitive species of the four tested. We conclude that a biomarker, such as the comet assay, can give reliable and useable data from which to infer sensitivity differences between species.

MO 269

Determining chemical effects on earthworms at the population level through the use of energy budget and individual based modelling

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Standard OECD test methods for measuring the toxicity of pesticides on earthworms use optimal laboratory conditions to record specific endpoints e.g. mortality, growth and reproduction. These data are compared to exposure values in accordance with EU guidelines for registration of agrochemicals, and the resulting risk quotients are compared to arbitrary safety factors for acute and sublethal risks to earthworms. When these triggers are not passed, field trials are required. Field trials are expensive, time-consuming and variable, providing coarse information about population-level effects. Mechanistic population modelling of organism responses to chemicals has the potential to act as a reliable intermediate stage in higher tier risk assessments and can provide greater resolution to help identify key parameters driving population-level effects. Energy budget models relate individual life cycle processes to one another through energy and mass conservation. Organisms uptake resources from their environment and expend assimilated energy on maintenance, growth and reproduction. The allocation of energy to these different subsystems depends on a combination of environment- and organism-specific conditions. Here we present a general energy budget model, based on broadly accepted fundamental principles. The model is applied within an individual-based model (IBM) framework, which allows individuals to interact with their simulated environment, giving an insight into life cycle processes affected under variable environments and chemical exposure. This enables ecotoxicological inferences to be made about population dynamics under realistic field conditions, as the biology of individuals relates to the ecology of populations through metabolism. Standard toxicity data, including NOEC and EC50 values, can be used as input data to the model, highlighting its usefulness as a method in higher tier risk assessments. We use data for the OECD recommended earthworm species *Eisenia fetida* to validate the model for use in predicting ecotoxicological effects. We discuss the potential of the model in decision-making for pesticide regulation and environmental management, particularly in comparison to alternative modelling techniques. Future studies aim to utilize the flexibility of the model to extrapolate population-level ecotoxicological effects between the varied ecological niches of different earthworm species.

MO 270

Ecotoxicity of phenanthrene and its nitrogen-containing analogues to the earthworm *Eisenia fetida* in soil

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Major sources of these homocyclic and N-heterocyclic polycyclic aromatic hydrocarbons (PAHs) are found in the environment being discharged from petrogenic, pyrogenic and biogenic sources. The toxicity of soil amended with phenanthrene a three ringed PAH and its nitrogen heterocyclic aromatics (NHAs) analogues were assessed on the earthworm *Eisenia fetida*. The effects were studied over a 21-day incubation, during which health and mortality were assessed. During the 60 day incubation, LC50 and EC50 ranged from nd-606 mg/kg and 4.8-574 mg/kg respectively. NOEC levels were derived from EC10 values: estimated NOECs ranged from 0.01 to 204 mg/kg. LC50 and EC50 results were compared to other studies from literature and our results differed significantly. The results of this study showed that NHAs were more toxic in soil than homocyclic PAHs (with aging). This can be attributed to their higher solubilities and lower K_{ow}s. Based on visible pathological changes, NHAs were not only carcinogenic, mutagenic, teratogenic and genotoxic, they may also have been clastogenic to soil organisms especially benzo(h)quinoline. These differences might be useful in soil contamination / risk assessment procedures and also provides basis for regulatory action.

MO 271

Toxicity to the earthworm *Eisenia fetida* of two transformation products of amines used in carbon capture technology

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The aim of carbon capture and storage technology is to minimize greenhouse gas emissions through a reaction between amines and effluents from gas power plants. During the overall process of CO₂ capture, amines and their transformation products might escape to the environment through emissions, leakage, and as solid waste. Although amines in some applications are known to represent a health risk, there is still a lack of knowledge on environmental hazards and risks related to amines and derivatives used in CO₂ capture. The aim of this study was to assess the acute and chronic toxicity to the earthworm *Eisenia fetida* of two transformation products of amines, dimethylnitramine (DMNA) and 2-(nitroamine) ethanol (NAE), following the OECD guidelines 207 and 222.

Acute tests demonstrated that neither DMNA nor NAE induced acute toxicity in earthworms, even at a maximum concentration of 1000 mg/kg dry soil. Therefore we conducted a reproduction test at 1000 mg/kg only, as recommended by the OECD guideline 222. At 1000 mg/kg, both DMNA and NAE impaired the reproduction of *Eisenia fetida*, although NAE was more toxic than DMNA. In comparison to control worms, the production of cocoons was reduced by 30% and 70% by DMNA and NAE, respectively. The production of juveniles was reduced by 20% and 80% by DMNA and NAE, respectively. NAE induced a reduction in hatchability by 35%. In contrast, DMNA did not reduce hatchability but delayed hatching, as shown by the smaller size of juveniles. Finally, the number of juveniles per cocoon was slightly higher (although not statistically significant) when worms were exposed to DMNA. Evidence of reproductive toxicity at a single high concentration suggests assessing the effects of these compounds at several lower concentrations in order to get the EC_x required for risk assessment.

MO 272

New earthworm endpoints based on coelomocytes

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Earthworms are used as test organisms in environmental pollution assessment. Used in studies of acute and chronic toxicity determining how response variables such as mortality, growth and reproduction. In addition the group offers ability to make assessments of biomarkers of oxidative stress and genotoxicity. In the last case coelomocytes isolated by extrusion of the coelomic liquid are frequently used. Among them there are three main groups with different functions: eleocytes, amebocytes and granulocytes. The relative proportions of these cell types in the body are related to nutritional and physiological status. The cultivation of these animals in the laboratory is relatively simple and is a means to obtain cells in a non-invasive or destructive method. In this work we explored a set of indices that allow us to recognize the trophic status of individuals prior and during their use in ecotoxicological studies. This set of endpoints include:

Whole Body Coelomocytes Index (WBCI): $\log_{10} (Cn/wwf)$ where Cn is total coelomocytes number average per individual / mL of celomic fluid; and wwf is wet weight without feces. Absolute Trophic Index Earthworm (ATIE): (En/Cn) where En is total eleocytes number average per individual / mL of celomic fluid. Relative Trophic Index Earthworm (RTIE): $ATIE/wwf$.

MO 273

Effects of the use of onshore drilling cuttings as soil amendment on reproduction of *Eisenia andrei*

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The onshore procedures of an oil drilling well generate a great amount of drilling cuttings that are composed of rocks, sand, oil residues and drilling fluid. The cuttings volume produced by an oil well, estimated from 0.6 to 6 barrels, depends on reservoir depth, diameter, geological features and drilling fluid type. The drilling cuttings are treated to recover the fluid, using different equipment, as cuttings dryer and centrifuge. A common drilling fluid in Brazil contains barium sulfate (Ba SO₄), what requires an appropriate disposal or safely utilization. One alternative use is as soil conditioner to increase pH in acidic soils. However, the cuttings have other potential contaminants as arsenic, lead, molybdenum, zinc and sodium that can act in a synergistic harmful way to soil organisms. The objective of this study is to test mixtures of drilling cuttings and soil to provide information about the potential toxic effects of this residue to soil non-target organisms. Reproduction assays with *Eisenia andrei* were performed using two types of cuttings: from the drier and from centrifuge, with artificial OCDE soil (10% organic matter content). The barium doses tested were: 300, 900, 1500, 2100, 2700 e 3000 mg Ba.soil kg⁻¹, which correspond to critical values determined by Brazilian law. Results demonstrated that the waste from the cuttings drier (EC 50 = 114 mg Ba.soil kg⁻¹) had a more deleterious effect to *E. andrei* reproduction than the waste originated in centrifuge (EC 50 = 797 mg Ba.soil kg⁻¹). A more detailed ecotoxicological study has to be carried out to ensure the environmental safety of the cuttings as soil conditioner in agriculture.

MO 274

Methodological improvements in the study of pollutant-induced toxicity on the earthworm *Eisenia andrei*: the effects of chromium(VI) on coelomocytes physiology

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Over the last decades, the widespread use of chromium by industries has led to numerous cases of environmental contamination by this element. In mammal cells, Cr(VI) is reduced to Cr(III), a process during which reactive Cr(V/IV) intermediates and reactive oxygen species (ROS) are generated. Cr(VI) is widely known to cause toxic and genotoxic effects in vertebrates. Relatively less investigated are the effects of Cr(VI) on invertebrates, particularly on the edaphic organisms. *In vitro* and *in vivo* studies were performed on *Eisenia andrei* to investigate the biological effects of environmentally relevant concentrations of Cr(VI). In this study, we show the results obtained on coelomocytes of earthworms exposed for 1 and 3 days to different sublethal concentrations of Cr(VI) using the filter paper test. Coelomocytes are the cells circulating in the coelomic fluid and playing an important physiological role in the immune defence of earthworms. Cr(VI)-induced oxidative stress was investigated by evaluating the radical production using fluorescent probes, the lysosomal accumulation of lipofuscin and the oxidative damage to DNA. Moreover, to highlight changes in cellular physiology, mitochondrial activity and lysosomal membrane stability were analysed. Pathological reactions involving the lysosomal system are often linked to augmented autophagic sequestration of cellular components. *In situ* FITC diacetate labeling of intracellular proteins was performed to assess the transport into autophagic degradative compartments of these proteins. In addition, the leakage of lactate dehydrogenase in the coelomic fluid was determined to reveal cell membrane damage. Finally, the immune response of coelomocytes was studied assessing the phagocytic activity of the cells. The results demonstrated significant changes in the different parameters evaluated on coelomocytes of treated earthworms. Cr(VI) provoked, just 1 day after the exposure to lower doses, an increase in ROS levels, generating in the cells oxidative stress conditions leading to lipid peroxidation with lysosomal lipofuscin accumulation and oxidative damage to DNA. Cr(VI) also caused alterations of intracellular compartments till to reduce, at the highest dose and at the longer exposure time, the immune response of the cells. Overall, the data demonstrated that oxidative stress is an important factor involved in Cr(VI)-induced toxicity and genotoxicity in *E. andrei* coelomocytes.

MO 275

An exposure regime that brings more realism in the testing of plant protection products in soil?

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A modified exposure regime for Plant Protection Products (PPP) in soil eco-toxicological test systems (e.g. earthworm, collembola, or predatory mite reproduction test) is proposed in order to achieve more realism in the risk assessment. Beside active substances, the toxicity of PPP is often influenced by highly volatile solvents in formulations (i.e. in emulsion concentrates), which degrade fast and occur only in the top soil layer. In the risk assessment the Predicted environmental concentrations in soil (PECsoil) are compared to the endpoint in the laboratory test and a safe use is indicated if an assessment factor of 5 is surpassed. For persistent active substances (a.s.) the PECsoil consists of the plateau concentration (only the a.s.) and the initial concentration (the a.s. within the product). However the potential effects of the formulation inerts within the end use product are limited in space and time. Thus, the risk of PPP to soil organisms may not be correctly reflected if a) the NOEC is related to a PECsoil calculated for 0-1 cm soil depth and b) if the end use product is evenly distributed in the soil layer to establish the test concentration in the laboratory test.

In this study the effects of a PPP (fungicide; EC formulation with 2 persistent a.s.) on the reproduction of *Eisenia fetida* (OECD, 222) will be determined according to the currently valid test design and compared to a more realistic exposure scenario. The realistic exposure regime considers a plateau concentration (adjusted with the technical grade a.s.) at 0-5 cm soil depth. The test organisms are inoculated and the end use product is applied by overspray application. Finally, the total concentrations of the a.s. in the treatment groups can be calculated as the sum of two components: a) the plateau concentration and b) the calculated concentration from the overspray application, considering a soil bulk density of 1.5 g/cm³ and a soil depth of 0-2.5 cm or 0-1 cm, depending on the KOC-values of the a.s.

This modified test design considers two aspects improving the realism of exposure of PPP in soil eco-toxicological test systems: 1) a more realistic exposure of soil organisms to formulation inerts and 2) a more realistic distribution of the PPP in the uppermost top soil layer, considering the specific sorption properties of the a.s. This modification would improve the realism without losing the conservativeness necessary in a tier I test system in the registration process for PPP.

MO 276

Introducing the 'ResOrgRisk' Project: environmental risk assessment of the use of organic residues as soil amendments

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The amount of organic residues (ORs) generated worldwide is increasing and environmentally sound strategies for their disposal and recycling must be developed and optimized. Council Directive 1999/31/EC has set down strict mandatory targets for reduction in the amount of ORs reaching landfill; by keeping organic matter away from landfills, their nutrients can be recycled, less methane is emitted at the landfill, and leachates produced are less polluted. Recycling ORs (e.g. sewage sludge, compost, agro-food industrial wastes) in agriculture is considered a way of maintaining or restoring the quality of soils, enlarging the slow cycling soil organic carbon pool. However, a wide variety of undesired traits, such as heavy metals and organic contaminants, can have adverse effects on the environment. In fact, the Proposal for a Soil Framework Directive recognized that "soil degradation or soil improvements have a major impact on other areas, ([3DOTS]) such as surface waters and groundwater, human health, climate change, protection of nature and biodiversity, and food safety". Taking that in account, the aim of the Project "ResOrgRisk" is to assess the environmental risk of the use of ORs as soil amendments, evidencing their benefits and constraints, and emphasizing which tests are more trustworthy to assess their quality and the environmental risk of their application.

The study will rely on integrated results obtained by a multidisciplinary team based on:

- (1) Chemical, biochemical, microbial and ecotoxicological characterization of the ORs, of soils amended with ORs, and of their leachates/extracts;
 - (2) Ecotoxicological and ecological evaluation of effects and fate of pollutants derived from the application of different ORs to agricultural soil using TME's (Terrestrial Model Ecosystems) experiments;
 - (3) Evaluation of chemical, biochemical, microbial, ecotoxicological and ecological effects of the application of ORs to soils in field experiments.
- Classical and instrumental chemical analysis will be used to quantify physico-chemical characteristics of the materials. Ecotoxicity tests will be used to provide an integrated measurement of both environmental and toxicological bioavailability of the contaminants, and their detrimental effects in the ecosystem. The effects in soil microbial communities will be assessed evaluating microbial indicators, and using molecular methodologies.

MO 277

Validation of the Czech approach to dredged sediments ecotoxicity assessment

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Research is focused on the actual environmental issue - a dredged sediment application on soils and preceding evaluation of their ecotoxicity to prevent possible ecological risks. High volumes of sediments are removed from rivers and ponds routinely. Although they may have a lot of positive properties (e.g. high nutrient levels) there are reasonable suspicions about contaminants present accumulated sediments. Therefore, an assessment scheme is necessary which helps to decide, if the land application will be allowed for every dredged sediment. This assessment cannot be based only on chemical analyses and should be accompanied by ecotoxicological testing. In the Czech Republic, novel directive 257/2009 Coll. determines the limits for the dredged sediments to be applied on agricultural soils. There are also 4 ecotoxicological bioassays suggested in the directive. In our study, we verified practicability and reasonability of ecotoxicological testing of dredged sediments using thirty-six sediment samples and wide battery of ecotoxicity bioassays, both for solid and eluate samples. The aim was to compare directive bioassays with other soil bioassays (for example, faster and more practical ones) and to compare bioassays of eluates with the soil bioassays.

MO 278

Selection of relevant ecotoxicity tests to assess the effects of biosolids used in agriculture

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In modern agriculture and in accordance with environmental sustainability, biosolids are applied on fields. These materials are used as fertilizers, to maintain a high production rate of plants. But if they are a source of nutrients, they can also be potentially a source of pollutants. As a consequence they can affect plants, soil fauna and finally humans. In this study, environmental effects of different biosolids (sewage sludge, manure, composted materials [3DOTS]) were studied using ecotoxicity tests on soil and aquatic organisms. The aim of this work was to establish a test battery to assess the effects of biosolids, by comparing their respective sensitivities towards these materials. Organisms composing the biotest battery and their respective acute and/or chronic endpoints are the followings:

- algal growth inhibition of *Pseudokirchneriella subcapitata* (NF EN ISO 8692)
- inhibition of growth and reproduction of the nematode *Caenorhabditis elegans* for solid and liquid media (ISO 10872)
- inhibition of the mobility of the crustacean *Daphnia magna* (NF EN ISO 6341)
- inhibition of population growth of the rotifer *Brachionus calyciflorus* (NF ISO 20666)
- inhibition of light emission of the bacteria *Vibrio fischeri* (NF EN ISO 11348-3)
- inhibition of root growth, emergence and growth of higher plants (ISO 11269-1 and ISO 11269-2)
- avoidance and reproduction inhibition of the earthworm *Eisenia fetida* (ISO 17512 and ISO 111268-2)

All of these bioassays were conducted using their respective standardized protocol, excepted for *C. elegans* soil tests which were carried out using the method detailed in another work (in Huguiet *et al.*, Applicability of the *C. elegans* survival, growth and reproduction test to assess the effect of solid wastes; same conference topic). Concerning samples preparation, standard soils (ISO and/or LUFA) were mixed with biosolids at defined doses (application rate, and e.g. 2 times, 5 times, 10 times, 20 times the

application rate) to determine toxicity thresholds. For liquid samples, water extracts of the mixtures were obtained following the standard EN 14735. Results of a limed sludge, a cow manure, ashes and a compost obtained from domestic wastes will be presented and discussed in details.

MO 279

Effects assessment of biosolids soil amendments on terrestrial organisms

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Use of municipal solid waste (MSW) and sewage sludge composts is a common practice to improve the chemical and biological characteristics of impoverished soils by supplying soil organic carbon content and nutrients. Nonetheless, they not only influence soil fertility, but may also be a route of entry of emerging pollutants to consequently affect soil organisms, plants and microorganisms. Consequently, there is increasing concern for the presence of these pollutants and the need to assess their environmental risk. This work aims to identify the effects of biosolids directly or indirectly on terrestrial organisms and plants, and aquatic organisms, respectively, in order to assess environmental risks and to provide information which may be useful for decision-making support systems on both the national and international regulatory scale.

A terrestrial microcosm was carried out with four biosolids (MSW composts, and sewage sludge: compost or thermal drying sludge) obtained from different Spanish solid urban waste treatment plants to assess the effects on organisms. Biosolids application rates were calculated by considering the N requirement of plants and were added to control the soil used to fill the microcosm columns. After 21 exposure days, the effects on soil invertebrates, plants and microorganisms were assessed. Acute toxicity tests (*Daphnia magna*, *Chlorella vulgaris*) were also performed on leachates.

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MO 280

Analytical and ecotoxicological characterisation of the biosolid samples collected from Spanish wastewater and municipal solid waste treatment plants

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Biosolids are end products of wastewater and municipal solid waste (MSW) treatments processes and are, therefore, rich in nutrients and organic matter. Regulations on the presence of pathogens, nutrients and metals in biosolids for use as agricultural soil amendments are clear; however, the contents of organic compounds, such as pharmaceutical, personal care products (PCPs), endocrine disrupting compounds (EDCs) or persistent organic pollutants (POPs), are not yet regulated. Indeed, Spain and other EU Member States have been considering reviewing the two European Directives on applications of composted MSW (Directive 86/278/EEC) and sludge WWTPs (2nd Draft of Biological Treatment of Biodegradable Waste, 2001) in soil for agricultural purposes to establish new limits for the pollutants first considered and to increase the number of analysed substances as regards new emerging organic compounds. To assess the potential environmental risk of biosolids, it is necessary to know their chemical composition and their effects. This study aims to obtain information about the analytical and ecotoxicological assessment of 16 biosolids collected from different Spanish wastewater and MSW treatment plants as a previous step for use in microcosms and field studies. The results of the analysed samples show similar ranges to those found in references from other works using biosolids collected in other countries.

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MO 281

Ecotoxicological fate and effects of German river sediments

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Sediments are well-known to act as a sink and secondary source for environmental pollutants. The joint research project DanTox, funded by the German Federal Ministry of Education and Research (<http://www.bio5.rwth-aachen.de/DanTox>, 2009 - 2012) aims to develop combined vertebrate-based sediment contact assays to investigate teratogenicity, neurotoxicity, genotoxicity, mutagenicity and Ah-receptor agonists as well as to analyze the gene expression (microarray and qRT-PCR) in embryos of the zebrafish (*Danio rerio*). For this purpose, three model sediments were selected, two from the Rhine River (Altrip and Ehrenbreitstein) and one from the outer Vering Canal in Hamburg. First, the sediments have been ecotoxicologically characterized with standardized routinely applied bioassays. This is important for ranking the results achieved with the newly developed methods in the context of routinely applied assays. The poster presents results from the bioassay battery applied. Toxicity of sediment soxhlet extracts transferred into DMSO (20 g mL⁻¹) has been determined in the Algae growth inhibition test (OECD 201, 2006), the *Daphnia* acute toxicity test (OECD 202, 2004), the Fish acute toxicity test (OECD 203, 1992), the Fish egg test (ISO 15088, 2007), the Lemna growth inhibition test (ISO 20079, 2005), the Luminescent Bacteria test (ISO 11348, 2007), the Ames test (ISO 16240, 2005) and the umuC test (ISO 13829, 2000). Freeze dried sediments have been investigated in the Fish egg sediment contact assay. Genotoxicity was evaluated in the Comet assay with zebrafish eggs exposed for 48h post fertilization. In addition, toxicity of freeze dried sediments towards bacteria was investigated using the Arthrobacter contact test (ISO/DIS 10871, 2009). Algae turned out to be the most sensitive species. EC₅₀ values for all three sediments were in the range of 1 mg SEQ/L (sediment equivalent). *Daphnia* and fish were at least 10 times less sensitive. Two sediment extracts (Altrip and Vering Canal) were found to be mutagenic in the Ames test. One sediment (Ehrenbreitstein) turned out to be genotoxic in the umuC test. The results obtained so far do not indicate genotoxic effects with the Comet assay performed with zebrafish eggs. For several assays, DMSO concentration in the test solution was limiting the determination of ECx values. Here, only limit tests with the highest possible DMSO concentration could be performed. Analytical data are available for all sediments tested.

MO 282

State of the art of soil ecotoxicology standards in Brazil: challenges and perspectives

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Soil ecotoxicology in Brazil had a conceptual mark in 1988 when it was published by the extinguished Special Office of Environment (SEMA) the Handbook of Tests for the Ecotoxicity Evaluation of Chemicals. In that document it was described a single method for soil organism toxicity evaluation: a method (Norm X31-250, 1984) using earthworm (*Eisenia fetida*) based on AFNOR (ASSOCIATION FRANÇAISE DE NORMALIZATION). This method was applied during several years for pesticides registry and the study of pesticides ecotoxicity was also the major interest in soil ecotoxicology of the Brazilian researches during the last years. In 2002 it was created the Special Committee of Ecotoxicological Analysis of ABNT (Brazilian Association of Technical Standards), an organization member of ISO - International Organization for Standardization - responsible for the development of Brazilian Standards in many areas. The Committee of Ecotoxicological Analysis was created based on the increasing demand related to quality and new tests, for example, using soil organisms. In 2004 an increasing in soil ecotoxicological studies was noted in Brazil mainly because of the rising of academic and research groups in Brazil together with Germany and Portugal. So, in 2006 ABNT Committee wrote the first Brazilian Standard related to terrestrial ecotoxicology and soil quality (ABNT NBR 15537:2007, acute toxicity test with earthworm), based on the translation of ISO 11268-1:2003. Nowadays there are six Brazilian Standards published (tests with earthworms, collembolan, and soil flora; guidance for ecotoxicological characterization of soil and soil materials) based on ISO Standards (ISO 11268-1:2003, ISO 17512-1:2008, ISO 11267:1999, ISO 11269-2:2005, ISO 17616:2008, ISO 15799:2003). The work will continue and the group intends to translate ISO Standards that could be applied on contaminated sites, an increasing problem in Brazil. These standards will be the basis for the development of new researches and tropical findings that would be incorporated in the next revisions. The members of ABNT Committee of Ecotoxicological Analysis believe that this is just the first step because a lot need to be done but the availability of standards written in portuguese and acquired with local money could be an important way for the development of soil ecotoxicology studies in Brazil.

MO 283

Derivation of soil screening levels (SSLs) for Uranium, Cooper and Cadmium using a natural reference soil

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Aimed in evaluating and managing land contaminated with metals, many countries have been adopting generic quality standards, the soil screening values (SSV). SSVs are particularly useful for the first tier of Ecological Risk Assessment processes applied to contaminated sites, allowing decision-making in this initial stage of assessment, based on the chemical line of evidence. Due to the influence of soil properties in the toxicity of metals, SSVs derived based on specific soils could not be appropriate for site-specific assessments. Hence, a Portuguese natural soil (PTRS1), representing one of the dominant types of soil from a granitic region (cambisol), was characterized and used as substrate for ecotoxicological tests aimed at deriving SSVs for national assessments of contaminated sites. An extensive chemical characterization was initially done in terms of pseudo-total metals, PAHs, PCBs and pesticide contents. The performance of invertebrate (*Eisenia andrei*, *Folsomia candida*, *Enchytraeus crypticus*) and plants species (*Avena sativa*, *Lycopersicon esculentum*, *Zea mays*, *Lycopersicon esculentum*) was also tested to evaluate the performance of test species in this natural soil. After this deep characterization, the soil was used to perform sub-lethal ecotoxicological tests with the same species of invertebrates and plants aimed at deriving NOEC, LOEC, EC20 and EC50 values for uranium, copper and cadmium. *Eisenia andrei* proved to be less sensitive than *Folsomia candida* to uranium contamination. For plants, since concentrations till 1000 mg Kg⁻¹ were tested, we can say that uranium does not yield a toxic effect in the endpoints evaluated in these species. NOEC values of 101.69 mg Kg⁻¹ and EC50 values of 131.23 mg Kg⁻¹ were recorded for copper in the reproduction of the *E. Andrei*. *Folsomia candida* showed to be less sensitive than *E. andrei* to copper when reproduction was assessed (EC50 163.3 mg Kg⁻¹). All species revealed to be high sensitive to cadmium contamination. The EC20 values estimated using the logistic model were remarkably lower than the NOEC values, hence they were selected for the derivation of more protective soil screening values, which were determined integrating all the ecotoxicological data obtained.

MO 284

Environmental risk assessment in the São Domingos Mine area (Portugal). Tier I: screening phase

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The present study constitutes the Tier 1 of the site-specific ecological risk assessment (ERA) of the São Domingos Mine (South of Portugal), integrating information from the chemical line of evidence (metal analysis and calculation of toxic pressures) and the ecotoxicological line of evidence (*Vibrio fischeri* solid-phase test, *Folsomia candida* avoidance test and *Avena sativa* seed germination test), which were integrated to calculate risk values. A total of 62 soil samples were collected along six transects perpendicular to a central axis (CA) which was coincident with the inactivated railway line that was used for ore transport across the area; at each transect samples were taken at 0, 20, 50, 150, 400, and 1000 m distant from the CA, both on left and right sides. First, a multivariate factor analysis was run on various soil properties (silt/clay, fine sand, coarse sand, and organic matter contents, conductivity, water holding capacity, and pH) to group the soils. Four groups of soils were defined indicating the need to use a multi-reference approach. To comply with the latter three additional soil samples were collected (at 15 to 20 Km from de CA), to be used as reference material, together with a soil from one of the transect points.

Total metal and arsenic concentrations showed high heterogeneity among samples with most points along the CA exceeding the benchmark values. Results showed that the ecotoxicological tests used were adequate to assess the ecotoxicological potential of the soils collected. As predicted, integrated risk values decreased with increasing distance from the CA. The importance of using a multiple test battery to screen the toxicity of soil as Tier 1 of a site-specific ERA was demonstrated since it allowed clarifying some false positives or false negatives revealed by chemical analysis on its own. However, the risk uncertainty still persisting at some points highlighted the need for a more detailed evaluation, which will constitute the next stage of the risk assessment (Tier 2).

MO 285

The impact of red mud on reproduction of the collembolan *Folsomia candida*

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Red mud is a residue generated from the refining of bauxite for alumina production (Al_2O_3) by the Bayer process. The main features of this residue is the high alkalinity, high salt contents and the clay texture. Brazil is the third largest producer of alumina, resulting in an estimated red mud production up to 16 million tons each year. The disposal of this residue is done in large sealed reservoirs that become inappropriate for any kind of use. The treatment and utilization of a high volume of the red mud waste as an alternative to conventional disposal has been a challenge for the alumina industry around the world. In Brazil there is a proposal to process the waste to reduce its alkalinity and sodium contents, what enables its return to the original mine sites. This will require a variety of tests, including soil ecotoxicological evaluation to ensure environmental safety. The objective of this study is to test mixtures of red mud and soil to provide information about the potential toxic effects of this residue to soil non-target organisms. Reproduction assays with *Folsomia candida* were performed using two types of mixtures: with artificial OCDE soil (5% organic matter content) and with a natural soil from Poços de Caldas municipality, Minas Gerais state, Brazil. The concentrations tested were: 0, 2.5, 5, 10, 20, 30 and 40% of residue (w:w basis). Results demonstrated that the red mud strongly inhibited the reproduction of *F. candida*. No juveniles were observed at concentrations of 30% and higher for artificial and natural soils. The high sodium content ($Na^+ = 49.19 \text{ cmol} \cdot \text{dm}^{-3}$) is probably the most deleterious factor to Collembola reproduction. The EC50 was 9.36% for OCDE and only 1.36% for natural soil. This difference indicates that the properties of the natural soil increase the toxicity of the red mud and that strong protective measures should be taken when encapsulating this residue.

MO 286

Assessing biological activity of soil organisms in no-till soils under different agricultural practices using the bait lamina method

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In agriculture, application of fertilizers and phytosanitary products varies depending on the cultivation methods used. Sustainable farming practices such as zero tillage increase the amount and variety of life in the soil but may require the use of herbicides. In the present work, the impact of different fertilizer and herbicide treatments on the biological activity of soil organisms of a no-till soil was compared. Using the bait lamina test, the changes in overall feeding activity of the soil biocenosis were measured in an agricultural brown soil (15% clay, 3% humus) with and without application of glyphosate for two types of fertilizers. The experiment was conducted at different exposure times, immediately and several weeks after the herbicide application, at different application rates and with different crops (corn, winter barley). The results of the bait lamina test show that according to the exposure parameters and application rates of the herbicide, the biological activity of the soil organisms tends to be higher in soil where glyphosate was applied. The bait lamina results were completed by physico-chemical analyses and by reproduction tests with Collembolans on the collected field samples.

MO 287

Effects of contaminated soils from North Africa on enchytraeids and predatory mites in standard laboratory tests. II. The case of a Phosphogypsum plant near Sfax (Tunisia)

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Mining activity is an important economic activity in several NATO and Mediterranean Dialogue Countries. Within their territory derelict or active mining explorations represent serious risks to surrounding ecosystems; nevertheless engineered-based remediation processes are usually too expensive to be an option for the reclamation of these areas. Thus, in order to find a more eco-friendly solution for mining areas, mainly related with uranium and phosphorus exploration, experts in Ecotoxicology, Chemistry, Plants Physiology, Microbiology and Molecular Biology from Portugal, Germany, Tunisia and Morocco, have joined together in a project funded by North Atlantic Treaty Organization (NATO). As part of this project the risk of contaminated soils to selected soil organisms has been assessed. In this contribution, it is asked: Does the contaminated soil of a still active phosphogypsum plant located in Sfax (Tunisia) affect the reproduction of enchytraeids (*Enchytraeus bigeminus*) and predatory mites (*Hypoaspis aculeifer*)? For this purpose soil samples were taken at 15 plots along five transects encircling the main storage area of phosphogypsum. These soils were characterized pedologically (e.g., the pH of all soils was in the range between 6.0 and 8.5) and chemically. Few plots showed meaningful metal contamination (mainly copper) but all soils contained high concentrations of hydrocarbons and salt. The reproduction tests with the enchytraeids and mites were performed following standard laboratory test protocols according to OECD guidelines 220 (2004) and 226 (2008). The predatory mites were less affected (at 8 plots the reproduction was almost similar to the control), while no or very few juveniles were found at the remaining plots. The number of juveniles of *E. bigeminus* was lower than the control at all plots but almost zero at 11 out of 15 plots. These results are probably caused by a complex interaction between soil parameters, contaminants and the salt content.

MO 288

Assessing pesticide use impacts on soil compartment at a Brazilian potato and strawberry producer region

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Interviews with potato and strawberry producers from Bom Repouso, MG, Brazil, showed intense and inappropriate utilization of pesticides. Local soil samples presented residue of aldrin, heptachlor, heptachlor epoxide, chlorpyrifos and carbofuran (potato soils); aldrin, heptachlor epoxide, endosulfan-I, carbofuran and abamectin (strawberry soil); and aldrin, heptachlor, heptachlor epoxide, endosulfan-II, λ -BHC, chlorpyrifos, abamectin and carbofuran (preserved forest soils). Retention function tests with *D. rerio* showed low acute toxicity of soils percolates, but organisms presented lower growth and gill histopathological alterations. Direct effects on soil organisms of a commercial formulation of abamectin, the most mentioned product, were also analyzed, through acute, chronic (reproduction) and behavioral (avoidance) tests with *E. andrei* in a tropical artificial (TAS) and a natural (NS) soils, in addition to an multi-species assay. The LC50; 14d for *E. andrei* in TAS tests was 7.64 mg abamectin/kg of soil. Chronic test showed significant reduction of earthworms survival (28 days) with 10.5 mg abamectin/kg of TAS. Morphological (threadlike and colorless posterior region; constrictions on different body regions; fragmentation and loss of segments) and behavioral alterations (lethargy or slowness to respond to mechanical stimuli) and weight loss occurred, and were correlated to the increasing pesticide concentrations. Average number of juveniles was 33 (Control); 3 (0.875 mg of abamectin/kg of TAS) and 0 (other treatments). Earthworms avoided contaminated soils, with significant difference from control, from 1.75 mg abamectin/kg of TAS. There was no significant response of the earthworms to any parameter in SN tests. Multi-species tests on SN soil showed significant effects on density of organisms, higher in control, which showed higher diversity and richness indexes for edaphic fauna, and small dominance index, compared to treatment with abamectin.

MO 289

Collembola community response to petroleum hydrocarbon contaminants in sub-Antarctic soils

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A series of fuel spills (diesels and lubricating oils) have occurred on sub-Antarctic Macquarie Island associated with fuel storage and power generation for the Island's research station. The Australian Antarctic Division is currently bioremediating these sites through the addition of air and nutrients to the soil. To contribute to the development of appropriate remediation targets, this study examines responses of Collembola to Special Antarctic Blend (SAB) diesel. As organic carbon plays a crucial role in determining bioavailability of organic contaminants in soils, Collembola community response to SAB was examined in 30 plots (1 m²) across a natural organic carbon gradient. Seven pairs of soil cores (75 mm wide x 70 mm deep) were sampled from each plot and spiked with SAB by injection (7 concentrations, 0 - 10,000 mg/kg) and incubated under field-simulated conditions (8°C, 16 h light / 8 h dark) for 15 days. At the end of the exposure period, surviving Collembola were extracted using a temperature gradient

extraction cabinet. Collembola were counted and identified, and matched cores were analysed for physical and chemical properties. Community assemblage response to SAB contamination and interactions with TOC and other physical and chemical variables were determined. The Collembola species richness decreased with increasing SAB concentration, with the Shannon-diversity index decreasing from 1.5 to 0.02. Sensitive and tolerant species within the community assemblage were able to be identified for future research. EC50 values for the Collembolan community response to SAB were calculated. This work contributes to a multinational project investigating hydrocarbon contamination in cold region soils aimed at developing site specific clean-up and remediation guidelines.

MO 290

Spatial distribution of micro-arthropods in agricultural ecosystems

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Information on species distribution and abundance of individual groups of micro-arthropods is available. The best researched group are the collembola. However, even for this group there is not much knowledge on the spatial distribution on small plots. For the semi field study approach (terrestrial model ecosystem TME) a grass site is used with the smallest sampling area possible. In field studies individual plot sizes of 3 m x 7 m or bigger are used.

The aim of this publication is to compare different sampling schemes and their potential for the evaluation of micro-arthropod communities in field trials. Sampling approaches are presented that differ in the mean distance between individual soil core samples. The species abundance, diversity and heterogeneity are evaluated. The results are discussed with respect to field study test designs, sampling schemes and the minimum number of samples that are required to yield a robust dataset.

MO 291

Seasonal dynamics of soil micro-arthropod populations in agricultural ecosystems

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The structural and functional protection goals for soil are given in the EU directive for the registration of plant protection products. In the past the impact of a plant protection product on the soil's ability to turn over organic matter was tested with litter bag studies. Recently the structure of the micro-arthropod community has been the focus for a future directive.

The main challenge for field tests with micro-arthropods is the agroecosystem were the tests take place. Reasons for this are the low content of organic matter in the soil, the high degree of soil disturbance (tillage) and the reduced diversity in cultivated fields. Collembola species in the agroecosystem are adapted to the changing environment through a high reproductive potential.

Since the abundance of micro-arthropods is a primary endpoint in field studies it is important to have an idea about seasonal population changes over the period of a year. In this publication the seasonal changes in abundance and structure of micro-arthropod communities in two fields are presented over one year. Results are discussed in terms of test design and sampling scheme.

MO 292

Effects of different land uses on soil microarthropod biodiversity in a Mediterranean urban context

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The majority of human activities frequently lead to the conversion of natural habitats to anthropogenic land uses. Land-use change is an important form of global pressure affecting biodiversity. Unfortunately, little is known about causes and consequences of soil biodiversity loss, although soil biodiversity affects the main regulatory services of the ecosystem.

The present study aimed to analyse biodiversity of soil microarthropods in soils with different human impact, in order to highlight how different land-uses affect soil arthropod communities. In addition, the soils were characterized for metal and PAH contents, to evaluate if different land uses cause differences in kind and degree of pollution. Finally, the content of metals and PAHs were related to different taxa of soil arthropods to individuate the taxa more sensitive/tolerant to different pollutants. To this purpose, 18 soil samples, grouped in 4 different typologies (natural, residential, industrial and agricultural), were collected in Naples and surroundings (Southern Italy). The soils were analysed for physical-chemical properties (pH, water holding capacity, bulk density, porosity, organic matter content, content of nutrients, metals, PAHs). To evaluate the arthropod biodiversity, the organisms were extracted using Macfadyen method. Abundance of Mites (identified at the sub-order level) and abundance and diversity of Collembola (identified at the species level) were assessed. Preliminary results highlighted that two taxa (Collembola and Acarina) were extremely widespread and abundant in all the investigated soils. Arthropod communities largely differ among the soil typologies.

MO 293

Year-round responses of soil invertebrate communities to pesticide applications in mediterranean apple orchards

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Apple orchards are highly manipulated crops in which large amounts of pesticides are applied. The aim of the present work is a better understanding of soil invertebrate responses to pesticide use taking account year-round dynamics. The originality of this work is the study of both (i) the within-year gradient and (ii) the between pest management strategies gradient, i.e. abandoned, organic, IPM (integrated pest management) or conventional. Pest managements are characterised by difference in the nature of pesticide (organic orchards) and in the intensity of application (IPM vs conventional).

Arthropods were sampled during fall, spring and summer in 20 apple orchards with different management strategies using six pitfalls in each orchard. In spring, orchards with organic practices had the highest abundance and abandoned orchards the highest taxonomic diversity. Some taxonomic groups present a negative response (isopoda, homoptera, arachnea) or a positive response (carabidae) with the increasing use of synthetic pesticides. These differences were less marked in autumn, which could be a sign of resilience (last pesticide applications occurred in August).

We concluded that not all arthropod families have the same sensitivity to pesticide management. The next step is to determine if difference in sensitivity occurred as well for natural enemies and other kinds of arthropods species.

MO 294

Effects of the nitramine explosive CL-20 on the soil invertebrate community in a sandy loam soil

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We investigated the effects of nitramine explosive CL-20 on soil invertebrates in Sassafra sandy loam (SSL) soil using a 12-week soil microcosm assay with the indigenous soil microinvertebrate community. In these studies, freshly-collected SSL soil was amended with CL-20 and thoroughly mixed to prepare individual analytically determined treatment concentrations 0 (carrier control, acetone), 108, 479, 870, 2355, 4635, 7020, and 10300 mg/kg. The selected concentration range of CL-20 adequately assessed the concentration-response relationships for total microarthropods, as well as for individual microarthropod groups in SSL soil, and allowed us to determine group-specific ecotoxicological benchmarks for CL-20. The overall composition of microarthropod community in SSL soil was not affected by exposure to CL-20, based on the number of taxonomic groups present in the individual treatments after 12 weeks. However, community structure analysis revealed greater sensitivity to CL-20 by predatory mesostigmatid mites. Microarthropod and nematode communities showed contrasting sensitivities to CL-20 in SSL soil. Total numbers of nematodes were either unaffected or significantly ($p < 0.05$) increased in CL-20 treatments compared with control. Only predator group among nematodes was consistently adversely affected by exposure to CL-20. The abundance of predatory nematodes decreased in a concentration-dependent manner throughout the 12-week exposure. Overall toxicity data for soil microarthropods and predatory nematodes established in this microcosm assay were comparable with mortality data established in our standardized single-species toxicity tests with the soil invertebrates earthworm *Eisenia fetida* (ISO 11268-2:1998), potworm *Enchytraeus crypticus* (ISO 16387:2004), and collembolan *Folsomia candida* (ISO 11267:1998) exposed to CL-20 in similar SSL soil. Microcosm assay with corresponding community structure analysis can provide the means for validating the ecotoxicity data from standardized laboratory tests, both complimenting and expanding upon the ecotoxicological significance of data from standardized single-species toxicity tests.

MO 295

Microarthropod communities in lead contaminated forest soils at active and abandoned shooting ranges

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As a part of a larger ecosystem-scale investigation, microarthropod communities at two shooting range sites with different contamination history and at one uncontaminated site were studied. The three study sites locate in a same pine forest ecosystem; one site situates right behind an active shotgun shooting range and another site locates in a position where shotgun shooting terminated over 20 years ago. The amount of lead pellets in the soil of both of the contaminated study sites is equal, reaching up to four kilograms of pellets per 1 m². The overall toxicity of the organic soil layer was higher at the active shooting site than at the abandoned site. In the active shooting range, lead posed negative effects on Collembola, Oribatida, Mesostigmata, Prostigmata and total microarthropod abundances, whereas at the abandoned site the effects were clear in total number of microarthropods, Prostigmata and some separate groups at lower taxonomical level. The microarthropod community structures of the uncontaminated and abandoned shooting sites were quite similar, whereas the microarthropod community at the active shooting site was different from the others. These findings suggest the recovery of the soil system due to accumulation of litter on soil surface after shooting is closed. However, when the microarthropods were sampled separately in fermentation layer and humus layer of the soil, the humus proved to be more toxic at abandoned shooting site than at active shooting site. Total microarthropod, Oribatida and Mesostigmata abundances were lower in the humus layer at the abandoned shooting site than at the uncontaminated site, whereas the abundances of any higher microarthropod taxa in the humus at the active shooting site did not differ from those at the uncontaminated site. Thus, although the soil biota at the upper soil layer appear to recover after cessation of shooting, the dissolution of Pb from the pellets deeper in the soil continues to pose a risk for microarthropod communities.

MO 296

What to do when there are too many specimens: two strategies for sub-sampling in Off-crop studies for non-target arthropods

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Over the last four years protocols have been developed to test the influence of plant protection products on arthropods in off-crop areas. However, for some sampling methods, especially suction sampling, in off-crop areas high numbers of arthropods together with high amounts of undesired material (i.e. fine plant material, seeds, litter and soil) are sampled at the same time. This leads in turn to a tremendous work load for sorting and taxonomical determination of the specimens, which rapidly exceeds an acceptable time period for practical and economical purposes. Therefore, procedures need to be developed to efficiently and effectively use the samples with high specimen numbers and a high pollution. Here, two different sub-sampling methods were used for Vortis suction samples from an off-crop field study with high individual numbers (e.g. > 15 000 individuals per sample). The first method uses a sample divider in four steps. The divider is originally constructed for creating sub-samples of plankton samples. The second method was additionally to set a time limit for sorting the arthropods that are not micro arthropods out of the sample. The aim was to take out at least 80% of the arthropods from the sample before sub-division. As a following step the subsamples were divided by the sample divider before counting the numerous collembolans and mites. To assess the performance of both methods the coefficient of variance for different taxa identified was calculated and compared. There was also an assessment of the sample time for the two methods.

MO 297

Computer controlled high gradient extractor for soil organisms

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Soil micro-arthropods, such as Oribatida and Collembola, are important components of the soil decomposer system. This makes the soil micro-arthropod community a valuable indicator of soil quality. Therefore, there will be probably a higher focus on possible impact of plant protection products on soil micro-arthropod communities in the future. For different field study types soil micro-arthropods have to be removed from the soil in order to determine species. The most common method according to ISO 23611 for extraction of soil micro-arthropods from respective samples is the high gradient extractor according to Macfayden (1961).

The principle of this extraction method is to create an artificial temperature gradient between the canister in which the samples have been placed (hot) and the collecting device below (cold). The presentation will show a highly sophisticated computer controlled extractor constructed by RIFCON GmbH. In this modified high gradient extractor the temperature can be regulated stepless and monitored in all different compartments, which enables a repeatable and adaptable extraction of soil micro-arthropods. The efficiency of the extractor is verified with Collembola species (*Folsomia candida* and *Heteromurus nitidus*) in different soil types and soil humidity regimes. At least 80% of *F. candida* and 96% of *H. nitidus* were recovered from soil cores.

MO 298

Use of anaerobic digested-based composts as soil organic amendments: effect on the soil biological status

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Climate changes together with organic matter and plant nutrient loss, low water infiltration and water holding capacity accelerate soil degradation. This is a serious problem for farmers and food suppliers and also for the reclamation of landscapes for nature conservation. Moreover, intensive livestock production has led to a generation of great amounts of animal manures and slurries that can constitute an environmental risk, if they are not managed properly. Consequently, there is a need for more environmentally sound methods for the treatment and utilisation of animal manure. Anaerobic digestion constitutes one of the main alternatives for managing it and is based on the anaerobic conversion of organic matter, obtaining biogas and a digested substrate called digestate. Although the latter presents a high fertilising value, this material shows some characteristics that make necessary to treat it prior to its direct soil application. One option is to separate the digestate into a liquid and a solid fraction, the latter can be composted to obtain valuable and marketable end-products, which can be used with agricultural purposes or for soil restoration. Within this context, different studies have shown that compost application increases soil organic matter content, improving physical, chemical and biological soil properties and thus, preventing soil erosion. The general aim of this work was to evaluate if the addition of two anaerobic digestate-based composts to a soil with a low C content could improve the overall microbiological activity and the C pool conservation. For this purpose, two composts (C1, mainly composed by cattle manure anaerobic digestate and C2, mainly composed by pig slurry anaerobic digestate), at two different rates (30 t/ha and 60 t/ha respectively) were incorporated into a semiarid soil from central Italy. Two other additionally treatments were also considered: the unamended soil (control) and a mineral fertilised soil. Subsequently, several plants of rosemary (*Rosmarinus officinalis*) were planted on these soils. The efficiency of the treatments was evaluated by analyzing soil physico-chemical and microbiological properties, such as soil respiration, microbial biomass and soil bacterial community activities, such as dehydrogenase and bacterial viability.

MO 299

Evaluation of interactive toxic effects of binary metal mixtures to the bioluminescent fungus *Gerronema viridilucens*

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Fungal-based assays can be used to assess the toxicity of environmental samples of polluted areas. Toxicity studies with binary mixtures of metals are more realistic than those with single metals as interactions between the species can interfere with the toxicities. Currently, there is no work on the combined toxic effect of metal binary mixtures to fungi, despite the importance of these organisms in soil nutrient cycling. This work reports the use of a fungal-based luminescent assay using the Brazilian bioluminescent fungus *Gerronema viridilucens* to assess the toxicity of binary mixtures of metal cations (Ca^{2+} , Cd^{2+} , Cu^{2+} and Zn^{2+}). The toxic unit concept was used to calculate the EC50 values of binary mixtures ($\text{EC50}_{\text{mixC}}$). Experimental EC50 values ($\text{EC50}_{\text{mixE}}$) were obtained from bioluminescence inhibition vs. log(cation concentration) curves and were compared with $\text{EC50}_{\text{mixC}}$ to determine the nature of interactions (antagonistic, additive, greater than additive, or synergistic). The single metal cation toxicity was found to be $\text{Cd}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Ca}^{2+}$. The interactive effects were as following: synergistic for $\text{Cu}^{2+}+\text{Cd}^{2+}$, between synergistic and greater than additive for $\text{Cu}^{2+}+\text{Zn}^{2+}$, between additive and greater than additive for $\text{Zn}^{2+}+\text{Ca}^{2+}$, between additive and antagonistic for $\text{Cu}^{2+}+\text{Cd}^{2+}$ and $\text{Zn}^{2+}+\text{Cd}^{2+}$, and antagonistic for $\text{Ca}^{2+}+\text{Cd}^{2+}$.

MO 300

Soil adenosine triphosphate (ATP) as a marker for assessing ecotoxicology of crude oil pollution in soil

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Crude oil pollution on land can cause serious damage to soil by affecting both its biophysical and biochemical properties. Microbial populations (and microbial diversity) in the soil provide adequate nutrients and stored energy (ATP) for primary productivities. Hence, in this study, the effect of terrestrial oil pollution on the ATP content of the soil and changes in microbial diversity in our terrestrial agricultural soil ecosystem were studied in soil macrocosms polluted with Bony light crude oil at different concentrations. In order to add value to this evaluation method, the mark-up of ATP content was assessed by evaluating the ameliorating effect of some bioremediation strategies exemplified by the use of cow and poultry dung and inorganic fertilizer (NPK). The results of this study show that both the physicochemical properties and ATP content as well as microbial diversity of the soil were negatively altered significantly ($p < 0.05$). Soil pH in water decreased from 6.55 ± 0.05 to 5.85 ± 0.05 and 5.75 ± 0.05 at 0.1 % and 0.5 % contamination respectively. Similarly, in KCl medium, the soil pH decreased significantly ($p < 0.05$) from 5.95 ± 0.05 to 4.95 ± 0.05 and 4.75 ± 0.05 at 0.1 % and 0.5 % crude oil contamination respectively. Contamination of soil by crude oil (0.1 %) decreased the ATP content from 0.44 mg/100g to 0.22 mg/100g after 7 days of exposure; from 0.55 mg/100g to 0.33 mg/100g after 14 days and from 0.61 mg/100g to 0.44 mg/100g after 28 days of exposure. Addition of cow dung, poultry dung and NPK to the polluted soil increased the ATP level as against the untreated polluted soil samples. The cow and poultry dung with high microbial biomass and diversity marked up the ATP content of the amended soil. Hence soil ATP can be used as a reliable marker to assess the impact of crude oil pollution on soil ecosystem productivity and microbial diversity of terrestrial agricultural soil ecosystem.

MO 301

Adverse effects of fungicides on ectomycorrhizal mycelium growth - impact of quinoxyfen, boscalid, tebuconazole and azoxystrobin

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Ectomycorrhizal fungi (ECM) are symbionts of many tree species such as European beech (*Fagus sylvatica* L.). In Germany about one third of the landscape is forested, the European beech dominating large areas. Mycorrhizal plants have an increased and thereby more efficient water and nutrient uptake and are better adapted to the specific challenges of climate change, such as drought stress [1].

Studies have shown that fungicides on the one hand stimulate and on the other hand inhibit mycorrhization [2,3]. However, the impact of fungicides on the mycorrhizal symbiosis is poorly understood. This is especially true for new and modern active compounds. First results of laboratory experiments presented on the poster were performed to choose fungicides to be used in field experiments.

The impact of authorized fungicides used in agriculture and forestry on the ECM fungi *Pisolithus arhizus*, *Hebeloma crustuliniforme*, *Lyophyllum* and *Cenococcum geophilum* was investigated. In a first step active ingredients quinoxyfen, boscalid, tebuconazole and azoxystrobin were examined in in-vitro experiments under laboratory conditions. In a consecutive step corresponding commercial fungicide formulations were used.

For this purpose, serial dilutions were prepared from 0.1% to 200% of the application rate used in agriculture and forestry together with controls at each dilution step.

Tests were conducted in Petri dishes as well as with sterilized soils to approximate natural conditions.

For the plate tests mycelium was taken and placed on the treated medium. The test tubes were incubated for 29 days at 25°C in the dark. The evaluation and quantification was performed by determination of the radial growth of mycelium corrected by results from the control group.

In all disk tests tebuconazole caused the strongest inhibition whereas the lowest inhibition of mycelial growth was found for quinoxyfen. For *Pisolithus arhizus* an inhibition was found even at 0.1 % application rate for three fungicides. For *Cenococcum geophilum* an inhibition was found at 10% application rate using tebuconazole. Interestingly an irreversible damage of ECM was found. Hence, ECM with inhibited growth after 29 days shows adverse effects on mycelial growth even after re-inoculation on untreated culture medium.

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 [3] Laatikainen,et al.(2002) Microbiol. Res. 157,127-137

MO 302

Selective removal of high molecular weight PAHs in crude oil and tar contaminated soils using biodiesel

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An observation that biodiesel amendment can enhance the selective removal of benzo(a)pyrene has been made in soils spiked with crude oil and coal tar. Results from laboratory microcosm experiments of tar spiked soils showed that after 60 days, this pollutant was reduced by 91% in biodiesel amended samples as compared to 17% and 29% reductions in the control and nutrient amended microcosms, respectively. Perylene was reduced by 70% and anthracene was almost completely mineralised in the biodiesel amended experiments. However, there was no detected depletion in phenanthrene in the biodiesel amended soils as opposed to that in the control (91%), and nutrient amended (85%) experiments, even up to a 180-day degradation period. Fluoranthene and pyrene were also not depleted in the biodiesel amended experiments. A similar trend was observed for the crude oil spiked experiments which showed 52% reduction of benzo(a)pyrene in the biodiesel amended samples after 60 days. However, the degradation of fluoranthene, pyrene, and phenanthrene was substantially inhibited in these samples as compared to the other treatments. The six ring compounds indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene were observed to be more degraded in the treatments with biodiesel for both crude oil and tar contaminated soils. These findings suggest that biodiesel can preferentially degrade higher molecular PAHs by a selective degradation mechanism during bioremediation. Further work to investigate this process and also assess soil toxicity changes with treatments are being conducted.

MO 303

The contribution of bacterial cell wall fragments to the formation of soil organic matter - a case study from a glacier forefield

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Recently, stocks of soil organic matter (SOM) have been shown to decrease in European soils and also worldwide, which compromises soil fertility and enhances emissions of carbon dioxide and other, even worse green-house gases, to the atmosphere. However, the general structure of SOM, and thereby the mechanisms behind its genesis and loss, remain unclear.

In this framework, microbial biomass is generally regarded to be of low importance for SOM formation. In particular on freshly exposed surfaces, however, bacteria colonize barren mineral surfaces faster than fungi or higher plants. Moreover, recent results indicate that bacterial cell wall fragments frequently occur on soil mineral surfaces and also accompany the microbial colonization of previously clean and sterile activated carbon surfaces after incubation in groundwater. Hence, we hypothesized that, at least, in the initial stages of soil formation bacteria and their fragments may play an important role in particulate SOM formation bearing in mind that most dead organic matter entering the soil is processed by bacteria.

This hypothesis was proven by tracing the development of SOM in a chronosequence with samples from the forefield of a receding glacier (Damma-glacier, Canton Uri, Switzerland) by scanning electron microscopy and other methods. The initially barren mineral surfaces have been shown to be rapidly covered with microbial residues as soil age increases. Moreover, this data compares well to growing C/N-ratios, water contact angles and fatty acid contents in earlier deglaciated samples.

MO 304

Bioavailability and effects of cadmium, copper and zinc contaminations on microbial communities and respiration activity of soils in SE-Georgia

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The study area is located about 80 km south of Tbilisi in southeast Georgia in the Mashavera valley. Intensive agricultural land use is limited by low annual precipitation (504 mm) and hot, arid summer months. Accordingly, vegetable gardens, vineyards and orchards as well as arable land are irrigated with water of the Mashavera River. The water is heavily burdened with suspended particles, which show very high concentrations of sulfidic metals (Cu, Zn, Cd) and come from slag heap erosion and a flotation plant of a non-ferrous metal mine. For this reason the irrigated soils in the Mashavera valley are highly contaminated with these trace metals. Aim of the study was to test the use of soil microbial parameters for the evaluation of the habitat function of soils contaminated with trace metals.

Overall 51 soil samples were collected of the Mashavera valley in March 2010. Habitat quality was evaluated by determination of pH-value, organic matter, soil texture and total as well as bioavailable (aqua regia, EDTA-, NH₄NO₃-extraction) trace metal concentrations. Total contents ranged between 284 mg kg⁻¹ and 1193 mg kg⁻¹ for Cu, between 303 mg kg⁻¹ and 973 mg kg⁻¹ for Zn, and between 1.4 mg kg⁻¹ and 5.9 mg kg⁻¹ for Cd. Basal and substrate induced respiration of the microorganisms were assessed by carbon dioxide production determined with an infrared gas analyser. Low respiration rates were measured in samples with high trace metal contents. Indicating a reduced microbial activity in soils irrigated with contaminated Mashavera water. The analysis of microbial community structure by polymerase chain reaction - single strand conformation polymorphism (PCR-SSCP) shows differences in the structural diversity of soil bacteria between contaminated and uncontaminated soils. Due to the exposure a shift of the native microbial community structure is shown by a decrease in microbial diversity. Cloning for the phylogenetic classification is still in process. First results will be presented.

MO 305

Assessing the effect of metal pollution on soil functioning in forest and grassland ecosystems in Antwerp, Belgium

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In the suburb of Hoboken (Antwerp-Belgium), UPMR (Umicore Precious Metal Refining) runs one of the world's largest precious metal recycling units. Although the plant has implemented substantial modernization since the 1970's, the legacy of 122 years of historic pollution is still present; Pb, As and Cd levels in the soil increase with proximity to the factory. In highly polluted smelter locations the decomposition of litter and thus nutrient cycling is impaired due to the almost complete absence of litter decomposers in the soil and litter layer. Endemic soil microbial communities vary from site to site; therefore a set of tests with different assessment endpoints is needed. The bait lamina test developed by Von Törne, (1990) measures the feeding activity of the decomposer community and consists of vertically inserting 16-hole-bearing plastic strips filled with a bait substrate into the soil. Bait removal is directly linked to decomposition activity. An alternative method based on substrate induced respiration will also be applied to support the results of the bait lamina test. The MicroResp[TRADEMARK] system consists of a 96 well 'deepwell' plate in which moisture corrected soil samples (at the soil's field capacity for water) amended with various carbon substrates are placed. The plate is separated from a 96 well microtiterplate containing a colorimetric CO₂ detection gel by a PTFE lined porous rubber seal. The system is incubated at 25°C for 6 hours followed by spectrophotometry readings carried out at 570nm. The results will be interpreted in relation to the soil physicochemical parameters of each test site.

MO 306

Biodiversity of soil microfungi and the role of melanin pigment in biomonitoring

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We known numerous examples of fungal structures such as spores and spores that are extremely resistant to harsh environmental conditions and it is significant that most of these resistant structures contain black pigments which have been described as melanin. Microfungi were isolated from soils in the Karkonosze and Bieszczady Mountains and the Upper Silesia Region in Poland. Among dominant soil microfungi was isolated such species with genera *Aspergillus* as: *A. flavus*, *A. fumigatus*, *A. humicola* and *A. versicolor*; among *Cladosporium* such as *C. cladosporioides*, *C. breviscompactum* and *C. herbarum*; among *Penicillium* such as: *P. canescens*, *P. frequentans*, *P. lanosum*, *P. nigricans* and *P. ochraceum*; among *Trichoderma* - *T. album* and *T. viride*. Isolated melanin polymers were of high molecular weight, were decolorized by oxidizing agents, were insoluble in organic solvents, and gave a positive reaction for polyphenols. On the basis of their chemical and spectral properties, the melanin from this fungi have been described as similar to "indole" melanin and belonged to eumelanin group.

The location of granules with melanin was investigated in the *Cladosporium cladosporioides* cells. Cadmium, cuprum and zinc form discrete black granules and electron microscopy analysis indicated that these metals were also associated with cell walls and intracellular structures. Localization of lead granules was clearly evident, which were found in cell walls and at intracellular structures, most likely-vacuoles, which have an important role in the regulation of the cytosolic concentration of metal ions.

Explaining the role of melanin would certainly contribute to understanding the protective function of dark-pigmented fungi on soil pollutants. It is a problem of a great importance especially as far as degraded soil by heavy metals is contaminated. In the natural soil environment are another dark-pigmented soil fungi species such as *Aspergillus* sp. and *Penicillium* sp., too. Their wide occupation in all of soil environments may be used in biomonitoring of heavy metals and melanin pigment is the useful ecotoxicological biomarker.

MO 307

Understanding binding mechanisms of active pharmaceutical ingredients to sewage sludge using a surrogate solid phase extraction approach

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Over the past decade there has been a growing concern over the fate of active pharmaceutical ingredients (APIs) that are released into the aquatic environment. Some APIs can be effectively biodegraded in waste water treatment plants but others can be sorbed onto sludges that are often subsequently used as fertilisers or disposed of as landfill. In order make an accurate risk assessment it is important to know how a given API will be distributed between the aqueous or solid phases within a treatment plant. A sorption test to determine the partitioning (determination of the K_d value) of an API in sewage sludge is already available (OPPTS 835.1110), but this method is long and fastidious.

Predictions of K_d based on Log K_{ow} have been attempted, but these are not very reliable, especially for ionisable compounds, such as many APIs of interest. Therefore, better predictive models are urgently needed that include binding mechanisms in addition to the hydrophobic interaction. As activated sewage sludge is a complex matrix and hard to fully characterise we used ten commercially available solid phase extraction (SPE) cartridges with different chemistries to try to reproduce these binding processes. Various eluents with different organic modifiers and different buffers at a range of pH were tested. The results of these experiments, where the sorption mechanism is known, were compared to results obtained using 'in house' prepared cartridges of sludge at different sludge/silica ratios.

MO 308

Risk assessment of pesticides to soil organism: exposure analyses of two insecticides in soil layers during a sampling period of one year

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The spatial and temporal behavior of pesticides in the soil environment depend on properties of the substance (e.g. degradation, leaching, sorption), the soil and the climate. To study the effects of the chemicals on the soil fauna, the spatial and temporal distribution of soil organisms is also important. Within the project "Risk assessment of pesticides to soil organism under real conditions" investigations to the metabolism and leaching of pesticides are synchronized with studies on their potential effect on soil organisms. Two insecticides, imidacloprid and lindane, both persistent in soil, but differing in the leaching behavior were separately applied to Terrestrial Model Ecosystems (TMEs) in two concentration each: 7,5 kg/ha and 20 kg/ha (lindane) and 0,75 kg/ha and 2 kg/ha (imidacloprid). The distribution of the pesticides in the soil profile (0-2,5 cm, 2,5-5 cm, 5-10 cm and 10-20 cm depth) during one year was analyzed.

We here present the analytical work. Lindane was extracted with ethyl acetate and the residues were determined by gas chromatography with electron-capture detection. Imidacloprid samples were extracted by microwave digestion in acetonitrile/water; after centrifugation the extract was analyzed with LC-MS/MS. Results of the currently performed chemical analyses will be presented. In another part of the project the effect of the insecticides to soil organisms on the population level are investigated and shown in a platform presentation.

MO 309

Understanding the relationship between extraction technique and bioavailability

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In addressing the significance of bound residues in environmental risk assessment, ECETOC concluded previously that non-extractable residues were characterized by a pragmatic extraction approach for determining whether they are extractable or not. There is now a need to develop a standard framework for soil and sediment extraction methods and to associate extractable fractions (such as freely dissolved, readily desorbed or reversibly desorbed) with a level of bioavailability and with appropriate test organisms for these compartments. A Task Force has reviewed extraction methodologies and how such methods correlate with bioavailability, identified those 'destructive' extraction techniques which result in loss of sample matrix integrity and also considered the state of knowledge regarding mechanisms of binding and their forces which would result in either extractable or non-extractable residues. The aim was to develop new approaches to screen the bioavailable fractions and validate such an approach. This supports a consistent interpretation of data and provides a transparent basis for assessing the potential risk of non-extractable residues. The Task Force will evaluate a framework for an intelligent extraction strategy and make recommendations for future research topics.

MO 310

Plant uptake of some commonly used pharmaceuticals

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Presence of different pharmaceuticals in sewage sludge is apparent, but there is still a lack of knowledge about the fate of pharmaceutical residues in the environment. Still, remarkable amounts of pharmaceuticals enter the soil via fertilizing with sewage sludge. As long as such a disposal practice is widely applied, a better knowledge on the fate and effects of chemicals in sludge treated soils is needed. Only a relatively small number of investigations have been published on the mobility and bioavailability of pharmaceuticals in the environment. Uptake of antimicrobials into vegetation is a major route for these substances into the food chain. In the current study uptake of ciprofloxacin, norfloxacin, ofloxacin, sulfadimethoxine and sulfamethoxazole was demonstrated in the case of lettuce, potato, carrot and wheat. Measureable residues of the studied compounds occurred in food plants. The uptake of fluoroquinolones and especially sulfonamides by plants like potato and carrot might pose health risk, as the detected levels of the studied pharmaceuticals were of considerable magnitude, if compared to their soil concentrations. As a rule, the uptake of antimicrobials was more efficient from sandy soil into underground parts on plants - roots and tubers. The concentrations of antimicrobials in plants exceeding the MRL values for meat and milk were seen only at antimicrobial soil concentrations equal to 1 mg/kg and 10 mg/kg. Such concentrations might be present in soil if sewage sludge treated by anaerobic digestion is used as a fertilizer. In Estonia the soil concentrations of antimicrobials are presumably lower, as only sewage sludge compost may be used for fertilization.

MO 311

Degradation of pharmaceuticals in sewage sludge compost

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Sewage treatment facilities do not remove pharmaceutical residues completely. Several antimicrobials do not decompose during aerobic activated sludge process or anaerobic digestion, and release in sewage effluents or adsorb to sewage sludge. The sewage sludge containing pharmaceutical residues is used as a fertilizer. Pharmaceuticals reach the soil where they can affect microorganisms and accumulate in plants.

Our recent study showed that some fluoroquinolones and sulfonamides were frequently present in Estonian sewage sludge and the composting technology played the major role of their degradation. In Tallinn, where composting with peat was used and mixing of the compost stacks lasted 12 months, all studied antimicrobials were degraded after a year. In Tartu, where composting with tree bark was used and mixing of the compost stacks took place during 8 months, the highest contents of ciprofloxacin, norfloxacin, ofloxacin after a year storage were 70, 64 and 8 µg/kg (dm) respectively. Fluoroquinolones have been described as photodegradable, therefore we suppose that exposure to the light due to frequent and long lasting mixing could be one of the reasons of their faster decomposition in Tallinn.

Plant experiments in greenhouse showed that potato, carrot and lettuce can uptake fluoroquinolones from soil with antimicrobial concentration 10 µg/kg (dm) (the trigger value for antimicrobial content in soil recommended by EMEA/CVMP). The mixture of sulfonamides and fluoroquinolones in soil can be toxic for wheat and lettuce plants, if antimicrobial concentration in soil reaches 10 mg/kg (dm). Antimicrobial content in plants grown at soil antimicrobial concentration 10 mg/kg exceeded the MRL for meat and milk (100 µg/kg). To prevent such a high antimicrobial concentration in soil, the sewage sludge composting has a remarkable importance.

To compare the influence of different sewage sludge composting technologies to the degradation rate of the fluoroquinolones and sulfonamides in it, some new experiments were performed. The sewage sludge was mixed with different materials as tree bark, peat, oil shale ash, litter and sawdust. The antimicrobial decomposition rate dependence on added material was estimated. Antimicrobials were extracted from compost samples using PLE, extracts were cleaned up by SPE and analyzed by LC-MS. The results will be presented. This work helps to optimize the sewage sludge composting technology to produce safe agricultural fertilizer.

MO 312

Prospects of miscanthus x giganteus for the rhizoremediation of PAH contaminated soils

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Phytoremediation of large surface area using crop cultivation appears as an attractive strategy to allow for long-term but economically viable site rehabilitation programs. The aim of this study was to characterize the phytoremediation potential of *Miscanthus x giganteus* for PAH polluted soils. For this purpose, three factors affecting bioremediation, namely the nature of soil contamination (spiked-phenanthrene and pyrene each at 100 mg/kg dry soil), bioaugmentation (with a PAH-degrading microflora) and planting (with *Miscanthus x giganteus*), were arranged according to a full factorial design in a pot experiment. Their effects (alone or in combination) were assessed on soil bacterial communities (total and degrading microflora enumeration, 16S rDNA molecular diversity analysis) and PAH dissipation. Results showed that contamination was the main factor positively influencing the development of PAH degraders. Phenanthrene disappearance was observed regardless of the presence of plants or initial bacterial inoculum whereas remaining pyrene contents were negatively affected by bioaugmentation and to a lesser extent by planting. The ability of plants to maintain a degrading rhizosphere microflora, thus favouring dissipation of the recalcitrant compound would deserve further investigations.

MO 313

Nano, bulk or complexed iron: what to use for soil remediation?

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Zero-valent Iron has a long tradition in soil remediation, due to its reductive properties and its sorption capacity. The latter substantially increases when iron is present in nanoparticulate form. Since at the same time the costs of remediation are considerably reduced, nano-iron has been used for remediation purposes. We are using an array of standard test systems for prospective hazard assessment of new substances, including nanoparticles. In an earlier study we found that nano-iron had a limited capacity to reduce the toxicity of cadmium to plants (*Lepidium sativum*, *Arabidopsis thaliana*) and caused avoidance by earthworms (*Eisenia fetida*), even when applied in uncontaminated soil. The tests also rendered *A. thaliana* way more sensitive than *L. sativum*. Currently we are conducting follow-up experiments in which we test the impact of iron powder and iron fertilizer on both plant species, earthworms and the toxicity of cadmium to them. First results clearly show that highly concentrated fertilizer is highly

toxic to plants and thus cannot be used for remediation purposes. The experiments will be finished in early spring so that we expect to answer our title question during the conference.

MO 314

Time-course changes of PAHs and their oxygenated derivatives in a PAH contaminated soil during full scale biological remediation

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Polycyclic aromatic hydrocarbons (PAHs) are fairly common in contaminated industrial areas, especially at old gasworks sites, gas stations and former wood impregnation facilities. U.S. Environmental Protection Agency (EPA) has listed 16 PAHs as priority pollutants. Because of their toxicity, PAH-contaminated areas are highly prioritised for remediation. Today, the generic guideline values of PAHs in contaminated soils are usually based on chemical analysis of the 16 priority PAHs, even though often hundreds of PAHs and PAH derivatives exist in the soils. Oxygenated PAH derivatives (oxy-PAHs) originate from combustion sources and are also formed during biological remediation of polycyclic aromatic hydrocarbons (PAHs). Oxy-PAHs are mutagenic, can induce aryl hydrocarbon receptor (AhR) mediated toxicity and are in addition more mobile than their parent PAHs and therefore of environmental concern. In contrast to the well monitored 16 priority PAHs, only little information is available regarding toxicity, levels and distribution of oxy-PAHs in the environment.

In the present study we will analyse levels of 26 PAHs and 12 oxy-PAH derivatives during a full scale biological remediation of a PAH contaminated soil using GC-MS. Our objectives are (1) to examine the changes in PAH and oxy-PAH levels during the remediation process (2) study the availability of the compounds over time using a mild methanol extraction and (3) study if the toxicity in the soil is reduced in proportion to the putative reduction of the PAHs, using the H4IIE-luc dioxin bioassay, based on rat hepatoma cells. Mass balance analysis will show how much of the AhR-mediated activities in the soil the GC-MS analysed compounds account for. The results will give information of levels of PAHs and oxy PAHs found in PAH contaminated soils, the degradability of these compounds, formation of oxy PAHs and changes in availability during a biological remediation.

MO 315

Ecoethic problems of erosion, salinization and alcalination of soils in Azerbaijan

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The exposure soil to different level of erosion is one of characteristic ecological problems of our Republic. But real disaster of land management in republic and in the world is the water erosion (31% of land) and wind erosion (deflation) which actively influence 34% of surface land. Relief of the territory plays an important part in erosion process and intensively influences to erosion process and gradually changes under its influence. At the result of agricultural activities 30 th. ha of land cover destructed, but 40% of land cover exposed to erosion processes, result of wrong land reclamation work shows its negative influence, in results underground water level and salinization of arable land increases. In arable regions of the country more harm gives irrigational erosion. In appropriation of mountain mass under not follow antierosional activities washout of the soil will decrease which shows negative influence on productivity and often causes take out of the plant from agricultural cultivation. Very often antierosional processes causes washout of the 100 t/ha soil, especially in irrigation season. Mountain and plain regions of Azerbaijan exposed to irrigational erosion. At the result of wrong irrigation activities productivity of the land decreases. Research works shows that total territory of lands exposing to erosion is 31444,7 th. ha in our Republic, which means 36,4% territory of Azerbaijan. Problem of salinization and alcalination are the most important problems among the ecoethic problems. Nowadays approximately 37000 th. ha soils from all existing land (8641506 ha) in Azerbaijan exposed to degradation. Soil salinization is wide spread in Azerbaijan. 60% of Kur-Araz plain occupying 2,2 million ha territory consists of average and full saline soil. Generally in the territory of our Republic total area of average and full saline soil is 1,3 mln ha. That is why these lands considered useless from the point of view of land reclamation and ecology. Knowing its negative dynamics is necessary to take otherwise actions, such as taking certain ecoethic measures (derange, washout, chemical melioration, necessary administrative and legal activities and etc). In the way of struggle against erosion, salinization and alcalination which became one of the important ecoethic problems for our soil and plant cover, complex measures are needed to be carried out.

MO 316

Ecological characterization of Mountain Shirvan Regions of Azerbaijan

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The composition of Mountain Shirvan economic and geographic region includes Agsu, Ismaili, Gobustan and Shamakha districts. Total area of the region is 613 thousand ha, population-273,4 thousand persons. The relief of the area is divided into upland and plain territories. On the plain territory climate is soft-hot and dry subtropical; on the upland places - the summer is some cool, the winter is rather cold and arid. Annual middle temperature of foothill-plain territory is 14,10C (Agsu), in upland territory - 10,50C. Temperature of the coldest month in Agsu is 3,30C, in Shamakha - 0,60C. Annual rainfall is 800 mm (Ismayilli) and 379mm in Maraza (Shamakha). Evaporation is 762mm in Ismaili, 872mm in Maraza, and 970mm in Agsu. 428 thousand hectare (69,8%) of general territory is upland and 185 thousand hectare (30,2%) is foothill-plain. Composition of the land cover: 37,2%-mountain chestnut; 11,4%-chestnut; 11,8% brown and brown mountain-forest; 3,3%-black; 8,2%-grey; 8,0%-alluvial-meadow; 2,7%- meadow-forest; 5,7%- mountain-meadow. Composition of land supply: useful lands for agriculture - 376769 ha (67,1%), from them arable - 37,5% (141,3 thousands hectares), perennial plants -2,5% (9,2 thousands hectares), hayland-1,2% (4,6 thousands hectares), pastures -57,1% (212,5 thousands hectares), Garden plot - 2,5 % (9191ha), forest -13,3% (81596ha). Irrigated lands useful for agriculture is 52,8 thousand hectare (14,2%). From vegetation - cereal-growing, potato-growing, vegetable-growing, vine-growing, melon-growing, fruits-growing, cotton-growing, feed-growing. In subalp are meadows: moderate-damp-cereal, mix-cereal, damp- forb meadow, mezophyl-bean and sedge, superfluous-humidified, subalpine criophyte and subalpine steppe meadows. The vegetation of the Alpine belt widespread within 2400-3200m above s.l. also is expressed by meadow and carpet elements. The vegetation at these heights is formed, mainly, on gentle slopes, dome-shaped tops, saddles. Data of the spent researches give representation about spatial laws of ecosystem distribution of Mountain Shirvan with different disturbs level and also data on change of typical structure of soils and specific structure of vegetation accordingly.

MO 317

Influence of anthropogenic factors on landscape complexes in mountains areas

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In modern age which the society is developing rapidly and has great technical strength, the relation between nature and human brought and dangerous state was beginning. Even recently the getting the nature is so strong that natural resource begins to exhaust more hastily. Now the guarding of natural resources, the use of them with suitable way, especially the guarding of animate resources and soils are considered as very important national and international problem. From this viewpoint, the solution of ecological problems pretends increasing of soils fertility with scientific bases to register them, estimating and monitoring. Part of our Republic regarding to Small Caucasus is one of the region which has natural complex and increasing the anthropogenic influences to the cover of soil. This district has according to soil climate condition or economical, geographical position to number suitable sides. Landscape complex of this area and cover of soil annually suffer to change under influence of anthropogenic factor (the weakening of agriculture's soil as for quality, expanding of the settlements, forest breaking) in last ten years. In Small Caucasus driving works such as "The ecological evaluating of the North-East slope of Small Caucasus" assume scientific and practical importance. So, general area of the investigation on the North-East slope of Small Caucasus is 433642,86 hectares and present large territory. There is a special place the soil erosion among the problems of the North-East slope of Small Caucasus. 21 soils subtype are calculation suffer to erosion have been determined. 25% s of the soil general area are undergone to erosion in the weak degree. The soils which suffer to erosion in the middle degree is 21 % s, it is less than the soils which suffer to erosion weakly. 75% soil spreading in the North-East slope of the Small Caucasus have been undergone or erosion apparently. This is the main ecological problem for the area which has been investigated not only for our Republic and also for region generally. Thus, on the weaken bases of fertility indicator of investigated soils taking into consideration the natural resources of district, for the purpose of restoration and extension of fertility and preventing negative process, complex agromeliorative, agrotechnical measures (shift sowing system, planting perennial herbs, correct pasture system) must be organized.

MO 318

Organochlorine pesticides in soils from the agricultural valley of Mexicali, Baja California, Mexico

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Mexicali valley is one of the most important agricultural areas in northwest Mexico. Is located in the borderline between Mexico and the State of California, in the United States. Due to the highly technified agriculture; organochlorine pesticides were extensively used in 60s, 70s and early 80s to enhance productivity. Even though most of the organochlorine pesticides are prohibited or restricted in their use in Mexico, there is little research about their current concentration levels, and how much they have spread in the area. In 2008, soils samples were collected for analysis of 24 pesticides (α -HCH, β -HCH, γ -HCH, δ -HCH, Heptachlor, Hectachlor epoxy, Aldrin, Dieldrin, Endrin, Endrin aldehyde, 4,4-DDE, 4,4-DDD, 4,4-DDT, 2,4-DDE, 2,4-DDD, 2,4-DDT, α -Chlordane, γ -Chlordane, Endosulfane I (alpha), Endosulfane II (beta), Endosulfane sulfate, Metoxychlor, Mirex, and total Toxaphene. Because several population centers are spread all over the valley, and are surrounded by crop land, human health risk was of the main concern. So, soils samples were collected in public areas located inside 25 of those towns. Concentration results indicated that all the organochlorine pesticides analyzed are widely spread in Mexicali valley, with DDTs accounting for the highest contribution to the total pesticides, mainly the 4,4-DDE (50-80%). The highest concentration for total DDT was 151.6 ng/g d.w., 8.1 ng/g d.w. for total HCHs and 1334 ng/g d.w. for total toxaphene. For most pesticides, surficial distribution pattern showed the mayor concentrations in the central area of the valley. Conventional ratios for DDTs indicate ancient application for this compound whereas HCH ratios indicate probable use of technical-HCH as the main source of this compound to the environment. Toxaphene results are important because basically there is lack of information about these compounds in the area. This work presents an overview of the most relevant results in order to enhance the interest of the government in this valley. Concern is raised because these and maybe other pollutants are being released constantly to the atmosphere as it has been confirmed by our passive samplers in the area.

MO 319

Environmental fate of heavy metals at small arms firing ranges

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Canadian military small arms firing range (SAFR) backstop berm soils contain high concentrations of Pb, Cu, Sb, and Zn, as a consequence of the bullet compositions consisting of a Pb-Sb core, and a Cu-Zn jacket. In order to characterize the vadose zone pore water total Pb, Cu, Sb, and Zn concentrations, three Teflon® gravimetric lysimeters (GL) were installed in the vadose zone of the backstop berm, at 30 cm and 150 cm depths. This was done in order to assess the potential for vertical migration of the metals from the berm to groundwater. The GL consisted of Teflon® cylindrical buckets filled with the in-place soil, in the bottom of which Teflon® tubes were installed. The tubes were connected to LDPE sampling bottles located in an access well. Results indicated that the Cu and Zn concentrations were below the drinking water thresholds prescribed by Health Canada (1 mg/L for Cu, and 5 mg/L for Zn) and that in many cases, Pb and Sb concentrations were above the thresholds (0.01 mg/L for Pb, and 0.006 mg/L for Sb). This indicates that a metal vertical migration occurred in the vadose zone, at levels over the acceptable drinking water criteria for Pb and Sb. Decreasing order of metal concentrations was Zn>Pb>Sb>Cu. Water infiltration rates were also monitored with a drain gauge, and used along with metal concentrations to calculate the annual total metal fluxes in the first 150 cm of the berm, and the annual metal leaching rates. Results indicated on one hand that only a small portion of the metals in the soil were mobilised in the vadose zone, as annual metal leaching rates were 0.0001% for Pb, 0.0005% for Cu, 0.003% for Sb, and 0.09% for Zn, and on the other that metals could remain in the backstop berms for extremely long period of times; e.g., several hundreds of thousands years for Pb to several thousands of years for Zn, highlighting the importance of finding ways to remediate such soils in order to limit metal mobility in SAFR backstop berms. The effects of various amendments on the environmental fate of Zn, Cu, Sb and Pb will also be described.

MO 320

Quantitative determination of PAHs in biochar - a prerequisite for its quality and safe application

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Biochar (BC) is pyrolyzed organic feedstock. It has become more and more important as soil conditioner and received broader attention as a possible measure against climate change. Polycyclic aromatic hydrocarbons (PAHs) are produced during BC production due to incomplete combustion. These PAHs will enter the environment when the BC is applied as soil conditioner to avoid leaching of nutrients, to increase microbial biomass and activity, to remediate the soil of organic pollutants, etc. Such positive effects are undermined if PAH exposure is concomitantly increased.

Methods specifically adapted for PAH analysis in BC are hardly available. Different solvents and extraction techniques have been used to determine the total concentration of PAHs in BC such as dichloromethane, methanol, acetone, hexane with Soxhlet, liquid extraction, pressurized fluid extraction, etc. Such approaches were often based on certified analytical procedures (e.g. ISO 38 414 using cyclohexane, DIN EN 15527 using petroleum ether) originally established for other matrices such as soil, and may lead to negligible or low total concentrations of PAH in BCs, or low recoveries of PAH internal standards. Hence, traditional methods may fall short to determine PAH in BC quantitatively. The aim of this study is therefore to provide an easy and validated method for the quantitative determination of PAHs in BCs to researchers, practitioners, and legislators.

Four BCs of different feedstocks were extracted with Soxhlet with two different extraction durations and with accelerated solvent extractor. Different solvents were used such as toluene, hexane, dichloromethane/acetone (1:1, v/v) and others and different cleanup steps were applied. Total concentrations were measured by gas chromatography-mass spectrometry (GC-MS).

The extraction results will be presented which include the best extraction solvent and technique, the optimal extraction duration, linearity, detection limits, method precision, sample representativeness and absolute and relative recoveries. Some implications concerning the amendment of BC will be critically evaluated.

We have developed a simple, robust and sensitive extraction method to quantitatively determine total concentrations of PAHs in BCs. This method may serve researchers, practitioners and legislators to optimize BC production with a view to minimize its PAH content, and to properly assess the environmental benefits and risks of this overall promising material.

MO 321

Fate of persistent organic pollutants in soils amended with sewage sludge: sampling and modelling results

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Sewage sludge (SS) application on agricultural soils is a managing practice of increasing use because of its benefits to soil and crops. However, SS has an extensive range of persistent organic pollutants (POPs) that may be transferred from soil to crops and reach the food chain.

The main objective of this work was to evaluate POPs fate in soils amended with sewage sludge. To accomplish this objective, the work was divided in two parts: samples evaluation and model development. First, 200 SS samples from 125 wastewater treatment plants all around Spain were collected and the concentrations of the POPs proposed in the directive (PAH, NP+NPes, PCDD/Fs, PCBs, LAS) and other emergent organic contaminants (PBDEs, PCNs, PFOS+PFOA) were measured. Also, controlled parcels with different soil texture, organic matter content, crops, and sewage sludge application rates were chosen. In each parcel, soil samples were measured for POPs.

Then, a probabilistic multimedia model to predict the accumulation of POPs in soils, as a consequence of SS application on agricultural soils, was developed. This dynamic model predicts the concentration of POPs in amended soils for different time spans, considering the following processes: volatilization, diffusion between compartments, leaching, reaction and deposition. The model input parameters are: sludge characteristics and application dose, soils characteristics (density, bioavailability, volume fraction of soil compartments air, water, organic matter and mineral matter) and pollutant properties (solubility, K_{ow} , K_{oc} , vapour pressure, H , persistency, and half life in soil). Two layers of soil were considered. This model was validated with data from the sampling campaign, considering SS characteristics and application rates, soil characteristics and POPs concentration in soil and SS. Results showed a good agreement between model predictions and field values.

MO 322

Alteration of the reproductive toxicity of Cadmium in the soil living Nematode C.elegans upon potential chemosensitization by HHCB

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1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran (HHCB or Galaxolide) is a bioaccumulative and environmentally prevalent synthetic musk compound contained in various consumer products (such as perfumes and detergents). From first toxicity studies on HHCB the European Commission (2008) concluded with no or negligible risks for humans and the ecosystem. Mechanistically HHCB could be characterized as a chemosensitizing compound and as a possible substrate and inhibitor of cellular efflux pumps (Luckenbach and Epel, 2005). Cellular efflux systems were shown to co-regulate the intracellular bioavailability of xenobiotics and other contaminants (Bard, 2000; Epel, 1998; Kurelec et al, 2000). Seizing the scientific debate on the potential toxicological implications of chemosensitization this study presents novel ecotoxicological data on the chemosensitization potential of HHCB on *Caenorhabditis elegans* (C.elegans) towards the heavy metal cadmium. The reproductive toxicity of the LOEC of cadmium (0.1 mg/L) was herewith potentiated by 27% under simultaneous exposure to the LOEC of HHCB (0.1 mg/L). Remarkably, a post-exposure chemosensitization effect was observed 12 to 96 h after a 12 h pre-exposure to 0.1 mg/L HHCB, reflected in a 17% to 60% higher reproductive toxicity of Cadmium at 0.1 to 10.0 mg/L. We assume that HHCB at low concentrations had potentially inhibited relevant efflux transporters of C.elegans (Broeks et al., 1995 and 1996) in a competitive manner, coinciding with an elevated intracellular bioavailability of Cadmium. The obtained results support related observations by Luckenbach and Epel (2005) and by Cheng and Zhou (2009). Overall the results appreciate the mechanistic complexity of mixtures of prevalent aquatic and soil contaminants and the manifold factors involved in their bioavailability and toxicity.

ET13P - Natural toxins and bioactive compounds

MO 326

Tetrodotoxin expansion in the temperate waters of the Atlantic Ocean new vectors and impact on marine ecosystems

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Tetrodotoxin (TTX) is a potent low weight marine toxin found in the warm waters of the Indic and Pacific Oceans. Intoxications are usually linked to the consumption of the puffer fish although TTX was already detected in several different taxa. The rise of water temperature due to climate change, and anthropogenic interventions are pointed as the main causes of the toxin spreading.

Benthic organisms such as mollusks and echinoderms, with different eating habits were collected monthly along the Portuguese coast since the summer of 2009 till the end of 2010.

The extraction and analysis techniques were optimized and TTX and analogues were detected for the first time in two intertidal gastropod species -*Gibbula umbilicalis* and *Monodonta lineata* by UPLC-MS/MS and LC-MS/MS.

These findings and their possible impact on marine communities are discussed taken into account the dynamics of the ecosystem and human health.

This work was made under the ATLANTOX project.

MO 327

Hydroxylated and methoxylated brominated diphenyl ethers: natural products or Anthropogenic Contaminants?

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Brominated Diphenyl Ethers (PBDEs) have been widely used as flame-retardants in the last few decades. Since their chemical structure is similar to PCBs, these chemicals may pose a significant threat to the environment and have emerged as contaminants of concern.

In recent years, however, structural analogs of PBDEs, including hydroxylated- and methoxylated- BDEs (OH-/MeO-BDEs) have been detected in the biota at different trophic levels. The wide spreading high concentration of OH-/MeO-BDEs in marine biota leads to the postulation that these compounds may be natural metabolite products of PBDEs of marine organisms rather than anthropogenic contaminants.

In this study, four species of plankton algae (two diatom species: *Thalassiosira pseudonana* and *Skeletonema costatum*; and two dinoflagellate species *Prorocentrum dentatum* and *Prorocentrum minimum*) were cultured in (a) Br free artificial sea water and (b) artificial seawater with isotope ⁸¹Br under laboratory conditions. Levels of 9 common MeO-BDEs analogues, 10 OH-BDEs analogues and 12 Brominated Phenol (BRPs) analogues were determined by GC-MS.

Mass balance of ⁸¹Br in the algal and water samples was constructed to test the hypothesis that OH-/MeO-BDEs are synthesized by the algae. Result of this study will be discussed and help us to differentiate and understand the anthropogenic and natural sources of hydroxylated and methoxylated BDEs found in the marine environment.

MO 328

Assessing the toxicity of two natural bioactive compounds applied in aquaculture using zebrafish embryos

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Losses in aquaculture production are often associated with predation by wild fish species in culture ponds. The rotenone (RO) and saponin (SA) are two piscicides plant-derived widely used to remove undesirable fish species in the ponds in aquaculture activities. In the present study the effects of RO and SA in zebrafish embryos were studied. A 144-h test was performed following the draft guideline Fish Embryo Toxicity (FET) test and several endpoints were analysed. Lethal endpoints included egg coagulation, non-development of somites, non-detachment of the tail, and absence of heart beat. Sub lethal endpoints included hatching, lack of body and eyes pigmentation, oedemas (yolk sac, pericardial) and tail malformation. The results show clear dose response effects of rotenone on zebrafish development with a 96 h-LC50 value of 16.8 µg/l. After 96 h of exposure to concentrations >10 µg/L of RO the embryos showed abnormal body pigmentation, loss of equilibrium, and spine deformities and at 20 µg/L embryos showed cardiac oedema. For SA a 96 h-LC50 value of 27900 µg/l was determined and no sub lethal effects were found for the concentrations tested. Thus, SA was much less toxic than RO for zebrafish embryos. Nowadays, the available data on RO and SA for fish control purposes indicate that it can be used safely. However due the very high toxicity of RO and uncertainties beyond the intended target fish to both compounds, effects on non-target organisms can be expected.

MO 329

Environmental responsibility, nematode management and collaboration between academia and industry

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Plant-parasitic nematodes account for annual losses in world agriculture that exceed (U.S. dollars) 11 billion. An extensive arsenal of tactics for their management exists; but, unfortunately the use of ozone-depleting, soil-contaminating, mammalian-toxic, broad-spectrum and expensive organophosphate, carbamate and fumigant nematicides remains the most widely utilized tool. This chemical management tactic choice is economics-driven in an era hypersensitive to escalating agricultural production costs. Widespread public awareness of the negative environmental consequences of over- and/or mis-usage of pesticides, especially nematicides, has resulted in legislation in America and abroad prohibiting or severely curtailing their production. A decade of university-based research with a novel colloidal mixture, formulated with near-inert sodium alginate as the primary ingredient, has produced an environmentally -responsible, low-rate and inexpensive material effective against a range of the most damaging phytoparasitic nematode species. Research to date had documented significant efficacy of this material against populations of reniform, root-knot, soybean cyst, sting, stunt, lesion, lance, spiral, ring and stubby-root nematodes. Subsequent yield enhancement resulting from management of these nematodes ranged from 23-60 percent on sugarcane, soybean, cotton and a wide range of vegetables including tomato, cucumber, eggplant, bell pepper, cabbage and carrot. Unlike most traditional broad-spectrum nematicides that are lethal to both pest and beneficial rhizosphere-inhabiting microorganisms, this material is nematode-specific. The activity of this as yet non-commercialized formulation results in large measure from the physical blockage of natural body openings of the nematode and also from interference with the tactile responses that lead to host-finding. Supporting evidence to be presented will include data from field, microplot and greenhouse trials comparing the density and distribution of phytonematodes and crop yields in soils treated with this alginate-based material versus traditional fumigant and non-fumigant nematicides. Additionally, data from in-vitro laboratory trials and light and electron microscopy studies will verify the mechanism of action. Collaboration between university and commercial scientists in the development of eco-responsible chemical management tools is essential for plant protection in twenty-first century agriculture.

MO 330

Chronic effect of boiling green tea on Swiss albino mice

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Boiling green tea is a common hot drinking in Libya. Usually, it boils for 20 to 30 minutes, to have it concentrated. Most people who drinking the green tea, have it three times daily. In this study we investigated the effect of boiling green tea on Swiss albino mice for chronic effect, for 21 days. The concentration were used daily, 0.1, 0.2 and 0.4 ml, orally to adult mice body weight, 26 ± 3 g which is three time more than amount that people drink daily. During the study, body weight, blood parameters (PCB), Cholesterol, triglyceride, sugar, and liver, heart, kidney and pancreas weight were measured. These doses have shown no effect on mortality. However, Body weights were decreased at 0.4ml comparison to control. RBC, PLI number as well as HGB and Cholesterol, triglyceride, sugar have no different with control group. However, WBC were increased at 0.2 and 0.4 ml. This study was first in Libya to show effect of boiling green tea, and can be repeated to show effects on reproduction for longer period as well as to investigate its effect on short time, on some biomarker.

MO 331

The Sacaca's study on the treatment of Hypertension, Rio Branco, Acre, Amazonia, Brazil

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INTRODUCTION: Modern science has gradually awakened interest in plants of the Amazon. The *Croton cajucara*, Benth (Euphorbiaceae), known popularly as sacaca seems to have different pharmacological properties. It is known that the poorest segments of society and older have a higher prevalence of hypertension (SH), and have as the only alternative medicines supplied by the Unified Health System, so, invest on the potential of medicinal plants is essential because it corroborates the ethical principles and international commitments, while promoting the generation of wealth with social inclusion. The study aimed to evaluate the effects of its use in the treatment of hypertension according to reports in the study population. **MATERIAL AND METHODS:** The study focused on people living in the city of Rio Branco, Acre. The questionnaire was used in 125 people in this population, and 25 people each SPS to carry out ethnobotanical survey of Sacaca. We used the randomizer program for the selection of each of the 25 people who were interviewed. All respondents signed a consent form Statistical analysis was performed SPSS, version 13.0 for Windows. The work was approved by the Ethics Committee at the Federal University of Acre. **RESULTS:** From the survey it was possible to profile the population studied, most of whom were aged over 60 years, female, attended high school, had incomes of up to three minimum wages, lived with more than four people, worked, did not consume alcohol, not smoke and were part of some religious group. It was observed that 60% of those interviewed knew the sacaca, 80% of these had their blood pressure controlled and monitored. No respondent indicated the use of medicinal plants through medical advice. Most received indication from friends. About 30% used sacaca for the treatment of illness. The population was unable to report on side effects or contraindications, which drew attention because cytotoxic and hepatotoxic effects have been proven. **CONCLUSION:** We did not observe the use of sacaca in the treatment of hypertension. However, there are therapeutic effects that can be used as adjuvants in the treatment of this disease. We emphasize the need for more scientific research in the north of this plant and its uses especially in the treatment of heart disease, since in 2025 Brazil will be the sixth country in the world in the elderly population (WHO).

KEY WORDS: Hypertension, Medicinal Plants, Sacaca.

MO 332

Ecotoxicological effect studies with mycotoxins of possible environmental concern

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Mycotoxins are naturally occurring secondary metabolites of fungi colonizing a variety of cereals, fruits, vegetables and organic material in the soil, but can also arise due to moist conditions during storage. They regularly contaminate our food and feed and, hence, their toxicity to humans and husbandry animals has been studied in detail.

Recently published data showed that the aquatic environment can also be exposed to mycotoxins. The identified main input sources of mycotoxins into the aquatic environment include 1) run-off and drainage water from fields cultivated with cereals, like wheat or corn, 2) manure application and excretion from grazing livestock and 3) human excretion via sewer systems. However, current ecotoxicological data on mycotoxins are scarce. Therefore, we applied an array of ecotoxicological tests to quantify their effects. The baseline toxicity was quantified with the bioluminescence inhibition test using the marine bacterium *Vibrio fischeri*. The specific modes of toxic action that were targeted with four additional bioassays included aspects of dioxin-like activity (AhR-CAFLUX-test), genotoxicity (umuC-test), phytotoxicity (IPAM-test) and glucocorticoid like activity (GR-test). Data from these tests will be presented and used for a first preliminary evaluation of the ecotoxicological relevance of mycotoxins.

MO 333

Occurrence of mycotoxins in the effluent of a waste water treatment plant

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Mycotoxins are naturally occurring secondary metabolites of fungi colonizing a variety of cereals, fruits, vegetables and organic material in the soil, but can also arise due to moist conditions during storage. Mycotoxins have been studied intensively for decades due to their occurrence in food and feed and, hence, their potential threat to human and animal health. Recently published data support the presumption that the aquatic environment can also be exposed to mycotoxins via human excretion due to the consumption of mycotoxin contaminated food stuff. For instance, deoxynivalenol (DON) was frequently observed in urine and was significantly associated with cereal intake. Accordingly, DON proved to be omnipresent in the primary effluent of several waste water treatment plants (WWTPs). Based on comparable or even higher amounts produced in wheat and maize, and similar or even higher aqueous solubilities, a larger number of mycotoxins is likely to enter the aquatic environment. This hypothesis was tested by application of a newly developed multi-residue screening method to quantify 33 mycotoxins in WWTP effluent samples. Data from this campaign will be presented.

MO 334

Occurrence of mycotoxins in Swiss surface waters

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Mycotoxins are naturally occurring secondary metabolites of fungi colonizing a variety of cereals, fruits, vegetables and organic material in the soil, but can also be formed due to moist conditions during storage. Mycotoxins have been studied intensively for decades due to their occurrence in food and feed and, hence, their potential threat to human and animal health. More recently, it has been shown that deoxynivalenol (DON) and zearalenone (ZON), two important mycotoxins produced by *Fusarium* spp., are emitted from fields cultivated with *Fusarium*-infected cereals or maize, or via human excretion and wastewater treatment plants, thereby acting as micropollutants in the aquatic environment, specifically surface waters. This far, no such data is available for any other mycotoxin, although, based on comparable or even higher amounts produced in wheat and maize, and similar or even higher aqueous solubilities, a larger number is likely to enter the aquatic environment. Therefore, we applied an earlier established analytical screening method to quantify 33 of the most prominent mycotoxins at trace level concentrations (i.e. ng/L) in different aqueous matrices to surface water. For this, flow-proportional samples were gathered weekly to biweekly at twelve sites from ten rivers across Switzerland since December 2009 till end of October 2011. Throughout the investigation period, four mycotoxins were detected regularly, with nivalenol being the most prominent mycotoxin in terms of frequency of occurrence, closely followed by DON which showed the highest mean concentration. Other compounds detected occasionally include beauvericin, and 3-acetyl-deoxynivalenol. Different approaches to elucidate the main contributors of mycotoxins in surface waters will be presented.

MO 335

Lipid soluble acyl conjugates of hydroxylated polybrominated diphenyl ethers (OH-PBDEs) in blue mussels from the Baltic Sea

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Algae and cyanobacteria in the Baltic Sea have been reported to produce high quantities of hydroxylated polybrominated diphenyl ethers (OH-PBDEs) (1). These are released into the surrounding ecosystem at the end of the algae's life cycles. Some of these compounds have been proven to disrupt the oxidative phosphorylation in exposed organisms (2), which causes a decreased production of adenosine triphosphate (ATP). This may lead to serious effects on the energy balance in the Baltic ecosystem. These compounds have also shown negative effects of the endocrine system through e.g. competitive binding to transthyretin (TTR) (3), and interactions with estrogen receptors (4).

The present study was undertaken to investigate the possible conjugation of OH-PBDEs, with lipophilic substrates (e.g. fatty acids), in mussels under natural conditions. Such conjugation could have the potential to work as a detoxification mechanism in mussels suffering from intense exposure.

The study revealed that as much as 50% of the total amount of OH-PBDEs in blue mussels can be bound as esters, and individual congeners can be conjugated in concentrations of up to 65 ng/g lipids.

The discovery that halogenated phenols, at least in mussels, can occur as lipid soluble neutral conjugates in high amounts, under natural conditions, is new. The repercussions of these findings may be that conventional analyses to assess the total amount of halogenated phenols, in e.g. mussels, systematically have underestimated the true levels.

Although the conjugation may relieve the toxic effects of the OH-PBDEs within the mussels, it is probable that these conjugates are hydrolysed in the digestive tract of predators feeding on these mussels, which will release the OH-PBDEs back to their free biologically active form. The blue mussel has a high ecological value in the Baltic Sea where it is the dominating specie and the main source of food for many other species, e.g. marine birds. Long-tailed ducks, with a body mass of about 1 kg, have to eat about 1 kg of blue mussels per day, and eiders up to 2.5 kg per day, just to stay alive in winter (5). This means that these birds are exposed to high amounts of both bound and free OH-PBDEs through their diet.

(1) Malmvärn et al. *Chemosphere* 2008, 72

(2) van Boxtel et al. *Environ. Sci. Technol.* 2008, 42

(3) Ucan-Marín et al. *Toxicol. Sci.* 2009, 107

(4) Meerts et al. *Environ. Health Perspect.* 2001, 109

(5) Bustnes and Systad *Waterbirds* 2001, 24

MO 336

Spectrophotometric determination of free gossypol in cottonseed by-products

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Gossypol is a natural herbicide found in the seeds of the cotton of the genus *Gossypium*, which is used as a protein source in diets for monogastric animals. This study aims to determine the level of gossypol in cotton matrices. The method used for the analysis procedure consists of a liquid-liquid extraction using organic solvents and spectrophotometric quantification using the second derivative spectra. The results obtained so far suggest that in the three matrices studied, the highest percentage of free gossypol is in cottonseed. The determination of free gossypol is important for purposes of food inspection.

MO 337

Novel tumour promoting metabolites in complex cyanobacterial samples (biomasses and exudates) characterized by in vitro assays

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Recently, production of novel cyanobacterial metabolites with tumour promoting activity had been reported (Toxicon 2009, 53:519-524). Goal of our present study was to investigate the tumour promoting activity of complex cyanobacterial samples including intracellular (extracts of biomass) and extracellular mixtures (exudates/spent-media).

Biomasses and exudates from 11 different cyanobacterial cultures forming toxic water blooms (*Microcystis aeruginosa*, *Aphanizomenon flos-aquae*, *Planktothrix agardhii*, *Anabaena flos-aquae*, *Cylindrospermopsis raciborskii* etc.) were collected. Tumor promoting potencies of the cyanobacterial samples were assessed using the assay for gap-junctional intercellular communication (GJIC) in rat liver stem-like cells WB-F344, where inhibition of GJIC is established as a marker of tumor promotion. Significant down-regulation of GJIC was caused by both intracellular and extracellular mixtures but effects differed among tested cyanobacteria and were not related to the content of known cyanotoxins such as microcystins or cylindrospermopsin. Exudate of *C. raciborskii* was selected for further characterization because of its strong GJIC-inhibition activity.

Semipreparative HPLC (C-18 column) with water/methanol gradient elution was used for fractionation. Two fractions with hydrophobic compounds strongly down-regulated GJIC and the LC-MS/MS analyses were used for detailed separation and characterization of the causative agent. A new small compound with molecular weight 320 appears to be responsible for observed tumor promoting potencies. The research was supported by the Czech National Science Foundation grant No. 524/08/0496 and by the project CETOCOEN (CZ.1.05/2.1.00/01.0001) from the European Regional Development Fund.

MO 338

Influence of light, cultivation and growth phase on cyanobacterial and algal production of metabolites with specific activity

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Cyanobacteria are known producers of wide spectrum of compounds with various effects such as skin irritation, gastrointestinal tract and respiratory distress, neurotoxicity, immunotoxicity or hepatotoxic, cancerogenic, genotoxic and mutagenic effects. Recent studies have shown also endocrine disruptive potential of specific cyanobacterial and algal strains, reported for natural strains as well as for laboratory cultures. Our previous papers reported activation of estrogenic receptor (intracellular nuclear receptor) using in vitro assays after exposure to both intracellular and extracellular mixtures of several cyanobacterial and algal samples. This estrogenic activity of compounds produced by cyanobacteria and algae could contribute to endocrine disruptive potential in surface waters. Aim of our study was to characterize impact of external factors such as light, cultivation conditions and growth phase on production of endocrine disruptive metabolites in selected cyanobacterial (*Microcystis aeruginosa* PCC 7806 and *Aphanizomenon gracile* RCX 06) and algal strains (*Scenedesmus quadricauda* CCALA 463) by use of in vitro reporter gene trans-activation assay with the ER-linked luciferase gene under control of the ERE (estrogen responsive element). At the same time, the production of well known cyanotoxin microcystin was assessed. Our results characterize dynamics and variability of production of endocrine disruptive compounds and microcystin during growth phase. The detailed characterization of the cultures enabled to quantify the production of studied metabolites relative to the cell count and dry weight of the biomass.

MO 339

The effects of oral administration of toxic cyanobacteria on multiple physiological, biochemical, hematological and immunological parameters in rats

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Cyanobacteria are known to produce numerous bioactive compounds including widely studied peptide hepatotoxins - microcystins (MCs). Aims of the present study were to provide a model simulation of a simple food chain for evaluation of impacts cyanobacterial toxins on the mammal (rat) physiology under different exposure scenarios.

Experimental rats were fed for 28 days with food containing fish meat with external additions of isolated microcystins, biomass of toxic cyanobacteria *Microcystis*, nontoxic cyanobacteria *Arthrospira* and also green alga *Chlorella*. Subgroups of the animals were also challenged with a model antigen KLH to investigated immune-related parameters. Although considerable amounts of microcystins were orally administered to rats, levels of MCs in the tissues were below the detection limit (1 ng/g fresh weight; based on the tandem LC/MS). Only traces of the conjugates of microcystins with cysteine and glutathione were detected in the rat's liver exposed to *Microcystis* biomass. The feed containing cyanobacterial biomass was found to have negative impacts on the weight gain and food conversion ratio of experimental rats. Interestingly, rats exposed to MCs had stimulated immune system (higher antibody responses to administered KLH antigen). Also modulations of certain lymphocyte subpopulations was recorded with the most interesting observation of stimulated numbers of NK cells in the groups exposed to isolated toxins. Biomarkers of the oxidative stress (glutathione levels, lipid peroxidation) did not show any differences, while several hematological parameters of blood were significantly modulated. Our study demonstrates that oral exposure to microcystins or cyanobacterial biomass may have different impacts on various less explored biochemical and immune parameters in experimental mammals.

MO 340

Protective role of N-Acetylcysteine against the oxidative damage induced by cylindrospermopsin in tilapia (*Oreochromis niloticus*)

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Cylindrospermopsin (CYN) is a cytotoxic cyanotoxin produced by several species of cyanobacteria. CYN consists of a tricyclic alkaloid with a high solubility in water, where it can be present approximately the 90% of the total CYN produced by cyanobacteria. Aquatic animals and plants can be exposed to CYN, and it is known that CYN is a potent synthesis inhibitor of protein and reduced glutathione (GSH). Moreover, recent studies have established that CYN produces oxidative damage through the generation of reactive oxygen species (ROS).

As the presence of CYN in freshwater systems could have negative effects on fish it would be interesting to find compounds able to counteract the toxicity observed. In this sense, N-Acetylcysteine (NAC) is a GSH precursor which can protect organs against the oxidative damage through two different mechanisms: by the stimulation of the GSH synthesis and by direct association to ROS. In the present work, we aimed to investigate the effects of pure CYN and CYN from a lyophilized *Aphanizomenon ovalisporum* culture on fish, and the protective role of a pre-treatment (7 days) with different doses of NAC. Both products (CYN and NAC) were administered together with the food. Fish were euthanized after 24 hours of the toxin exposure, and liver and kidney were extracted. For this purpose, we used different oxidative stress biomarkers such as lipid peroxidation, γ -glutamylcysteine synthetase (GCS) activity, and GSH levels. Results showed that CYN induced oxidative stress, as evidenced by the increase of lipid peroxidation, the decrease in GSH/GSSG, and the alteration of the GCS activity. Moreover, the exposure to cyanobacterial cells containing CYN induced higher toxic effects in comparison to pure CYN. NAC supplementation was effective in reducing the CYN induced toxicity, particularly at the highest dose employed (45 mg/fish/day). In conclusion, NAC can be considered a useful chemoprotectant in the prophylaxis of CYN-related oxidative stress induction in fish.

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MO 341

Preliminary study of biomarkers in mussel haemolymph after exposition to toxins extracted from *Microcystis aeruginosa* and *Lyngbya wollei*

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The freshwater cyanobacterium *Lyngbya wollei* is a dominant primary producer in many lakes and reservoir, whereas *Microcystis aeruginosa* can become a dominant cyanobacteria commonly associated with eutrophic conditions. Both cyanobacteria produced toxin, *M. aeruginosa* (MCYST) produced a potent specific hepatotoxin, whereas *L. wollei* (LYNGTX) produced an analogue to saxitoxin which is a neurotoxin. Selected immune parameters investigating haemolymph integrity and immunocompetency were analyzed after exposure of the two toxins by flow cytometry of non cytotoxic low concentrations of MCYST and LYNGTX. Exposure of freshly isolated mussel haemolymph to low concentrations of 2, 5, 10, 25, 50ng/ml for 18h periods resulted in a significant interaction term (concentration x type of toxins) of the two-ways ANOVA of haemocyte viability. Haemocytes exposed to MCYST tended to decrease their viability at the highest concentration whereas haemocytes exposed to LYNGTX tended to increase their viability at the highest concentration. However, the type of toxin was significantly different for phagocytosis activity. Haemocyte exposed to MCYST had a lower phagocytosis activity than haemocyte exposed to LYNGTX. Furthermore, the present study will investigate the effects of these toxins on the intracellular thiols production, reactive oxygen species (ROS) production, cyclooxygenase activity (COX) and nitrate production in mussel haemolymph. Preliminary results showed that haemocytes integrity and immunocompetence exhibited opposite reaction of these two types of toxins. A decrease of the phagocytic index is usually observed response to in vivo or in vitro exposures to toxic concentration of xenobiotics where MCYST seemed to have a similar effect. This preliminary study will require additional biomarkers to support the hypothesis of a toxic effect associated with the mats of filamentous *L. wollei*.

MO 342

Phosphatases and Esterases of *Limnoperna fortunei* (golden mussel) in presence of *Microcystis aeruginosa*

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Toxic blooms of cyanobacteria are reported in freshwater sources around the world, and the effects of these events in environments where there are filter feeding organisms can involve the accumulation of toxins and their transfer in the trophic chain. In order to study possible changes in mussels from some of the enzymatic activity and bioaccumulation of microcystins, we performed in vivo experiment with the species *Limnoperna fortunei* that were exposed to cells of *Microcystis aeruginosa* (strain NPLJ-4). After 48 hours of exposure, the cells were counted and assayed the total phosphatase and esterases, these at a fraction obtained by centrifugation at 10,000 x g. Specific activities of total phosphatase were inhibited by 90%, while those of carboxylesterase and acetylcholinesterase by 50% in this experiment of effect in vivo. Mussels exposed to higher concentrations in vivo (5,140,000 and 2,570,000 cells.mL⁻¹) accumulated microcystin. In vitro assays of enzymes soluble fractions *L.fortunei* with extracts of *M. aeruginosa* strain NPLJ-4, obtained with 100% methanol (Me100 - extract with microcystin) and 10% (Me10 - without microcystin extract) show that inhibition of phosphatases occurs with both extracts, whereas esterases are inhibited only with the Me10. The esterases of *L. fortunei* are similar to other species of molluscs as demonstrated in their IC50 in the presence of eserine. The inhibitions caused by the in vitro fraction without microcystin present new possibilities on the effects of exposure to *Microcystis aeruginosa*.

MO 343

Do mixture effects of metal stress (Cu) and natural stress (cyanobacterial toxins) add up in *Daphnia magna*?

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Current risk assessment of chemicals does not deal with mixture toxicity, which contrasts with ecology reality. The combined effect of stressors is not necessarily bound to the generic pattern of additivity but can instead result in their combined effect being either more or less toxic than would be expected from their individual toxicity (synergy or antagonism).

We investigated the combined and interactive effects of dissolved copper (Cu) and cyanobacteria in two *Daphnia magna* clones (Xinb3 and Iinb1). Several cyanobacterial species (*Microcystis aeruginosa*, *Cylindrospermopsis raciborskii*, *Anabaena* sp., *Nodularia* sp., *Oscillatoria* sp., *Aphanizomenon-flosaque*) were investigated, ensuring toxins with different Modes of Action (MoA). Semi-static 21-day life-table experiments were performed according to an extended second-order two factor central composite design, consisting of 24 treatments. Several life history parameters were recorded (survival, daphnid size, time to maturity, clutch sizes, total reproduction, and intrinsic rate of increase r_m). The experiment was performed. Significant deviations from additivity determined using the MIXTOX model (Jonker et al. 2005 Environ Toxicol Chem 24:2701-2713). The fitted model can be used to make predictions on the magnitude of increase or decrease of Cu toxicity in the presence of cyanobacteria.

MO 344

Interactive effects of combined chemical and natural stressors on *Daphnia pulex*

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In aquatic ecosystems *Daphnia* are exposed to a wide variety of both natural and chemical stressors. These stressors can cause interaction effects resulting in an increased impact on aquatic ecosystems. We exposed *Daphnia pulex* to binary combinations of cyanobacteria and insecticides. We selected three cyanobacteria differing in habitat and toxin production: *Cylindrospermopsis raciborskii* (cylindrospermopsin producer), *Nodularia* (nodularin producer) and *Oscillatoria* (anatoxin-a producer). These stressors were combined with 8 insecticides differing in mode of action: acetamiprid, carbaryl, chlorpyrifos, deltamethrin, endosulfan, fenoxycarb, tebufenpyrad and tetradifon. *Daphnia* were exposed for a period of 21 days to an insecticide, a cyanobacteria and their mixtures based on a modified central composite design. This design allowed for simultaneous testing of individual and combined effects as well as testing for different doses and ratios. Results were analysed with the mixtox model developed by Jonker (Jonker et al. 2005 Environ Toxicol Chem 24:2701-2713). The significance of model deviations for synergistic and antagonistic effects were determined through maximum likelihood, while regression analysis was performed to select the best reference model. The analysis resulted among others in additive effects for combinations of carbaryl with *Cylindrospermopsis*, while combinations of tebufenpyrad and *Cylindrospermopsis* demonstrated antagonistic effects on reproduction of *Daphnia*. Results suggest complicated interaction patterns between insecticides and harmful cyanobacteria potentially related to mode of action patterns.

MO 345

Physiological effects of a marine algal toxin on a primary consumer: tales of the unexpected

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Harmful algal blooms can cause serious health risks and economical losses due to the production of toxins. Okadaic acid (OA), a marine toxin produced by different species of dinoflagellates including *Prorocentrum lima*, accumulates in filter feeding shellfish. OA is known to inhibit the protein phosphatase enzymes in humans consuming contaminated shellfish, resulting in the diarrhetic shellfish poisoning syndrome.

Although the physiological effects of OA on mammals are relatively well understood, this is not the case for the effects on the main vector (i.e. shellfish). In this study, the effects of OA on the lysosomal membrane stability (LMS) of the haemocytes and on the protein phosphatase (PPase) activity of the hepatopancreas in the mussel *Mytilus edulis* were studied. In the first phase the mussels were dissected and the enzymes of the hepatopancreas were exposed to okadaic acid *in vitro* (conc. up to 672 µg OA/g hepatopancreas WW). This resulted in a significant reduction (4%) of the PPase activity, starting at a concentration of 26.2 ng OA/g hepatopancreas WW, with a maximum inhibition of approximately 15% at 168 ng/g hepatopancreas WW and higher. In the second phase, the mussels were exposed *in vivo* to toxin producing algae (*P. lima*). Two concentrations were tested: 2.85 µg OA/l (500 cells/ml) and 9.55 µg OA/l (1500 cells/ml). Okadaic acid accumulated in the mussels' hepatopancreas with a mean concentration of 220 ng/g WW (500 cells/ml) and 740 ng/g WW (1500 cells/ml). Based on the *in vitro* results a 15% reduction in PPase activity was expected. However, there was no significant reduction of the LMS or the PPase activity.

Two conclusions can be drawn from these results. First, *in vitro* there is a 15% reduction of the PPase activity this means that these enzymes are sensitive to OA exposure. Secondly, *in vivo* OA has no negative effect on the PPase activity or LMS. Apparently *M. edulis* is, at the concentrations tested, able to prevent the inhibition of protein phosphatase enzymes by OA.

MO 346

Oxidative stress responses produced in liver of tilapia by exposure to repeated doses of cylindrospermopsin from *Aphanizomenon ovalisporum*

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Cylindrospermopsin (CYN) is a tricyclic alkaloid, possessing a tricyclic guanidine moiety combined with hydroxymethyluracil. It is produced by several genera of cyanobacteria, growing worldwide in eutrophic freshwaters and it is easily transferred among different trophic levels causing damages to humans, animals and plants. It is well established its mechanism to block protein synthesis and, more recently, its ability to cause oxidative stress is being studied.

The aim of this work was to study the oxidative stress responses in liver of tilapia (*Oreochromis niloticus*) exposed to repeated doses of CYN by exposure to both a culture and lyophilized cells of *Aphanizomenon ovalisporum* (LEGE X-001). These parameters were: lipid and protein oxidation, and the enzymatic activities of Glutathione-S-transferase (GST), Glutathione Peroxidase (GPx), Catalase (CAT) and Superoxide dismutase (SOD).

In this study, nine groups of tilapia with 8 fish per group were established: 3 control groups, 3 groups exposed by immersion to an *A. ovalisporum* culture (10 µg CYN/L), and 3 groups exposed by immersion to lyophilized *A. ovalisporum* cells (10 µg CYN/L). The exposure to the toxin was carried out every two days within the periods of 8, 14 and 22 days, respectively. Afterwards, fish were sacrificed and the liver extracted for further determinations.

In general, major changes were observed in tilapia treated for 8 days and the alterations were more significant when tilapia were exposed to CYN produced by the culture cells, in comparison to the lyophilized cells of *Aphanizomenon ovalisporum*.

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MO 347

Development of a method for determination of CYN in lyophilized *Aphanizomenon ovalisporum* cells by LC-MS/MS: validation and application to real samples

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Harmful cyanobacterial blooms are occurring in eutrophic freshwater lakes and reservoirs throughout the world and can present a public safety hazard through contamination of drinking water supplies. There are over 40 species representing 20 genera from three cyanobacterial orders known to produce cyanotoxins which include both cyclic peptides and alkaloids such as cylindrospermopsin (CYN), produced by *Aphanizomenon ovalisporum* among other species. Therefore it is important to develop and validate a method to determine CYN at trace levels. The aim of this work was to develop an analytical procedure based on solvent extraction followed by a purification step with graphitized cartridges and LC-MS/MS for CYN determination from lyophilized cultures of *A. ovalisporum* (LEGE X-001). The extraction and purification steps were optimized using a two-level full factorial design with replications.

This report presents a sensitive, reproducible, accurate, and robust method for extraction and determination of CYN in lyophilized cells. The recoveries (83-94%) and intermediate precision values obtained (5.6-19.3 %), as well as the robustness of the method for the three factors considered, permit its validation. This method provides acceptable detection and quantification limits for environmental studies and proves its utility for monitoring CYN in lyophilized natural blooms samples, and also for routine human health assessment purposes in relation to the provisional guideline and TDI, representing an available instrument to advance in these studies.

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MO 348

Screening for cylindrospermopsin, anatoxin-a and saxitoxins in France

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Microcystins are the most intensively studied toxins and numerous reports on their occurrence exist all over the world. A WHO guideline value of 1 µg/L Microcystin-LR exists for drinking water, which has been adopted in several European guidelines (e.g. France). In contrast, little is known about the distribution of other hepatotoxins such as cylindrospermopsin (CYN), and the neurotoxins anatoxin-a (ATX) and saxitoxins (STX) in Europe. These are primarily produced by cyanobacteria of the Nostocale order. As they may be a problem for the safety of drinking water, Veolia carried out a research program on these cyanotoxins and their potential cyanobacteria producers in France from 2007 to 2011.

Ten freshwater reservoirs used for drinking water production were studied during the summers of 2007, 2008 and 2010. Mixed water samples from the water column of the epilimnion were collected. They were analysed for nutrients, chlorophyll-a, phytoplankton, CYN, ATX and STX.

Cyanobacteria were detected in 98% of the samples. WHO level 3 for raw water used for drinking water production (>100 000 cell/mL) was reached for 20-27% of the samples. In 2007, Oscillatoriales, dominated by Planktothrix agardhii, largely prevailed in deep water whereas Nostocales were more present in pre-reservoirs; Chroococcales were minor. In 2008, Oscillatoriales prevailed over all other orders in the pre-reservoirs or in deep waters. In 2010, Nostocales were primarily present in deep waters and Oscillatoriales in pre-reservoirs.

Among the species observed in the water samples, the following potential CYN, ATX-a, STX producers were observed: *Anabaena flos-aquae*, *Anabaena planctonica*, *Aphanizomenon flos-aquae*, *Aphanizomenon gracile* and *Aphanizomenon issatschenkoi*. ATX, CYN and/or STX were measured in 41% of the samples in 2007 and 2008. In 2010, 60% of the samples were positive for at least one of these toxins. Nevertheless, total concentrations remained low in the freshwater samples analysed (< 1 µg/L). The present investigation showed that cyanotoxins not yet investigated thoroughly in France, especially anatoxin-a, are widespread in French reservoirs. The raw water concentrations of CYN, ATX-a, and STX encountered in the 2007-2010 samples did not present any risk for drinking water production. Nevertheless, the presence of cyanobacteria can be highly variable over time and space and it is recommended to have specific monitoring strategies in reservoirs used for drinking water production.

MO 349

Sensitive determination of microcystins using online SPE from environmental waters

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Waterblooms of cyanobacteria (blue-green algae) can produce potent toxins that have become a severe problem for eutrophic aquatic environments. Hepatotoxins are among the primary toxins produced by these species growing in lakes, ponds, and rivers used as drinking water sources. Microcystin contamination of drinking water at low nanomolar concentrations is considered a risk factor

for cancer, and microcystin-LR has been associated with most of the incidents of toxicity involving microcystins. Therefore, the World Health Organization (WHO) has proposed a provisional guideline concentration of 1.0 µg/L for microcystin-LR in drinking water. The authors have reported a simple, fast, and effective target-cut on-line SPE method followed by HPLC with UV detection. This on-line SPE method is different from the typical one. The bound analyte on the SPE column is selectively eluted from the SPE column using a mobile phase gradient, just like the first dimension of a two-dimensional chromatography system. This reduces the number of interferences for sample analysis. Here, the target-cut on-line SPE method followed by HPLC with UV detection was applied to the determination of three microcystins (-LR, -RR, and -YR) in drinking, tap, and lake water. The three target analytes were co-eluted from the first column using chromatographic conditions that eliminated as many interferences as possible; then the analytes were sent to the analytical flow path and separated on the second column using the same type of stationary phase under different chromatographic conditions. This design takes advantage of the separation power of both columns and may eliminate interferences more efficiently than typical on- and off-line SPE methods. Sub-µg/L concentrations of microcystins-LR, -RR, and -YR spiked in water samples were determined, which exceeds the WHO requirement.

MO 350

Hazard assessment of microcystins produced by filamentous cyanobacteria in urban lakes using molecular and biological methods. A case study in Mexico City

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Formation of cyanobacterial blooms in water bodies with a high nutrient content (mostly P and N) is a frequent occurrence affecting freshwater quality at all latitudes. Bloom development is of great concern since cyanobacteria can biosynthesize a large number of secondary metabolites, some of which are toxic to aquatic biota and mammals, including humans; of these, microcystins (MCs) are the most abundant and well known. MCs are potent inhibitors of the protein phosphatases 1 and 2A. Zooplankton filter-feeders such as cladocerans are directly affected by MCs as a result of ingestion of cyanobacteria or through contact with intracellular products when cyanobacterial cells break up during and after blooms. In present study a total of 17 strains of filamentous cyanobacteria isolated from three eutrophied urban lakes in Mexico City were characterized using the microcystin synthetase region *mcyA*-Cd. Acute 48-h toxicity was evaluated in the aqueous crude extracts of different strains using the cladoceran *Daphnia magna* and total microcystin content was determined by ELISA. The *mcyA*-Cd region was amplified in 16 microcystin-producing strains but microcystins were only identified in eight strains with values ranging from 0.1422 to 2.772 $\mu\text{g L}^{-1}$. Nevertheless, all aqueous crude extracts induced acute toxicity in *D. magna* neonates, with LC_{50} values ranging from 363.91 to 741.8 mg L^{-1} (dry weight). The strains with the highest acute toxicity were *Pseudanabaena mucicola*, *Planktolyngbya* sp. and *Spirulina* sp. The toxicity observed in non-microcystin-producing strains may be induced by cyclic peptides other than microcystins (anabaenopeptins, microviridins and cyclamides). The analytical methods used in the present study (PCR and ELISA) were useful and reliable for determining MC production and its potential contribution to the acute toxic effects observed in *D. magna*. The results obtained warn of the toxigenic potential of filamentous cyanobacteria, since though *Microcystis* spp. are frequently predominant in blooms, other toxins and intracellular metabolites released by filamentous cyanobacteria may induce toxicity on aquatic organisms and potentially also in humans.

MO 351

Occurrence of harmful algae and related toxins in the Po River Delta (Northern Adriatic Sea, Italy)

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An integrated approach for the structural identification and quantification of algal toxins in water and mussel samples from the Po river delta (Northern Adriatic Sea) was developed by applying a combination of analytical techniques such as Optical and Scanning Electron Microscopy (OM, SEM), and Liquid Chromatography coupled with High Resolution Time of Flight Mass Spectrometry (HPLC-HR-TOF-MS). The proposed approach was applied to the investigation of potential occurrence and distribution of harmful algae and their related toxins in the above mentioned coastal area.

The performed sampling sessions showed that potentially harmful algae such as *Dinophysis caudata*, *D. mitra*, *D. sacculus*, *P. minimum*, *Protoceratium* sp., etc. were present during the summer period in the investigated area. Nevertheless, quantitative observations demonstrated that their abundances were always significantly below the conventional limit (~200 cells/L) for which poisoning events could occur. On the other hand, the HPLC-HR-TOF-MS analysis of collected mussels revealed a strong (over regulatory limits) contamination by free Okadaic Acid (OA), while concentration levels of Pectenotoxins (PTXs) and Yessotoxins (YTXs), concurrently analyzed, were instead below these limits. The adopted extraction/purification procedures permitted to distinguish the contributions of free toxins from those due to their various esterified forms identified to mussel contamination, and to determine the concentration levels in total edible tissue, as well in the hepatopancreas. The high resolution of the Mass Spectrometer permitted also to identify new toxins esters not previously reported by literature.

MO 352

Two new microginins congeners in Brazilian strains of Microcystis sp. (CYANOBACTERIA)

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Microcystis species are the most common bloom-forming cyanobacteria in several countries and are related to significant health problem due to the production of over 60 chemical forms of microcystins. Numerous new bioactive compounds known as cyanopeptides (CNPT) were identified as cyanobacteria secondary metabolites. Among these compounds there are aeruginosins, anabaenopeptins and nostophycins, commonly described as protein inhibitors. More than 20 variants of microginins, which comprise a peptide group, were already found in *Microcystis* strains and natural samples. Despite several works have been published about CNPT produced by cyanobacteria, few of them are related to by Brazilian strains production. In this study microginins and microcystins diversity and their variation during growth of three *Microcystis* sp. strains (LTPNA01, LTPNA08 and LTPNA09) isolated from a southeast Brazilian reservoir were investigated. The strains were maintained in ASM-1 medium, 24±2 °C, with a 14:10h of light:dark cycle and photon flux of 40 $\mu\text{mol photons.m}^{-2}.\text{s}^{-1}$, during 15 days, in triplicate. Samples were collected every 3 days for cell counting and CNPT analysis. Compounds were extracted from dried cells using methanol 90% and analyzed by HPLC-DAD and LC-MS/MS. Microcystins and microginins were monitored at 238 nm and 225 nm, respectively. Standard solutions of Mcyst-RR and Mcyst-LR were employed for quantification. Compound identity was confirmed by high accuracy mass spectrometry. New Microginins were identified by the characteristic gas phase fragmentation behavior for this class of peptides. Cells of *Microcystis* sp. grew exponentially until the 6th day of culturing (LPTNA01, $R^2=0.9834\pm0.002$; LTPNA08, $R^2=0.9897\pm0.011$; LTPNA09, $R^2=0.9876\pm0.051$). Growth rate of LTPNA01 ($\text{rn}=0.9183\pm0.003$) was higher than LTPNA08 ($\text{rn}=0.8059\pm0.012$) and LTPNA09 ($\text{rn}=0.8111\pm0.031$; Dunn test, $p<0.001$). The cell content of microginins and microcystins have not shown significant variation along the evaluated growing period (KW test, $p>0.05$). The two new microginins congeners presented the peptide sequence Adha-Val-Iso/Leu-HTy-Tyr and MeAdha-Val-Iso/Leu-HTy-Tyr and were named as FD1 and FD2, respectively.

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Degradation of microcystins by polymeric nanofiber membranes with encapsulated tetraphenylporphyrine

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Cyanobacterias cause various problems in freshwater ecosystems such as reduction of the oxygen content and production of cyanotoxins. The most common group of cyanotoxins are microcystins. They inhibit protein phosphatase and they may act as hepatotoxic and tumor promoting agents. To degrade microcystins in drinking waters, several methods based on oxidation have been suggested (e.g. using of chlorine or ozone). In the present study we investigated photodegradation of microcystins by polymeric nanofiber membranes with encapsulated meso-tetraphenylporphyrine (TPP). TPP is a photosensitizer which produces singlet oxygen ($^1\text{O}_2$) with big quantum yield after irradiation by visible light. Singlet oxygen is a strong oxidizing agent, and it can effectively degrade microcystins. Nanofiber membranes have large surface that assures contact with microcystins in water, they are stable, don't release any harmful substances into the surroundings and they can be used repeatedly without substantial decrease in efficiency. It appears to be an ideal carrier for a photosensitizer such as TPP encapsulated into the structure of nanofibers. We have tested nanofiber membranes from polystyrene, sulfonated polystyrene, polyurethane, polyamide 6 and polycaprolactone for their ability to adsorb microcystins and to produce $^1\text{O}_2$. The rates of microcystins photodegradation by nanofiber membranes with TPP were studied. The degradation products were characterized chemically (using LC-MS/MS) and toxicologically (testing effects in vitro). The existing results indicate potential of studied nanomaterials to photodegrade microcystins which can be used in technological applications. The final results of the study will be discussed.

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Acclimatisation strategies in gastropods to environmental pollutants: comparison of a strong invasive to a declining endogenous species

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Long term surveys in western France (Marais de Brière, Grand Lieu and Combours Lake), evidenced declining populations of indigenous gastropod species, e.g., *Lymnea stagnalis*, whereas the invasive *Physa acuta* became the single occurring gastropod species. The environmental stress enforcing these alarming biodiversity decreases is hypothesised to be a combination of anthropogenic pollution (e.g., pesticides) together with recurrent and dense cyanobacterial blooms. Due to eutrophication of freshwaters, the frequency of cyanobacteria proliferations is increasing worldwide, with from 40 to 75% of cyanobacterial blooms producing hepatotoxins [e.g. microcystins (MCs)]. MCs constitute a real threat for target organisms as gastropods (intoxication by absorption of toxic cyanobacteria or dissolved toxins). Physiological reactions of biotransformation and oxidative defence on the other hand enable organisms to withstand and excrete harmful substances. The capacity to perform these reactions is species specific and might contribute to the persistence of the invasive *Physa acuta*. The lower susceptibility may be facilitated by better cellular protection mechanisms: this is including the way of storage of accumulated toxin; the efficiency of biotransformation enzymes that transform the toxic compound into a less toxic one; activity of antioxidant enzymes mending or preventing oxidative damage; and excretion mechanisms. Therefore, this physiological capacity of *Physa acuta* will be compared to the indigenous species *Lymnea stagnalis* under exposure to a mixture of non MC-producing and MC-producing cyanobacteria *Planktothrix argardii* with or without a common pesticide used in Brittany (glyphosate in Round Up suspension). It seems likely that exposure to the one affects the reaction to the other, due to similar pathways for detoxification (glutathione S-transferase) and antioxidant response (enzymes and peroxidation markers). In the environment, exposure occurs to mixtures of pollutants, this scenario is hence focus of the proposed work. Results are in process and we only expose hypothesis. Short and long term reactions of biotransformation and antioxidant response during these periods of exposure and depuration enable assessing of differences of the two species in efficiency in protection against the ecotoxicological stressors.

MO 357

Study of the pro-oxidant capacity of anatoxin-a(s)-containing extract in mice exposed to a sub-lethal dose

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Anatoxin-a(s) (antx-a(s)) is a cyanobacterial neurotoxin whose principal mechanism of action is the irreversible inhibition of the enzymatic activity of acetylcholinesterase (AChE). Because of the molecule's instability and the lack of an analytical standard, the occurrence of this toxin in freshwater reservoirs and the knowledge of the whole events related to its toxicity are scarce.

In the last decade, research related to organophosphates insecticides capability to induce oxidative stress in humans and animals has been profuse. Considering that antx-a(s) is the unique organophosphate produced by cyanobacteria currently known; the main of this work was to investigate the biochemical mechanism related to the pro-oxidant capacity of antx-a(s)-containing extracts. In order to achieve the objective, there was determined the activity of cholinesterase and antioxidant enzymes such as acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase (SOD) in

Swiss mice blood. Group of 9 or 10 animals were treated intraperitoneally (ip) with 20 mg.kg⁻¹ of antx-a(s)-containing extract and sacrificed after 24 h, 48 h, 7 and 14 days of treatment by cardiac puncture.

The AChE and BuChE activities in mice treated with antx-a(s)-containing extract stayed inhibited more than 55% during 48 h (n=9, p<0,001). Normal activity of both enzymes was observed after seven days of treatment. By the other hand, changes in the antioxidant enzymes activities only began after the second day of treatment. Initially, both CAT and GPx showed lower activity than the control group after 48 h. Among those enzymes, GPx showed the highest decreased activity (n=9, p<0,001). After seven days, while the antx-a(s)-containing extract promoted increasing of CAT and GR activities, GPx activity remained deeply decreased (n=10, p<0,01). SOD activity did not show any statistical difference related to the control during all the treatments. Activity of all the evaluated enzymes was completely recovered after fourteen days. From the obtained results, it can be concluded that the antx-a(s) extract demonstrated its pro-oxidant capacity in mice at sub-lethal levels. This fact was proved through the unbalance on the activity of the antioxidant enzymatic defense system.

MO 358

Physiological effects of phytotoxins in plants - laboratorial studies to evaluate the impact of toxic cyanobacteria in agriculture, food quality and human health

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Toxic cyanobacteria outbreaks are recognized as emerging environmental threats worldwide. The process occurs in inland water systems as in some coastal areas and is becoming widespread mostly as a consequence of human activity, leading to nutrient input increases and degradation of water quality. The cyclic heptapeptide microcystin (MC) is geographically the most widely distributed phytotoxin in freshwaters. This toxin has been directly related to cases of human poisoning and to a prevalence of cancer induction in populations with chronic exposure to MC. Cylindrospermopsin (CYN) is another secondary metabolite produced by cyanobacteria. It is a tricyclic alkaloid with bioactivity. These compounds have shown to produce effects in a variety of aquatic organisms, vertebrates and mammals. Reports regarding variations in the animal behavior, growth rates, mortality, oxidative stress induction is contributing to a better understanding of the impacts of these toxins in the environment and animal health. Recent investigation supports the hypothesis of accumulation of MC and CYN in plant tissues. Nevertheless a major gap lies in the physiological response of plants to phytotoxins and the mechanisms of uptake and translocation of these molecules from roots to shoot. This knowledge is essential to evaluate the impact of the use of contaminated waters in agriculture, food quality and human health. In this presentation we will discuss preliminary findings regarding the interaction of *Lycopersicon esculentum* and *Oriza sativa* plants with MC and CYN. Hydroponic cultures have been established and plants exposed to either isolated toxins or cyanobacterial cell extracts with concentrations varying between 1 µg/L and 300 µg/L. Short term exposures to both toxins does not alter the quantum yield of PSII in *O. sativa* and in *L. esculentum* after 15 days of exposure or the growth rate of plants. Nevertheless proteomic analyses displayed variations in protein expression in *L. esculentum* leaves suggesting for a biochemical response of plants. Analysis of the activity of antioxidant enzymes is being undertaken to estimate the oxidative stress in plants in response to the phytotoxins. Moreover HPLC and mass spectrometry techniques are being applied to quantify MC and CYN in plant tissues. We expect with this additional data to gather new insights in the toxicity of phytotoxins in these plant species and in the role of bioaccumulation in food quality and human health safety.

ET16P - Quantitative Structure Activity Relationship (QSARs) and similar models for predicting the toxicity of chemicals, mixtures and combined stress

MO 361

Environmental impact analysis of 10 active pharmaceutical ingredients with QSARs

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The environmental risk assessment of an active pharmaceutical ingredient (API) follows a stepwise approach, with a base set of studies on aquatic toxicology and fate as a start. This base set can be generated in standard tests, but at first the potential effects on aquatic organisms and the environmental fate can be calculated to screen for possible alerts. Quantitative structure activity relationships (QSAR) are methods for estimating intrinsic environmental (hazardous) properties of chemicals, while reducing time, monetary cost and animal testing. In this study QSAR models are used to calculate physical-chemical properties, acute and chronic ecotoxicity and environmental fate of 10 APIs from multiple therapeutic categories.

Calculations are performed in EPISuite. Model estimations are discussed and compared with measured data.

(Q)SARs show to be a reliable tool for screening environmental properties of APIs. All the test result on (non) ready biodegradability were confirmed by the predictions of the Biowin model. All the ecotoxic substances were identified by Ecosar. The acute toxicity is in line with measured values (74%) or overestimated (26%) by the model. For the chronic toxicity, the lowest chronic value determines the PNEC and hence the outcome of the risk characterisation. The calculated lowest chronic value for each API is in line with the measured value or lower. For the Ecosar classes considered for these 10 APIs, the main parameter in the algorithm is the log Kow which is inversely related to the ecotoxicity value. For the APIs with only covalent bonds the results with measured and calculated ecotoxicity are in the same order of magnitude. It can be argued for the salts to provisionally use the measured log Kow in order to refine the overestimation and avoid underestimation of the ecotoxicity by the model. However further research is needed on this subject.

As part of an intelligent testing strategy (ITS) within risk assessment, QSAR predictions can deliver added value to other non-test methods such as in vitro tests, read across, grouping and the weight of evidence approach, in order to minimise animal testing by focusing on those endpoints that may be of concern and to secure a high level of safety for man and the environment.

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MO 362

Externally predictive QSAR models: thresholds of acceptance by various external validation criteria and critical inspection of scatter plots

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The evaluation of linear regression QSAR models performances, both in fitting and external prediction, is of pivotal importance. In the last decade different external validation parameters have been proposed: Q^2_{F1} (Shi), Q^2_{F2} (Schuurmann), Q^2_{F3} (Todeschini), averaged r^2_m (Roy) and the Golbraikh-Tropsha (GT) method. Recently, the concordance correlation coefficient (CCC, Lin) has been proposed by our group as an external validation parameter to be used in QSAR studies. In our recent work, published in 2011 on JCIM, we have shown that, comparing with the commonly used acceptance thresholds ($Q^2_{F1}=0.6$, averaged $r^2_m=0.5$), the concordance correlation coefficient threshold value (0.85) is usually the most restrictive in the acceptance of QSAR models as externally predictive. This fact suggested that the CCC could be used as the preferred validation parameter in a precautionary approach, if the aim of QSAR developers is to have the smallest differences among the experimental data and the predictions of the external data set.

In this new work, we have studied and compared the general trends of the various criteria in dependence of different possible bias in the external data distributions (scale and location shifts), by means of a wide range of different simulated scenarios. This study highlighted, also by visual inspections of the experimental vs. predicted plots, some problems related to a few criteria; in particular, averaged r^2_m values, if based on the proposed cut-off, could be prone to accept also not predictive models. This analysis allowed also to propose recalibrated, and inter-comparable, new thresholds for each criteria in the definition of a QSAR model as externally predictive. Two additional relevant topics emerged from the analysis of the results: 1) the scatter plot of the external predictions must always be evaluated and 2) the root mean squared error (RMSE) must also be calculated, as it is usually done in the good QSAR practice. In fact, we have verified that the sensitivity of the various validation criteria to RMSE often differs. An additional important topic, here considered and applicable only to CCC, was to check by hypothesis test if the value of the calculated CCC is statistically significant. This procedure allowed, consequently, to determine also the minimum acceptable size of the external data set, an important point in QSAR studies, where the data set sizes are often small.

MO 363

Collection screening of QSAR models for REACH

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REACH is the Regulation on Registration, Evaluation, Authorization and Restriction of Chemicals. This legislation promotes the use of non-testing methods (NTM), which are all the approaches used to predict the effects of chemical compounds without the use of the real chemical compound. These methods include Quantitative Structure-Activity Relationship (QSAR) models and read-across. Before making an animal experiment the industry should verify if alternative methods exist. However, so far there is a deep gap of knowledge on which methods are available and can be used in practice.

In this context a LIFE project, named ANTARES, aims to reduce this gap assessing NTM as an alternative approach for the REACH legislation.

Here we will present the screening study on existing implemented QSAR models, both commercial or freely available through the internet. Almost 40 REACH endpoints are in principle covered by 250 QSAR models, 70 of them freely available. The full list of the QSAR models is available on <http://www.antares-life.eu/index.php?sec=modelist>. The QSAR models cover endpoints for physico-chemical, environmental, ecotoxicological and toxicological properties. Within ANTARES we are evaluating in details the models for these eight endpoints: Partition coefficient n-octanol/water; Carcinogenicity study; In-Vitro gene mutation study in bacteria; Acute toxicity-by oral route; Short-term toxicity testing on invertebrates/Daphnia; Short-term toxicity testing on fish; Ready biodegradability; Bioconcentration factor. In this way we cover all the areas of the effects, giving preference to endpoints where more models and data exist. Within ANTARES we are evaluating the performances of these models with external test sets using specific criteria. Results will be discussed.

The possibility to have checked and validated non-testing methods will provide a huge benefit, reducing the costs and time needed to get the information on chemicals, and saving animals. The use of these methods will reduce the animal tests not only for their use by chemical industry, but in the other sectors too.

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MO 364

QSAR classification models of the terrestrial toxicity of (benzo) triazoles: predictive tools for hazard and risk assessment

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Triazoles and benzo-triazoles (TAZ/BTAZ) are potentially hazardous chemicals that adversely affect humans and other non-target species, and are on the list of substances of very high concern (SVHC) in the European regulation of chemicals REACH. TAZ/BTAZ are synthetic molecules, widely used in various industrial processes, as well as deicing agents, pharmaceuticals and pesticides. Because of their wide use they have been found distributed throughout the environment. The amount of experimental data available for these molecules is insufficient for a comprehensive characterization of their environmental and toxicological profile and they have been included among the four classes of chemicals studied in the European FP7 Project CADASTER (Case studies on the Development and Application of in-Silico Techniques for Environmental hazard and Risk assessment).

In this study quantitative structure-activity relationships (QSAR) were used to model different endpoints of interest to define the potential toxicological profile of over 50 TAZ/BTAZ, with experimental acute toxicity data available for three key organisms of the terrestrial ecosystem. The studied end-points were: Honeybees 48 hour LD₅₀, Earthworm 14 day LC₅₀, Bird oral-LD₅₀.

Different theoretical molecular descriptors were calculated by different proprietary and freely available online software (DRAGON 5.5 and PADEL-Descriptor 2.6). The endpoints of interest were modeled by classification (*k*-Nearest Neighbors (*k*-NN)) and the best modeling variables were selected by Genetic Algorithm. The predictivity of the best models was validated by calculating the parameters sensitivity (Sn), specificity (Sp) and the non error rate (NER). External validation was also performed, depending on the dimension of the studied experimental datasets. All the proposed models have good statistical performance in fitting and in external prediction with Sn%, Sp% and NER%, calculated for the three endpoints, never below 85%, 75% and 81% respectively (full models). The reliability of the predictions was always checked by the leverage approach in order to verify the chemical applicability domain of the models.

In conclusion, the proposed models, which were developed in line with the 'OECD principles for the validation of QSARs for regulatory purposes', are useful tools applicable to fill the data gaps and to support the activity of regulators with additional information to be used in terrestrial Hazard and Risk Assessment procedures.

MO 365

The effect of molecular chain architecture on polyelectrolyte toxicity

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Polyelectrolytes are applied in a wide range of industrial sectors, from pulp and paper mills to waterworks, and hence risk assessment for this sort of substances is of major interest.

The functional performance, for example flocculant action, of polyelectrolytes may be maximised by manipulating parameters such as the molecular weight, the polymer charge density and the molecules' chain architecture. In this context, understanding how these variables affect the toxicity of polyelectrolytes towards aquatic organisms is very important. In fact, if reliable structure-activity relationships (SAR) become available, early risk assessment may be incorporated into the first steps of the development process instead of being postponed to later stages when the product is close to get into the market. Ultimately SAR may assist the optimisation of the overall product performance through the maximisation of the product functionality and the minimisation of the environmental impacts.

The dependence of polymer toxicity on molecular weight and charge density has been fairly investigated. While the molecules' chain architecture is a useful design variable, significantly affecting the product functionality, little is known about the way this parameter affects toxicity.

In this study, preliminary data elucidating the relationship between polymer chain architecture and toxic effects on aquatic organisms are provided. The responses elicited by cationic polyelectrolytes with different branching degree on *Daphnia magna* and *Pseudokirchneriella subcapitata* were assessed in standard laboratory bioassays. The toxicity of the chemicals to the Asian clam *Corbicula fluminea*, a biofiltering bivalve that is potentially controlled by polyelectrolytes, was also tested. The results show that the molecules' chain architecture significantly affects polyelectrolytes' toxicity.

MO 366

Development and validation of QSARs for chronic narcosis to fish

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Vertebrate testing requirements under REACH are encouraged to be avoided by use of alternative non-testing approaches such as QSARs. However, robust QSARs predicting chronic ecotoxicity to fish are largely lacking. The Ecological Structure Activity Relationships (ECOSAR) Class Program is a computerized predictive system that estimates aquatic toxicity. ECOSAR uses a number of log Kow based QSARs to estimate the acute and chronic toxicity of organic compounds for several structural classes. In case acute data sets are used, acute to chronic ratio's (ACR) are used to predict chronic toxicity to fish. Although ECOSAR scores good on several OECD criteria, the chronic QSARs in ECOSAR are not fully compliant with OECD criteria in the framework of REACH and/or CLP. This poster will present the development of a chronic ecotoxicity QSAR to fish for nonpolar and polar narcotics. These QSARs were built on a database of carefully screened toxicity values, considering only chronic exposure durations and relevant endpoints. After statistical multivariate diagnostic modeling, mechanistically relevant descriptors were selected to develop a multivariate regression model using the OECD Toolbox. These QSARs have been tested and were compliant with the OECD principles for the validation of a QSAR.

MO 367

Extension and validation of the target lipid model for deriving predicted no effect concentrations for hydrocarbons

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The hydrocarbon block method enables environmental risk assessment of complex petroleum substances to be performed. To implement this approach, predicted no effect concentrations (PNECs) intended to protect aquatic, marine, benthic and soil organisms need to be established for representative hydrocarbon structures that are used to simulate the complex substance composition. The target lipid model (TLM) provides a quantitative framework for deriving PNECs for hydrocarbons using the HC5 statistical extrapolation procedure. However, the TLM does not correctly predict the lack of observed chronic effects for poorly water soluble hydrocarbons. This limits the current applicability of the TLM since low solubility hydrocarbons are often constituents of many petroleum substances. To expand the TLM domain to include these hydrocarbons, chronic algal and daphnid tests were performed for selected C11-C16 aliphatic hydrocarbons. The aqueous solubility of each hydrocarbon was first determined using the slow-stir test method. Chronic 'limit' tests were then performed at a single exposure concentration corresponding to the aqueous solubility limit using vapor saturation and passive dosing techniques. Results indicated measured aqueous solubilities ranged from 0.3 to 2.5 micrograms/liter. Chronic effects were not observed for hydrocarbons with measured water solubility below 5 micrograms/liter. To estimate aqueous PNECs and discriminate toxic from non-toxic structures, the equation used in the TLM to estimate partitioning to target lipid was modified. The modified TLM was then evaluated using test data from this study and additional chronic effects data reported in the literature for aliphatic and aromatic hydrocarbons, ethers and alcohols. This compilation yielded over 260 experimental chronic values for 92 substances and 30 test species. Comparison of chronic data to predictions derived from the modified TLM confirmed that the HC5 method provides the level of protection expected. Therefore, this work extends the range of TLM applicability to poorly soluble hydrocarbons in support of risk assessment of complex petroleum substances using the hydrocarbon block method.

MO 368

QSAR models for aquatic toxicity of triazoles and benzotriazoles: WP3 results within the FP7 European Project CADASTER

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Quantitative structure-activity relationships (QSARs) are useful tools to predict unknown activities/properties for existing and not yet synthesized chemicals. The development and validation of QSAR models to be used as support tools for the application of the EU-REACH regulation, is one of the topics of the FP7 European project CADASTER (Case studies on the Development and Application of in-Silico Techniques for Environmental hazard and Risk assessment).

This poster summarizes the QSAR models developed by partners within the CADASTER project for the aquatic toxicity of triazoles and benzotriazoles (TAZ/BTAZ).

These chemicals are synthetic molecules with industrial and pharmaceutical uses. TAZ/BTAZ have been found distributed throughout the environment, mainly in water compartments, and are cause of concern due to their possible effects mainly on aquatic organisms. The modeling study presented in this poster involved different project partners in universities and research institutes across Europe (University of Insubria, Linnaeus University, IVL Swedish Environmental Research Institute, Ideaconsult Ltd., and University of Plovdiv). The presented QSARs were realized by different modeling approaches (Multiple linear regression by MLR-OLS or PLSR) starting from theoretical molecular descriptors calculated by different commercial (DRAGON) and freely available (PADEL-Descriptor, CADASTER Online) software.

The studied end-points were: LC₅₀ in *Onchorynchus mykiss*, EC₅₀ in *Daphnia magna*, and EC₅₀ in the algae *Pseudokirchneriella subcapitata*. The proposed models were checked for their robustness, external predictivity and applicability domain, in agreement with OECD principles for the validation of QSARs for regulatory purposes. The best models have good statistical performance in fitting and in external prediction with R² and Q² ranges, calculated for the three endpoints, of 0.75 - 0.85 and 0.70 - 0.81

respectively, for MLR-OLS models, and 0.93 - 0.99, 0.79 - 0.88 for PLSR models. The structural applicability domain to TAZ/BTAZ in ECHA preregistration list and the comparison of some models with ECOSAR predictions was also performed for the Insubria models. The external predictivity of the models was compared by analysis of the Root Mean Squared Error (RMSE) and of other statistical parameters such as different external Q^2 or the Concordance Correlation Coefficient (CCC) recently proposed, by the Insubria group, for its use in QSAR validation.

MO 369

Assessment of alternative approaches to hazard identification of substances

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The REACH Regulation stipulates that the principles of replacement, reduction and refinement of the use of animals in procedures should be fully taken into account in the design of the test methods, in particular when appropriate validated methods become available. REACH additionally promotes the use of (Q)SARS ((Quantitative) Structure Activity Relationships) because they contribute to the reduction of tests with vertebrates. The concept and use of alternative testing and (Q)SARs are therefore strongly embedded in the REACH Regulation and guidance documents. In particular, alternative testing methods and (Q)SARS may be applied where information is scarce, as is likely to be the case for substances registered between 1 to 10 t/y. This poster will present an assessment of recent developments in the area of information generation for hazard identification, in particular focusing on alternative methods, such as (Q)SARs or in-vitro methods. The assessment is a consolidation of review papers, state-of-the-art papers and several recently finished/ongoing EU research projects on alternative approaches. Options will be presented for using the new developments to meet information requirements (including as information below or beyond the minimum information requirements) for substances registered between 1 and 10 tonnes.

MO 370

Prediction of Michael-acceptor protein reactivity from quantum chemical reaction barriers

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The so-called Michael acceptors, i.e. α,β -unsaturated carbonyls, are an important class of industrial chemicals with the potential to enter cells and react with endogenous macromolecules such as proteins and DNA. For this reason their toxicity toward the ciliate *Tetrahymena pyriformis* (logarithmic 50% growth impairment concentration after 48-h exposure, $\log EC_{50}$) exceeds the minimum or baseline toxicity as calculated from their hydrophobicity (logarithmic octanol-water partitioning coefficient, $\log K_{ow}$). It has been shown that their reactivity toward glutathione (GSH), quantified as logarithmic 2nd-order rate constant, $\log k_{GSH}$, can be employed to predict the toxicity component driven by their Michael-acceptor reactivity, making $\log k_{GSH}$ a key determinant in predicting their toxicity.

The reaction of the electrophilic Michael-acceptors with the thiol group of GSH or protein side chains can proceed via two mechanisms. Firstly, a direct addition to the olefinic double bond (subject to our previous study), or secondly, a conjugated 1,4-addition. In this work we present an extensive quantum chemical assessment of the 1,4-conjugated addition and the subsequent tautomerization step in simulated aqueous solution. For a set of 12 compounds, consisting of 4 aldehydes, 4 ketones, and 4 esters with diverse substitutions at the $C_{\alpha}C_{\beta}$ double bond, reactant, transition-state, and product complexes with two explicit water molecules were calculated at the DFT and MP2 levels of theory. While DFT calculations yield ambiguous results as to which of the two steps is rate-determining, MP2 calculations support the textbook view that tautomerization is fast and not rate-determining.

The correlation of the intrinsic reaction barriers, $\Delta E^\ddagger$, of the addition step with $\log k_{GSH}$ was explored, yielding a simple linear regression model with good statistics able to predict $\log k_{GSH}$. In a second step, a regression model for $\log EC_{50}$ was developed, employing both $\Delta E^\ddagger$ and $\log K_{ow}$ as descriptors. The derived models appear useful as in silico tools for screening the reactivity and toxicity of α,β -unsaturated carbonyls in the context of integrated testing strategies (ITS) for REACH, enabling an early waiving of animal tests for non-reactive toxicants.

This work was financially supported by the European Union project OSIRIS (Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Testing Information), contract no. GOCE-CT-2007-037017.

ET17P - Trait-based approaches in prospective and retrospective risk assessment

MO 371

Relative toxicity of metal cations to the macroalga *Gracilaria domingensis* in synthetic medium

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The potential use of Quantitative Ion-Character Relationships (QICARs) to investigate the toxicity of metals to algae has been underestimated when compared to the Quantitative Structure-Activity Relationships (QSARs) studies, commonly used for organic toxicants. In the present study, we determine the individual toxicity of mono-, bi- and trivalent metal ions (Li⁺, K⁺, Na⁺, Cd²⁺, Zn²⁺, Pb²⁺, Cu²⁺, Ni²⁺, Mn²⁺, Co²⁺, Ca²⁺, Mg²⁺, Sr²⁺ and La³⁺) to the red macroalga *Gracilaria domingensis* (Kütz.) Sonder ex Dickie (Gracilariales, Rhodophyta) originally from the Brazilian Coast and cultivated in synthetic seawater medium under optimized conditions. The median inhibitory concentration values (IC50) were determined from daily growth rate (DGR, $\mu g/d$) vs. concentration curves using a 48h-assay. The IC50 values were converted to free ions (ICF50) values using the software minimization of total equilibrium activity (MINTEQA2, U.S. Environmental Protection Agency), version 3.0. After speciation calculations of the metal ions, these were transformed for $\log ICF50$ values and the toxicity of the free metals were predicted using the QICAR approach. The following parameters were used: ionic radius (r), atomic number (AN), electronegativity (Xm), covalent index (Xm2r), first hydrolysis constant ($\log KOH$), softness index (σp), ion charge (Z), ionization potential (ΔIP), electrochemical potential (ΔEo) and ionic index (Z2/r). The genetic function approximation (GFA) was used to investigate the sample distribution and to identify the most favorable subsets of descriptors to QICAR models (100 random models and 5,000 interactions to evolution). The best correlation found was with AN, Xm2r and σp [$\log ICF50F = 1.00 \cdot 0.24(AN) + 0.52(Xm2r) + 1.30(AN \times \sigma p)$, adj-R2: 0.93, pred-R2: 0.91, LOF: 0.87]. This mathematical expression allowed the prediction of IC50 values for mono-, bi- and trivalent metal ions (Ag⁺, Cs⁺, Ba²⁺, Hg²⁺, Fe²⁺, Fe³⁺, Cr³⁺ and Al³⁺). Moreover, the correlation of IC50F with σp and Xm2r allowed us to infer that the toxicity mechanism might involve the interaction between the metal and sulfur-containing biological species, such as proteins and smaller organic molecules with groups -SH, RSH, RS and R2S.

MO 373

Using benthic macroinvertebrate biotraits to assess specific risk of stream ecological impairment under multiple pressures scenari

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The full accomplishment of the European WFD objectives has required from EU members three successive steps: (i) the evaluation of the actual ecological status of their water bodies, (ii) a diagnostic assessment of the risk to be impaired by different anthropogenic pressure types and (iii) the implementation of appropriate management and restoration programs based on the previous diagnostic conclusions. We already have proposed a new French WFD-compliant bioassessment index (I2M2) to replace the current IBGN index (step i). In this paper, we aimed at designing an innovative risk assessment tool working for most of French wadeable stream types in a context of multiple anthropogenic pressures (step ii). This tool, based on random forests, was built on macroinvertebrates biotraits, we built one random forest model for each of 10 water quality and each of 6 habitat pressure categories. We demonstrated that this approach was very promising in stream ecological risk assessment. Our predictive models gave consistent impairment risk (IR) assessment, even at moderate risk level, for several specific pressure categories, three exhibited correct classification rate (CCR) higher than 0.70 and eight others had a CCR between 0.60 and 0.70. Moreover, most of the observed relationships between trait category utilization and IR could be explained through a mechanistic action of human-induced pressures on invertebrate assemblages, that is a highly recommended feature for diagnostic tools. To our knowledge, this diagnostic tool, composed of 16 random forest models, is the first macroinvertebrate-based functional diagnostic tool that address efficiently the problem of diagnostic in multiple pressure scenarii (including water quality and habitat degradations) at large spatial scale.

MO 374

Comparison of the species sensitivity distribution (SSD) and SPEAR index in the retrospective risk assessment of pesticides

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Various methods to evaluate impacts of contamination of aquatic ecosystems have been suggested including specific bioindices such as saprobic index or SPEAR_{pesticides} index, which has been shown to reflect pesticide pollution. The SPEAR_{pesticides} index uses biomonitoring data on macroinvertebrates and classifies different species as 'at risk' or 'not at risk' according to their ecological traits. On the other side, Species Sensitivity Distribution (SSD) is a statistical method, which uses toxicity data (e.g. EC50 or NOEC) of different species or taxons, and it can be used for prediction of the 'potentially affected' fraction (PAF) of the aquatic community exposed to a certain concentration of specific compound. It can also be used to predict PAF of mixtures, i.e. multisubstance PAF (msPAF). In the present study we calculate msPAF values for 25 pesticides measured at localities in France, Germany and Finland (years 1998-2000), and compare the results with the bioindicator (SPEAR_{pesticides} index). Our objective was to compare performance of both approaches in the retrospective site-specific risk assessment of pesticides, and the preliminary results indicate complementarity of both SPEAR and SSD. The full outcomes of the exercise will be discussed in detail.

MO 375

Physiological sensitivity of freshwater macroinvertebrates to heavy metals

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Macroinvertebrate species traits, such as physiological sensitivity have successfully been introduced in trait-based bioassessment approaches. We ranked macroinvertebrate species according to their physiological sensitivity for heavy metals, using acute toxicity data. Rankings for each heavy metal, namely Cd, Cu, Cr, Ni, Pb, Zn, and Hg were developed by normalizing each heavy metal distribution function based on all available species data in standardized laboratory conditions. Rankings for different heavy metals

on the species level were reasonably well correlated pairwise ($0.50 < r < 0.73$) and showed no significant difference for all heavy metals. Thus, an aggregated heavy metal ranking was developed (S_{metal}), which assigns physiological sensitivity values to macroinvertebrates taxa. Considering the high variation, especially for higher taxonomic levels i.e. order, it is recommended to use S-values of the genus or species level for meaningful analysis. In terms of taxonomic ranking, crustaceans were found to be the most sensitive organisms, with species in the order of Cladocera being three orders of magnitude more sensitive than the most sensitive insects of the order of Trichoptera. By contrasts, molluscs covered a wide range of sensitivities, with Bivalvia being on an average one order of magnitude more sensitive than Gastropods. Finally, physiological sensitivity represents a promising trait that would help identify the effects of heavy metal pollution in lotic ecosystems, therefore improving practices in ecological risk assessment.

MO 376

Identifying traits from arthropod community responses to insecticides

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In earlier work we analysed NTA field studies performed with the same active substance but in different cropping systems and different regions to provide a first insight into the importance of geographical gradient for the response of non-target arthropod communities to insecticide exposure. Using a variety of statistical techniques we investigated phenomena related to effects on community structure. Here we present an extended meta-analysis that includes studies with additional active substances and additional locations. The focus of this analysis was at the population rather than at the community level. In particular we asked whether populations that respond to insecticide exposure share traits. The objective of this investigation was to assess whether traits related to population response could be expected to relate to latitude.

For each study we calculated direct impact on populations sufficiently represented in the samples as the ratio of population densities in treatment and control groups at the first sampling moment after treatment. Duration of effects was categorized using the time to reach densities as in conditions of non-exposure. Analysis at the community level was performed using Principal Response Curves analyses (PRC). This analysis involved combination of PRC-axes. Additional multivariate techniques were used to investigate which autecological traits correlate to population level phenomena as initial impact and recovery potential. Furthermore, we investigated the prevalence of traits in communities at different latitudes. Finally, the relevance of the SPEAR evaluation method (Liess & Beketov, 2011) for terrestrial arthropod ecosystems was discussed from the perspective of using ecological traits for prospective or retrospective risk assessment in terrestrial ecosystems, similar to established methods used in aquatic systems.

MO 377

Application of a lentic SPEAR index to pond mesocosm experiments : why community assemblage characteristics and season matter in ERA

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An adapted version of SPEAR metrics for use in lentic experimental systems, LSPEAR (Lentic SPEcies At Risk), has recently been proposed. In this study, LSPEAR was applied to two different pond mesocosm experiments to check if the invertebrate communities hosted by the systems were vulnerable and if this index could track their responses to pesticides. Data from two experiments previously run in 9-m³ outdoor tanks were used. During the first experiment, the ponds were allowed to mature for 1 year. Pond snails and *Asellus aquaticus* were introduced but asellids did not successfully settled. Eight ponds were then treated with deltamethrin and eight were kept as untreated controls. Four units within both the treated and untreated groups were covered with lids just after treatment to prevent external recolonization. The second experiment was performed after a six-month maturation period during which invertebrates collected from uncontaminated ponds were introduced. Among these, different crustacean taxa successfully settled in the systems. Exposure scenarios were built based upon the modelling of transfer of pesticides applied on wheat and oilseed rape crops according to two crop protection programs. Different pyrethroid insecticides were applied, including deltamethrin. Abundance data were analysed using the multivariate Principal Response Curve (PRC) procedure. LSPEAR values in control and treated ponds were compared by ANOVA followed by post-hoc Tukey tests.

During the first experiment, PRC only detected a transient effect of deltamethrin on invertebrates in open ponds, followed by a quick recovery. A strong effect of treatment was shown in lid-covered ponds but without recovery. LSPEAR index partially anticipated the effects of deltamethrin in lid-covered ponds but not in open ones due to the mere absence of vulnerable taxa when the experiment started. During the second experiment, pyrethroids induced significant negative effects on invertebrates, especially crustaceans. LSPEAR index was able to forecast these effects. It therefore appears to provide complementary information to a posteriori assessment based on statistical analysis of abundance data. It can give a good a priori idea of the vulnerability of mesocosm invertebrate communities and be a good indicator of the exposure of a community to pesticides. The results also stress out the influence of community characteristics and season of exposure on the outcome of mesocosm experiments.

MO 378

Comparative sensitivity of detritivores and detritus processing to Cu

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Decomposition and sequestration of organic carbon are central ecosystem processes that depend on the interactions between functionally diverse microbial and invertebrate communities. Ecosystem services are, however, under continuous pressure. Therefore the aim of the present study was to evaluate how toxicants may affect the functional links in the benthic food-web and the services it is providing. To this purpose, the effects of the model toxicant Cu on functionally distinct macrofauna species (*Asellus aquaticus* and *Tubifex* spp.) and detritus processing were compared in microcosm experiments. Freeze dried, ground stinging nettle (*Urtica dioica*) served as detritus. After 5 days of exposure to Cu, detritus processing was determined by measuring LOI of the sediment and DOM content of the overlaying water. Comparing the dose-response relationships for the effects of Cu on macrofauna survival and detritus processing revealed that the ecosystem process was much more sensitive to Cu than survival of the invertebrates. Spatial-temporal redox profiles of the upper 10 mm of the sediment showed that this was explained by a decreased activity of the invertebrates resulting in lower detritus processing rates at sublethal Cu concentrations. We conclude that detritus processing is a sensitive parameter for ecological water quality and ecosystem functioning.

MO 379

Comparative assessment of the structural and functional effects of a fungicide in static and dynamic mesocosms

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A several month-long experiment was implemented to assess the relevance of various parameters for the identification of direct and indirect effects of pesticides in aquatic ecosystems including the study or recovery periods. The same protocol was applied to outdoor dynamic and static experimental systems to assess if comparable results were obtained in both types of cosms. It was anticipated that the recovery potential of lotic communities should be higher due to drift of organisms from upstream and that lentic communities should be more resistant to toxic effects due to the selection of taxa exhibiting various traits associated with a higher tolerance to stressful environments (e.g., drying or low oxygen concentration).

Outdoor streams (length: 40 m, depth: 50 cm) and ponds (volume: 7 m³, depth: 90 cm) were exposed to a dithiocarbamate fungicide, thiram. Two concentrations, corresponding to surface water contamination by drift from orchard treatment (nominal concentrations: 35 and 170 µg L⁻¹), were used, with two replicates per concentration. Four untreated cosms were used as controls. Streams were continuously exposed for three weeks whereas ponds were contaminated once a week for 4 weeks. Macroinvertebrate communities were repeatedly sampled in order to assess the structural effects of exposure using various parameters such as taxa abundances and diversity indices. Alder litter breakdown rate was chosen as a functional parameter and measured using litter bags during the exposure and recovery periods. In addition, ergosterol concentration on litter was used as a proxy for hyphomycetes biomass in order to assess the effects of the treatments on fungi. A strong effect of exposure to thiram on macroinvertebrates was shown in streams with a decrease in the abundance of various taxa such as gastropods and asellids. Midge larvae were indirectly positively favoured by treatments. These structural effects of thiram were associated with a significant decrease of litter breakdown rate. No recovery was observed up to two months after the end of the exposure period for both structural and functional parameters. The effects were less important in pond mesocosms with only a slight tendency to a decrease in the abundance of some groups and no effects on litter breakdown. Results are discussed using data of thiram concentration in water and sediment and information about biological and ecological traits of the organisms inhabiting both types of experimental systems.

MO 380

Life history characteristics of surrogate species used in ecological risk assessment: what is necessary for the protection of guilds?

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The ability of a population to recover from stressors such as pollutants, is dependent not only on exposure and innate susceptibility of individuals, but on the life history characteristics of the organism. Thus, species with certain life history characteristics (short generation times, early time to first production of offspring, and large numbers of offspring) should recover faster from stress than organisms with different characteristics if exposure and susceptibility are similar. The ecological risk assessment (ERA) process involves developing toxicological data for surrogate species. Surrogate species, often chosen because they are easy to culture, are supposed to be protective for many other species. For example, some regulatory agencies use toxicity data developed for one or two fish species to protect all fish and all amphibians. Because different, even closely related species can exhibit vastly different life history characteristics, surrogate species may not be representative of other species and may in fact result in under- or over-protection of the environment from chemical toxicants. We will show how population models can be used to determine the life history characteristics necessary to protect groups of organisms. We will discuss a mathematical approach using insect biological controls and pesticides to determine whether one species can act as a predictive surrogate species for others and what characteristics would make it protective. We will also present a generic model for the ERA of fish populations. The generic model was developed from five life history types and setup to be useful in a number of different management scenarios based on different environmental protection goals. A decision framework set up for three levels of population models (continuous, time limited, and varying exposure) and three levels of environmental protection will be presented. The approaches presented here may guide future use of population models and surrogate species in ERA.

MO 381

Behavioural response of juvenile rainbow trout during a short, low-dose exposure to a herbicide mixture

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Fish are capable of sensing water-borne chemicals at concentrations below the lowest observed effect concentrations, which in turn are often several orders of magnitude lower than at the reported onset of mortality. In this study we exposed juvenile rainbow trout (*Oncorhynchus mykiss*) to an environmentally-relevant low-dose mixture of three co-occurring pesticides - atrazine, linuron and metolachlor - during a 5-day test in controlled, flow-through conditions. Our hypothesis was that fish swimming activity, the use of the water column, and interactions between individuals would be modified due to exposure to the mixture.

Fish exposed to the mixture of pesticides were hypoactive and spent more time in the lower parts of the aquaria in comparison to non-exposed controls, reflecting inhibited swimming activity. Levels of aggression were comparable between the two treatments. In natural conditions, inadequate behavioural responses, such as excessive hypoactivity, could result in increased predation risk, as well as susceptibility to malnutrition through reduced foraging activity, with serious implications for survival.

Studies on the effect of pesticide mixtures are currently lacking, especially regarding the impact of environmentally realistic low doses on ecologically important and highly sensitive biological endpoints. Laboratory studies as the one presented here can contribute to the definition of different behaviours for use in routine risk and hazard assessment. The behavioural endpoints chosen here were easily observed, and within a short timeframe, simple to quantify, and of biological significance and ecological relevance.

MO 382

Ecosystem functions vs. functional groups: do they respond in the same way to stress?

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Understanding to what extent ecosystem functions respond to chemicals is a major challenge in ecotoxicology. Ecosystem-level experiments (micro- and mesocosms) mostly describe the responses of functional groups to stress. However, it is unsure if these responses are proportional to the responses of associated ecosystem functions. By combining carbon budget modelling with data from a standardized microcosm experiment with a known community composition, we quantified three ecosystem functions (net primary production, NPP; net mesozooplankton production, NZP; net bacterial production, NBP) at three copper concentrations and a control. Changes of these ecosystem functions with increasing chemical concentrations were not always proportional to the copper effects on the densities of the contributing functional groups. For example, copper treatments decreased mesozooplankton density by a factor 100 and increased phytoplankton density 10 to 100 times while increasing NZP and leaving NPP unaltered. In contrast, copper effects on microzooplankton density were comparable to those on the associated function (NBP). We illustrate that differences in the response of phytoplankton/mesozooplankton densities and the associated ecosystem functions to stress occur because functional rates (e.g. photosynthesis rates/ingestion rates) differ among copper treatments and in time. These variations can be explained by food web ecology but not by direct copper effects, indicating that ecological theory is needed to understand environmental effects of stressors.

MO 383

Effects of pesticide toxicity, salinity and other environmental variables on selected ecosystem functions in streams and the relevance for ecosystem services

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Effects of anthropogenic and environmental stressors on freshwater communities can propagate to ecosystem functions and may in turn impede ecosystem services. We investigated potential shifts in ecosystem functions that provide energy for freshwater ecosystems due to pesticides and salinity in 24 sites in streams of southeast Australia. First, effects on allochthonous organic matter (AOM) breakdown using three different substrates (leaves, cotton strips, wood sticks) in coarse and fine bags were investigated. Second, we examined effects on stream metabolism that delivers information on the ecosystem functions of gross primary production and ecosystem respiration. We found up to a fourfold reduction in AOM breakdown due to exposure to pesticides and salinity, where both stressors contributed approximately equally to the reduction. The effect was additive as, no interaction or correlation between the two stressors was found. Leaf breakdown responded strongly and exclusively to exposure to pesticides and salinity, whereas cotton strip breakdown was less sensitive and responded also to other stressors such as nutrients. No functional redundancy for the effects of pesticides and salinity on leaf breakdown was observed. For wood stick breakdown, no relationship to environmental gradients was found, however, the sample size was lower. We did not detect effects of pesticides or salinity on gross primary production or ecosystem respiration. A reduction in AOM breakdown by pesticides and salinity may impair the ecosystem services of food provision and possibly water purification. Hence, future studies should examine the spatial extent of these effects.

MO 384

Effects of SSRIs on aquatic predator-prey interactions

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Because pharmaceuticals and their metabolites have been detected in aquatic environments, there is potential for wildlife exposure and consequent effects on ecosystem structure and function. Not only is it important to examine sublethal effects such as changes in behavior of individuals, but it is also necessary to determine if these result in changes with broader ecological implications. This study focuses on the effects of pharmaceutical exposure on interactions between aquatic predators and prey, i.e. the European perch (*Perca fluviatilis*) and the small crustacean *Daphnia magna*. The effects of sertraline, a selective serotonin reuptake inhibitor (SSRI), on predator feeding rates were quantified at different prey densities to examine changes in the predators' functional response. This research will allow for application of ecological models to predict whether changes in predator-prey interactions caused by pharmaceuticals can alter population structures of aquatic organisms, and potentially community structure in aquatic environments. By taking such potential ecological impacts into account along with traditional methods, researchers and policymakers can improve the assessment of potential environmental risks of pharmaceuticals.

MO 385

The ecosystem services triad: linking stakeholder engagement, biophysical models, and ecological production functions to develop indices of ecosystem services for biodiversity

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There is tremendous momentum in the regulatory, academic, and business communities to develop approaches for measuring and managing ecosystem services. These efforts are evolving at various landscape levels ranging from site, to watershed, to regions. Most of these approaches rely to some extent on developing linkages between ecological processes and products and their use by humans. These linkages, often termed, ecological production functions, aim to describe and identify ecological inputs and processes that result in production of outputs that are valued by humans in such a way as to render ecologically meaningful measurement endpoints as well as to allow for accurate elicitation of values and preferences among stakeholders. One challenge for ecologists as well as social scientists is to develop analytical frameworks that accurately portray relevant ecological processes in ways that are easy to understand by non-scientists. Another challenge is to identify measurement endpoints that accurately represent ecological processes and functions, are easy and cost-effective to measure, and are good indicators of ecological changes over time. This poster will present results of a field demonstration of a decision support tool to quantify biodiversity-related ecosystem services at a U.S. Department of Defense site in the United States of America.

MO 386

Implementation of ecological risk assessment TRIAD for contaminated sites in the Basque Autonomous Community

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The Ecological Risk Assessment (ERA) methodology currently applied in the Basque Autonomous Community (BAC) is based on Potentially Affected Fraction (PAF %) based on toxicity data from literature and the concentration of contaminants on the site. However ecosystems are complex systems with multiple interactions and PAF may not be considered as a representative measure of ecological impact. With the aim of improving soil quality assessment methodologies in BAC, BERRILUR RTD project, pursues the implementation of TRIAD [1] as a proven ERA approach. Multiple expert groups from the Basque University and Research Centers are committed in BERRILUR gathering expertise from different disciplines. The first phase of BERRILUR has consisted in adapting the existing TRIAD approach to BAC regional conditions such as soil type, soil use and expert's knowledge.

Results and information gathered were combined and three comparative tables were generated, one per LoE. In total, 18 chemical, 35 ecotoxicological and 62 ecological tests have been classified and prioritized. In each table the tests are classified according to the following scheme: (i) name of the test, (ii) organization that carries out the test, (iii) taxonomic group and organism used in the test, (iv) type of contaminant (M: Metal, O: organic and T: all), (v) scoring of each test in each tier and finally, (vi) its corresponding ranking. So far results of the classification process show strong coherence, i.e. cheaper, quicker and highly standardized tests are ahead in tier 1, while the score decreases in tier 2 and 3. On the contrary the tests in the latter positions of tier 1 are best positioned in tier 3.

Regarding sensitivity of the tests towards nature of pollutant, slight differences could be appreciated only through chemical tests. The species used for testing consisted essentially in: microorganisms, plants, earthworms and slug. It should be noted that colembola, nematodes, arthropods and enchytraeids were not used in any test. So far, this means that available tests are not applicable to every existing soil function. It is therefore that we raise here the need to progress beyond the state of the art in by developing new testing tools and obtaining empirical data to assess specific replicability. In the second stage of the project the above methodology will be tested on a closed landfill in order to assess the ecological risk and define the possible soil uses and/or necessary remediation measures.

MO 387

Ecosystem services and environmental decision making: seeking order in complexity

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While there are still great uncertainties about the links between ecosystem biophysical drivers, biotic community structure and ecosystem functions and services, the concept of ecosystem services (ESS) is being applied in a rapidly expanding number of decision, management and policy frameworks. The majority of these frameworks can be referred to as net environmental benefit analyses (NEBA) or net ecosystem service analyses (NESA) as they seek to understand the net effect that various actions may have on ESS. As emergent properties of ecosystems, ESS are integrators of effects from multiple stressors and biophysical interactions at a range of spatial and temporal scales. ESS thus have the potential to provide a holistic approach using a "common currency" between environmental issues, helping us join up various programmes for a more integrated and sustainable management of the environment. However, how ESS within various frameworks are applied, defined, quantified, modelled and valued ranges widely between applications, potentially hindering their roles as cross-sectoral tools. For example, ESS can be transposed as assessment endpoints for "traditional" EcoRA measurement endpoints; they can be assigned indices such as habitat suitability for comparative assessment; they can be quantified as measurable material or product flows; or they can be mapped based upon landscape uses, each with very different scientific and decision implications. This paper will critically review how these issues are or could be addressed in a broad range of case studies in which ESS play a role, including examples for the assessment of environmental damage, sustainable remediation, International Finance Corporation Performance Standards, dredged material management, Integrated Coastal Zone, fisheries and landscape management, Plant Protection Products Registration, international environmental impact assessment, international due diligence, environmental insurance, and more strategic environmental decision making at catchment and landscape levels. Key assumptions, tools, similarities and differences will be identified, and opportunities for greater scientific relevance, clarity, transparency and read-across will be identified. Finally, the question of how we can ensure that current and evolving scientific understanding of ecosystem function and services in complex systems are relevant to and considered in wide-ranging environmental decision making and policy will be discussed.

MO 388

DASEES: a tripartite decision analysis framework to achieve sustainable environment, Economy & society growth and management goals

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Many of Societies management and growth decisions are often made without a balanced consideration of pertinent factors from environmental, economic and societal perspectives. All three of these areas are key players in many of the decisions facing societies as they strive to operate in a more sustainable manner. Typically decisions are made either considering one or two of these perspectives while ignoring the third, or the weighting for one or two of the perspectives vastly outweigh the second or third consideration. Far too frequently the process either ignores or does not adequately factor in consideration of the roles that ecosystem services play in a sustainable system. Most decision-makers do not currently have access to useful or usable methods and approaches when they are presented with economic and social welfare choices that will have significant ecosystem impacts. The goal of the USEPA Ecosystem Services Research Program (ESRP) Decision Support Framework (DSF) Team is to help provide that access by identifying and developing effective and user-friendly decision methods and approaches that empower decision-makers to explicitly, routinely, and substantively incorporate ecosystem services into their decision-making process. The goal of this framework is to provide decision-makers with an understanding of potential outcomes and effects of their planned decisions on economic, social, and ecological systems in order to promote more balanced and sustainable solutions. To this end the ESRP DSF team is developing an open-source, web-based decision analysis framework called DASEES: Decision Analysis for a Sustainable Environment, Economy and Society. DASEES integrates guidance and decision support tools to implement a five step iterative Bayesian decision process designed to: 1 - Understand the decision context; 2 - Define Objectives; 3 - Develop Options; 4 - Evaluate Options; 5 - Take Action. DASEES will be developed with stakeholder and decision-maker input, through case studies, to ensure the guidance, tools, and templates meet user needs and facilitate the incorporation of economic, societal and ecosystem services in the decision-making process.

MO 389

Acute exposure of common carp (*Cyprinus carpio* L.) fry to copper sulfate: a histological study

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One of the most common chemicals used in carp aquaculture is copper sulfate. It is regularly employed as a bath treatment against parasites as well as a fungicide and a herbicide. When used as a pesticide in plant treatment it can reach streams and rivers by surface runoff. It is known that copper sulfate is toxic, even in small quantities, and its use in fish farming has to be controlled. If used improperly, copper sulfate can cause severe damage to fish vital organs. In this experiment, 2 months old carp fry were exposed to increasing concentrations of copper sulfate (lower doses: 0.5 mg/L; 1 mg/L; higher doses: 2 mg/L; 4 mg/L) in aquaria for 1, 6, 12 and 24 hours. Fish fry were sampled, formalin-fixed, whole mounted in paraffin moulds and cut longitudinally for microscopic observations. The slides were stained histochemically with PAS (periodic acid-Schiff) or with propidium iodide and observed with light microscope and fluorescent microscope, respectively. Histological analysis showed various alterations in different organs of copper sulfate-exposed fry, especially the eye, the gills, the skin and the kidney. The structure of the eye was altered, it was partly shrunk and its function appeared to be impaired at higher doses. In the gills, complete fusion of secondary lamellae and necrosis of gill tissue were visible after exposure to higher doses of copper sulfate and longer exposure periods (6, 12 and 24 hours), while lower doses induced less severe alterations: hyperemia, hyperplasia, hypertrophy, as well as lifting of the respiratory epithelium (after 12 and 24 hours). Most common change in the kidney was dilatation and degeneration of glomeruli, which was seen at higher doses and longer exposure periods (12 and 24 hours). Fragmentation of nuclei in skin cells was increased, indicating elevated level of apoptosis in the skin of copper sulfate-exposed fry. The liver and the intestine did not sustain histopathological changes and appear normal in all treatments. These results are consistent with earlier studies on the toxic effects of copper sulfate on fish, indicating dose-dependent alterations in the vital organs.

MO 390

Zebra mussel parasites: potentially useful bioindicators of freshwater quality?

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Parasites are major components of aquatic ecosystems in terms of diversity, ubiquity and key role in food web structure. This implies that they can be a reliable tool in assessing the effects of ecosystem disturbances, as an alternative or complement to macro-invertebrate approaches. Their value as bioindicators of water quality has been documented with fish hosts, but few studies have focused on bivalves, particularly freshwater species. These hosts have the advantage of being less mobile and therefore provide information on the site-specific quality. In this context, we inventoried and analysed the composition and the structure of parasite communities of zebra mussel populations at twelve sites in France and the United States, characterized by different levels of contamination. Our specific goal was to determine if zebra mussel parasites could be useful as bioindicators of freshwater quality. Each host population was characterized for parasite infection following standard histological methods. We used multivariate analyses and descriptive modelling to study parasite communities in relationship with environmental quality. Our results indicated that sites of different chemical contamination levels exhibited different parasite communities characterized by different trematode species and parasite associations. The contamination promoted infections by microparasites (i.e. bacteria and ciliates), whereas it prevented those by macroparasites (i.e. trematodes). For example, the occurrence of intracellular bacteria, Rickettsiales-like organisms, was positively correlated with the nickel and chromium concentrations, and sampling indicated the absence of digenean trematode parasites at a highly contaminated site. Moreover, the use of multivariate analyses and modelling are promising tools to study zebra mussel parasite communities related to pollution

MO 391

Microcosm studies for evaluating potential of microbial community to degrade pollutants in soil and water ecosystems

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The ability of soil and water to recover from chemical contamination is primarily dependent on the presence of a microbial community with the ability to remove it.

Furthermore the microbial community characteristics of an ecosystem can indicate changes in resource availability and the presence of pollution. In this way, the microbial community represents an important key to understanding the impacts of environmental and anthropogenic factors on ecosystems.

Pollution can influence soil and water quality and productivity, but little is known on the effects on microbial communities, and consequent impacts on its functioning. Soil and water microcosm studies enable the studying, under controlled conditions, the effects of selective pressures, such as the xenobiotic occurrence, on natural microbial communities.

We report the overall results of several microcosm degradation studies, using natural soil and water samples, in which the degradation of many chemicals (including the parent compounds of pesticides and pharmaceuticals and their metabolites) in soil and water ecosystems has been evaluated. The natural bioremediation capability was evaluated comparing the microbiologically active microcosms treated with the parent compound (e.g. pesticide or pharmaceutical) with others previously sterilized. The disappearance time of 50% of the parent compound applied (DT50) was evaluated for each chemical in the presence/absence of microbial community. The overall results presented here show the key role of microorganisms in the degradation of the chemicals studied and encourage the use of microcosm approach for assessing more realistic environmental exposure scenarios and establishing the causal relationship between degradation and the role of microbial communities in chemical disappearance from the environment.

ET19P - Veterinary medicines in the environment: Basic research for risk analysis

MO 395

Risk assessment of veterinary medicines - lessons learned and research needs

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Since 2005 European legislation and guidelines ensure a harmonised environmental risk assessment for all new veterinary medicinal products. Information on fate and

effects of veterinary medicines and on their risk to the environment has increased in the past years. The presentation gives an aggregated overview of the results of the environmental risk assessments performed at the German Federal Environment Agency since the coming into force of the guidelines. Needs for future basic research are identified. A summary of ongoing guidance development is given.

MO 396

Behavior of steroids and veterinary antibiotics in soil: study of transfer and degradation in soil columns

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Antibiotics are more and more used for animal treatments and are excreted unchanged. So, these compounds can enter the environment through land application of manure. This phenomenon could induce a resistance to antibiotics in human or animal medicine. Steroids, which are endocrine disruptors are also found in the environment due to animals but also human wastes.

The presence, distribution, fate and impact of veterinary substances and hormones regularly introduced into the soil via land application are far from known and very poorly characterised at the present time. In this context, the project aims at the development of methodologies based on soil columns experiments, in order to characterise the main products of biotic and abiotic transformations and to evaluate the migration and/or retention of selected contaminants and their breakdown products.

For this purpose, 45 substances were selected including 11 hormonal steroids, 23 veterinary compounds and 11 other well-known human contaminants.

The surface of the columns were first spiked with the different compounds and then regularly watered with artificial rain (0.01 M CaCl₂ in distilled water). The leachates were regularly collected and analysed using a multi-residue method based on liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Several series of columns were set up for periods ranging between 0 and 30 days. After this, the columns were sectioned into 5 horizons that were analysed using an original method based on a sample extraction by QuEChERS followed by a purification by solid phase extraction and an analysis by LC-MS/MS. Then, the presence and distribution of contaminants in the different horizons and/or leachates were established. The influence of various parameters on this transfer was also examined such as the pluviometry and the composition of soil (clay, loam, sand and organic matter contents).

The degradation of the substances in soil was also studied. The kinetics of disappearance of the target contaminants were evaluated via modelling, by determining their lifetimes and degradation constants. On the other hand, the detection and identification of transformation products were considered by using complementary features of the coupling with two analysers: the time of flight (QqToF) and triple quadrupole (QqQ).

MO 397

Modelling environmental exposure from veterinary medicines with winPRZM-EXAMS

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The Pesticide Root Zone Model is used in the FOCUS Surface Water and Groundwater model software (winPRZM). Outside of these model frameworks, winPRZM linked with the Exposure Analysis Modelling System (EXAMS) may be used to estimate environmental concentrations of veterinary medicines associated with releases from beef cattle manure from pasture, feedlot, and fertilizer sources. This poster will show the enhancements to winPRZM for "applications" to feedlots and pasture. An example watershed scenario (based on the USEPA standard index reservoir scenario) will be shown with predicted exposure concentrations in the receiving water. The watershed scenario incorporated landscape metrics derived from the GIS analysis of watersheds (i.e., % manure application, % pasture land, and % feedlots in a watershed). Simulations were conducted for all three modes of manure "application" (pasture, manure, feedlot) and a combined mass (percent crop area adjusted) input into the waterbody to reflect a conservative proportion of each application source within one watershed.

MO 398

Temperature dependence of transformation and translocation processes of manure-amended sulfadiazine in soil

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Sulfadiazine (SDZ) is a widely used veterinary antibiotic. In pigs, it is partly metabolized to N-acetylsulfadiazine (Ac-SDZ) and 4-hydroxysulfadiazine (4OH-SDZ). Fresh manure applied to agricultural soils contains a mixture of these three compounds. A conceptual kinetic model describing concentration dynamics of SDZ and its metabolites in soil considers hydroxylation of SDZ, de-acetylation of Ac-SDZ, irreversible formation of non-extractable residues (NER) and reversible sequestration into a residual fraction.

In field experiments with manure-amended SDZ, a dominant influence of temperature on the environmental fate dynamics was observed. For an application of the model on the field scale, the effect of temperature on the rate constants needs to be quantified. Therefore, fate dynamics were investigated at two different temperatures (10°C and 20°C). To separately observe all transformation pathways in one experiment, SDZ, Ac-SDZ and 4OH-SDZ were each labelled with different C isotopes and spiked to manure, which was then homogeneously mixed with a silt loam soil. The batches were adjusted to 40% maximum water capacity and incubated in the dark for 150 days. Samples were sequentially extracted with (1) CaCl₂, (2) methanol/CaCl₂, and with (3) acetonitrile:water via accelerated solvent extraction (ASE). The extracts were analysed by HPLC-MS/MS. The kinetic model was fitted to the concentration data. Model parameters were optimized by a stochastic optimization algorithm.

All considered processes showed higher rate constants in the 20°C experiment. In principal, increasing temperature may exert different effects: (i) chemical and enzyme-catalyzed reactions are accelerated, (ii) microbiological processes (including growth) are enhanced, and (iii) equilibrium sorption coefficients are shifted leading to larger dissolved compound fractions. In accordance, de-acetylation of ¹³C-Ac-SDZ was several times more rapid at 20°C than at 10°C. The same tendency was observed for the slow hydroxylation of ¹⁴C-SDZ, but the effect was not exactly quantifiable. NER formation could only be determined indirectly, but likewise seemed to be enhanced considerably with increasing temperatures. Translocation into the residual fraction and back is assumed to be due to physical processes with less sensitivity to temperature. However, translocation also seemed to be positively correlated with temperature.

MO 399

Fate of ¹⁴C-sulfadiazine in soil and characterization of its non-extractable residues

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Antibiotics are important therapeutics for the treatment of bacterial diseases in humans and animals. Most antibacterial compounds are metabolized only incompletely by the medicated animals and humans. Therefore biologically active compounds, besides partially active metabolites, are excreted in urine and feces. Manure originating from livestock husbandry is commonly utilized as fertilizers in agriculture. Thus, residues of antibiotics present in manure have been directly released into the environment for decades.^[1]

Sulfadiazine (N1-[2-pyrimidinyl]-sulfanilamid) belongs to the class of sulfonamides and is one of the most widely-used antibacterial veterinary products. It exhibits a wide spectrum against Gram+ and Gram- bacteria by inhibiting the p-amino benzoic acid in the folic acid metabolism.^[2]

Non-extractable residues (NER) of xenobiotics in soil are discussed regarding their potential remobilization and bioavailability in the long term. In this study the fate of ¹⁴C-labeled sulfadiazine (SDZ) residues was studied in time-course experiments in soil. Silt loam was spiked with ¹⁴C-SDZ and amended with pig manure.

Balances of ¹⁴C are based on mineralization, extractable and non-extractable residues. The main focus of this study was the characterization of the non-extractable residues associated with humic substances.

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MO 400

A closer look on the non-extractable residues formed by SDZ and soil organic matter

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By application of the ¹⁴C-labeled veterinary drug Sulfadiazine (SDZ) to soil and incubation for 200 days the formation of non-extractable and/or bound residues with soil organic matter is studied. The study focuses on the alkaline extractable part of the soil organic matter which is referred to as humic acids. Extracted humic acids from the different soil samples are redissolved and separated utilizing size-exclusion-chromatography. Further analysis is carried out by radio-chromatography and high resolution mass spectrometry (HRMS).

The radioactivity balance study of the storage experiment shows that the organic and acidic extractable radioactivity decreases over time. In contrast to these findings the alkaline extractable radioactivity increases which means that the SDZ gets enriched in the humic acid fraction. The radio-chromatograms of the redissolved humic acids show two separated peaks. The first peak is broad and can be associated to SDZ which is somehow bound or trapped in the humic acids. The second peak is sharp and is identified by mass spectrometry as free SDZ. This shows that a fraction of the non-extractable sulfadiazine is not bound to the humic acids.

By quantification of the SDZ in these fractions and the evaluation of the results in context of the storage experiment the nature and dynamics of SDZ in soil can be characterized. This will lead to a further insight into the nature of binding of SDZ and in general xenobiotics to soil organic matter.

MO 401

Effects of amoxicillin, difloxacin and sulfadiazine on microorganisms and the nitrogen cycle in soil

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Veterinary drugs, e.g. antibiotics, enter the environment during fertilization with manure and may influence the soil organisms and nutrient cycles.

The present work summarizes information on the effects of different antibiotics added with pig manure on soil microorganisms and the nitrogen cycle pathways (potential

nitrogen mineralization, nitrification, and denitrification activity). The effects of amoxicillin (AMX; a beta-lactam), difloxacin (DIF; a fluoroquinolone) and sulfadiazine (SDZ; a sulfonamide) were investigated in batch experiments, a mesocosm trial under controlled moisture, temperature and light conditions and a field trial. In the batch experiments, antibiotic spiked manure was added to Ah material of an agricultural soil (Orthic Luvisol) at a ratio of 1:25 manure:soil (w/w). The antibiotic concentrations amounted to up to a final content of 100 mg kg⁻¹ soil. In the mesocosm and field trials, manures of DIF- and SDZ-treated pigs were used for fertilization and maize were cultivated on the soil during incubation. Analyses of the microbial activities were performed at the beginning and in distinct intervals after manure addition. The microbial biomass amount and all processes of the nitrogen cycle were stimulated by manure addition. The antibiotics inhibited the enhancement but the effect depended strongly on the kind of antibiotic. AMX and DIF showed only insignificant effects at medically caused concentrations. The effects at higher concentrations were less pronounced and only temporary. The reasons are the biological degradation of AMX and the strong and fast sorption of DIF to soil particles. In contrast, SDZ showed pronounced effects at medically caused concentrations over long time periods. Only, the nitrogen mineralization rate was inhibited temporarily. Potential nitrification and denitrification activities showed decreased activities (up to 30% inhibition) during the whole incubation time of the mesocosm and field trials. Considering the world wide applied amounts of veterinary antibiotics, particularly sulfonamides, these results suggest a significant risk by fertilization with antibiotic containing manure for crop production and fundamental soil functions.

MO 402

Sublethal effects of Ivermectin on dung beetles of the genus *Onthophagus* (*O. taurus*, *O. nuchicornis*)

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European legislation requires parasitocides to be tested for excreted residues using species of dung flies (OECD 228) and dung beetles (OECD GD 122). In the latter case, the beetle *Aphodius constans*, a dweller species has been approved as a test species for insecticidal residues in dung (OECD 2010). Larvae of dweller species develop in dung on the soil surface while larvae of tunnelers develop in dung buried in the soil by adult beetles. Therefore, results of toxicity tests using *A. constans* may not be relevant to tunnelers. Recently an international ring test was started using two tunneler species of the genus *Onthophagus* by the SETAC advisory group DOTS (Dung Organism Toxicity Test Standardization). The suitability of two species (*O. nuchicornis*, *O. taurus*) was tested using the parasiticide ivermectin as model substance. The new test covers more than one life stage and a sub-lethal endpoint, aiming to increase the sensitivity of the test. It has already been set-up at eight laboratories in five countries. Test concentrations were spiked into fresh cattle dung using acetone as a solvent. All concentrations are given as nominal values in mg a.i./ kg dung fresh weight (FW). Despite the fact that different concentrations were used in the tests with the two species, the resulting EC₅₀ values concerning the number of hatched beetles were almost the same. For example, EC₅₀ values of 31.3 (12.2 - 186.9) and 34.1 (13.9 - 85.1) µg a.i./ kg dung (FW) were determined at ECT GmbH. In comparison, EC₅₀ values of 100 - 176 µg a.i./ kg dung (FW) were determined in tests with *A. constans*. Thus, the new method is more sensitive than OECD test 2010. Future objectives include adoption of the new method as an OECD guideline to facilitate Phase II, Tier B testing when such tests are required

MO 403

Behavioural response of soil invertebrates to veterinary ionophore antibiotics

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Monensin and lasalocid are polyether ionophore antibiotics used in the poultry industry for treatment and prevention of coccidiosis. They enter the environment by the application of contaminated manure. They are therefore a potential threat to non-target soil invertebrates. In the present study, we were interested in potential behavioural responses of earthworms (*Eisenia andrei*) and woodlice (*Porcellio scaber*) to the two ionophores. The standardised earthworm avoidance test was used (ISO 17512-1:2008) and the same protocol was also adopted for use on isopods. Preliminary results indicate that the behavioural assay could be as sensitive as the standard toxicity tests. The environmental implications of avoidance behaviour should therefore be taken into account in risk assessment.

MO 404

Development of a guidance for testing transformation of veterinary medicinal products (VMP) in manure

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Results of a project to develop a test method to determine VMP transformation in manure are presented. Project aims are: adaptation of existing methods to study the fate of VMP and biocides under special emphasis on the complex matrix manure; method validation by use of an appropriate statistical evaluation tool and by intra- and inter-laboratory testing; development of Standard Operation Procedures; preliminary outline for an OECD-testguideline to be introduced into the OECD programme.

The project is subdivided into three subsequent phases:

1. Method development using cattle manure of different origin and the VMP salicylic acid. Method development includes an intra-laboratory validation. Fate of salicylic acid is described by dissipation of the parent, formation of characterized transformation products, NER, CO₂ and CH₄, mass balances.
2. Method refinement by using two further substances (paracetamol and a biocide) and pig manure.
3. Inter-laboratory validation.

The selected experimental setup is a suitable basis to study transformation of VMP and biocides in manure. The test design gives reliable and reproducible results. Statistical analyses yield minor variations in DT₅₀- and DT₉₀-values of the test substance. Also, quantification and characterization of transformation products as well as determination of formed NER, CO₂ and CH₄ is achieved reliably.

Still crucial points are: the influence of a static versus a flow-through incubation system on the ratio of formed CO₂ and CH₄, suitability of methods to determine the microbial activity (using ¹⁴C-glucose as reference substance in comparison to the fumigation method used for anaerobic transformation studies), and the influence of storage temperature and storage duration on the test manure.

MO 405

Co-extraction and -quantification of 16 veterinary antibiotics and the evaluation of their bioavailability in dairy manure

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Pharmaceutical products, most notably antibiotics are being applied on a large scale in animal husbandry. With close to 800t administered in 2005 they form the most avidly applied group of veterinary pharmaceuticals in Germany. Such quantities arise because antibiotics are being used not only for acute disease treatment, but are also administered prophylactically to prevent disease-expansion to an entire livestock. But the continuous feeding of antibiotics demonstrably leads to the dispersal of antibiotic substances in manure, soil and rural waterbodies thusly creating the selective pressure of constant subtherapeutic levels which are known to promote the spread of resistant and potentially pathogenic microorganisms. Accordingly the intermediate goal of this work was to develop a method for the co-extraction and subsequent quantification of 16 representatives of the most notable veterinary medicinal classes (350t tetracyclines, β-lactams 199.2t, 97.5t sulfonamides, 52.6t macrolides and 3.7t fluoroquinolones in 2005) in manure. The extraction of antibiotics from manure was done using a McIlvain buffer. Purification and concentrating of the analytes were accomplished through co-polymer-based solid phase extraction (SPE). The chromatographic determination, using UPLC-MS/MS, ran over a BEH C 18 column with an acetonitrile gradient. The antibacterial substances were identified from retention times and fragmentation pattern of each analyte using ESI-tandem quadrupole mass spectrometry and detection by MS / MS in MRM mode. The detection limits for the MS / MS in dairy manure were determined at 0.4 µg/kg. The recoveries between 28% and 158% were determined in dependence of the analyte and its concentration. For the investigation of the antimicrobial activity and bioavailability of the individual compounds an agar diffusion test was applied showing significant inhibition of the antibiotic potency under the influence of manure matrix in contrast to application of the pure substance. Further work will provide an extended method for the use on fatstock manure, due to a dramatically higher antibiotic consumption and correspondingly higher residues that can be expected.

MO 406

Determination of the veterinary antibiotic erythromycin's potential to bioconcentrate in the aquatic invertebrates, *Lumbriculus variegatus* and *Daphnia magna*, and evaluation of a surrogate model

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The bioavailability of ¹⁴C-radiolabeled erythromycin was evaluated in aquatic microcosm systems with and without the addition of pond sediment extraction disks and the aquatic worm, *Lumbriculus variegatus*, and using C8-Empore[TRADEMARK] disks as surrogate passive sampling devices for comparison. An aquatic microcosm was also examined using the crustacean *Daphnia magna* to assess the potential of erythromycin to bioconcentrate in non-target aquatic organisms. After 14 days of exposure, the worms, daphnids, and disks were extracted, and uptake of ¹⁴C-erythromycin residues in tissues was determined. Comparisons of relative uptake indicated a steady-state bioconcentration factor of 2.2 for *L. variegatus* compared to 18 for the disks in aquatic systems (reached between 3 and 7 days). The disk bioconcentration factor (BCF) was 8.2 times the worm bioconcentration factor without normalization to lipid and carbon content. In the pond sediment-containing microcosms, worms exposed to 0.05 and 0.5 ppm demonstrated a mean BCF of 0.04, compared to 0.13 for the 5-ppm treatment. The disks showed a peak "BCF" at day 3 in sediment-containing microcosms with no significant differences seen between concentrations and timepoints examined. The daphnids showed the highest erythromycin BCF at day 3, followed by a decrease in tissue content of the compound through day 14.

These organisms had higher BCF values at 0.005 ppm with a maximal BCF of 8 for 0.005 ppm at day 3, compared to the higher concentrations examined (0.05, 0.5 and 5). The higher BCF in daphnids in aquatic microcosms compared to the worms is potentially due to the daphnids being fed throughout the study, system type (renewal vs. static),

and/or tissue permeability. The comparative data presented could be used to evaluate antibiotic compounds in aquatic environments to better assess their bioavailability to non-target organisms within water and sediment.

MO 407

Potential environmental impacts of two veterinary ionophore antibiotics

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Monensin and lasalocid are polyether ionophore antibiotics used in the veterinary medicine for treatment and prevention of coccidiosis in broiler chickens and turkeys. They enter the environment by the application of contaminated manure and are therefore a potential threat to non-target soil invertebrates. The goal of our work is to ascertain what potential effects ionophore-contaminated manure could have in terrestrial ecosystems. Although earthworms (*Eisenia andrei*) are relatively susceptible to monensin and lasalocid and exhibit acute and chronic effects at low levels of ionophores, woodlice (*Porcellio scaber*) have not shown any effects to monensin or lasalocid even at very high concentrations and regardless of the route of exposure (soil or food). We were also interested in the effects of ionophore antibiotics on the bioaccumulation of cadmium and lead in woodlice and we examined potential behavioural response to contaminated soil.

The acute toxicity of monensin and lasalocid is probably not environmentally relevant because PECs in soil are well below the obtained LC50 values. However, they could have some collateral effects as ionophores in their ability to facilitate cation transport across biological membranes. Mobile animals such as woodlice might be able to evade any harmful effects since they exhibit avoidance behaviour in the presence of ionophores.

MO 408

Determination of the antiparasitic pharmaceutical toltrazuril and two metabolites in environmental water, soil and animal manure

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Toltrazuril is an organohalogenic endoparasiticide pharmaceutical used in the acute and prophylactic treatment of livestock such as poultry, piglets, sheep and cattle against coccidiosis. Despite that toltrazuril has been used as veterinary pharmaceutical for more than a decade, little is known about its environmental occurrence and fate. Already in 2003 concerns about this compound was expressed and toltrazuril was considered as a high-priority pharmaceutical with respect to especially environmental and toxicological concerns. Toltrazuril is metabolized into two pharmacologically active metabolites; toltrazuril sulfoxide and toltrazuril sulfone.

In this work, we present a novel methodology able to determine toltrazuril, toltrazuril sulfoxide and toltrazuril sulfone in environmental water at low ng/L-level, utilizing LC-MS/MS in combination with solid-phase extraction (SPE). Soil and animal manure matrices were also possible to quantify at trace levels using pressurized liquid extraction (PLE) with an inverse and an integrated clean-up procedure. A field site study, investigating the occurrence of toltrazuril and its metabolites in manure, soil, run-off and drainage water is presented.

MO 409

Determination of tetracyclines and sulphonamides in swine wastewater

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The animal husbandry industry provides a stable source for livestock products. There is a huge demand for veterinary antibiotics in concentrated animal feeding operations (CAFOs) on treating and preventing diseases as well as on promoting growth. This practice may favor a selection of antibiotic-resistant genes. To evaluate the potential impact of emitting antibiotics on the environment, a good analytical method is desired for measuring veterinary antibiotics in environmental matrices.

Tetracyclines (TCs) and sulphonamides (SFs) are broad-spectrum antibacterial agents, and were once heavily used in animal husbandry and as feed additives. This study developed a method to determine three TCs and eight SFs in swine wastewater and river water using deuterium-labeled TC and SFs as internal standards. The samples of influent and effluent were prepared with solid-phase extraction (SPE), and were analyzed using UHPLC-MS/MS at positive electrospray ionization.

The recoveries of tetracyclines were 40% - 60% in influent and 50% - 100% in effluent, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) of tetracyclines in influent were 40 - 75 ng/L and 150 - 250 ng/L, respectively, which were both higher than those in effluent (12 ng/L - 28 ng/L and 40 ng/L - 90 ng/L).

Sulfamethazine (SMT) was found to be the highest among the analytes in both the influent (340 µg/L) and the effluent (450 µg/L) from a piggy farm at Tao Yuan, Taiwan. The similar concentrations of SMT in the influent and effluent suggested limited removal from the wastewater treatment processes. The emission of SMT was estimated to be about 5,450 mg per day. The concentrations of all analytes in most river waters were either not detectable or lower than the limits of quantitation, which indicated a good dilution of the emitted effluent. Chlortetracycline and SMT was observed in a downstream sample at 160 ng/L and 37 ng/L, respectively, although they were not detected upstream. This suggested that there might be other emission sources of these antibacterial agents.

This study provided a method for determining tetracyclines and sulphonamides simultaneously in swine wastewater and river water. The method could be utilized for investigating the distribution and fate of tetracyclines and sulphonamides

MO 410

Degradation products of Oxytetracycline in anaerobic digestion and its effects on biogas production and microbial community structures

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Oxytetracycline (OTC) is one of the important antibiotics used in animal husbandry which is poorly metabolized and excreted in form of liquid and solid manure. Antibiotic presence in manure may inhibit microbial activity thus inhibiting biogas production in manure digesters. In this study, change in microbial community structures due to presence of OTC was investigated along with degradation products of OTC and its inhibition on biogas production in anaerobic digestion. Blank manures were diluted with tap water and established manure slurries were spiked with 50, 100 and 200 mg/l OTC. Blank and spiked manure slurries were digested in 120 ml serum bottles at 37±1°C for 30 days. During digestion, biogas production, biogas and volatile fatty acid compositions were also monitored. After 30 days, degradation products of OTC was analyzed with LC-MS/MS which showed that OTC was mainly degraded to 4-epi-OTC at a ratio of 5-10% of initial concentration. Other degradation products were a-apo-OTC and b-apo-OTC which were present at 1-2% of initial parent concentration. According results, half life of OTC was less than 30 days. 190±3 l/kgVS biogas yield was obtained in the control microcosm where inhibitions of 41%, 57% and 61% were monitored in spiked microcosms, 50, 100 and 200 mg/l OTC, respectively. IC50 concentration of OTC was found to be 68 mg/l. Microbial communities in microcosms were characterized by 16S rDNA targeting clone library formation as changes in community structures were characterized by Denaturing Gradient Gel electrophoresis (DGGE). Bacterial clone library was made up *Firmicutes* by 82% (33% *Clostridiales* and 49% *Bacillales*) and *Proteobacteria* by 15% (14% *Acinetobacter*). *Methanosarcina mazei* (52%) and *Methanobacterium* spp. (46%) were found in archaeal clone library. DGGE analysis showed that similarity changes of bacterial communities were higher than similarity changes of archaeal communities and changes increased with increasing OTC concentration. Statistical analyses of clone libraries with environmental parameters were made by Canonical Correspondence Analysis (CCA). Analyses showed that *Bacillales* were affected negatively by increasing OTC concentration while some *Clostridiales* were affected positively. Inhibitory effect of OTC on *Methanosarcinales* was larger than on *Methanobacteriales*.

MO 411

Flubenzurons: risks and developmental effects on non-target species, a Norwegian perspective

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Chitin Synthesis inhibitors (CSI) are part of a class of insecticides known as Insect Growth Regulators (IGRs) which interfere with the hormonal system of insects. Flubenzurons are CSIs and the two which are most often used in aquaculture in Norway are diflubenzuron (DIF) (market name Releeze) and teflubenzuron (TEF) (market name Ektoban). In Norway there was a voluntary ban at the end of the 1990s, however, in recent years they have been reintroduced due to the resistance of sea lice to emamectin benzoate, the replacement product for flubenzurons. Throughout the world, the monitoring of new and emerging contaminants is of increasing concern and although the flubenzurons cannot be considered a new contaminant, as they have been around since the 1970s, their re-entry to the market place and their recent increased use has become of concern. Non-target species of ecological importance may be affected by the use of these compounds, especially crustaceans and arthropods. *Tisbe battagliai* was selected as the test species as it is a crustacean and its life mode is similar to those of the target species, the sea-lice.

Acute naupliar (< 3 hr old) single substance and mixture toxicity testing was conducted with TEF and DIF and the copepod *T.battalgiai*. A further 7 day developmental naupliar experimental design was developed to investigate the ability of the copepod to successfully develop through the 6 naupliar and 5 copepodid stages to adult copepod after exposure to DIF, TEF and a mixture. The exposure concentrations were based on environmentally relevant levels employed and measured in Norwegian sediment and waters near fish farms treated with these insecticides. The results on development rate indicated significant impediment of copepod development at ng/L with the two compounds. Based on these results, recommendations on improvements in study design and ease of use are made, in addition to recommendations to regulatory authorities regarding the continued use of these pesticides.

MO 412

Assessment of tetracyclines toxicity with two Lemna minor assays

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Tetracyclines are used as antibacterial agents in veterinary medicine including aquacultures. The duckweed *Lemna minor* plays an important role in aquatic ecosystems. It is an important food resource for many fish and birds (notably ducks). *L. minor* is used as a model organism for higher water plant (ISO 20079:2006). The sensitivity of *L. minor* strain taken from fish aquaculture was evaluated with two assays. A microplate assay was conducted according to standard ISO protocol with some modifications.

The field samples needed for semifield assay were taken using the Kajak sediment core sampler. In the semifield assay special cylinders were used, which contained the 5 cm sediment layer and 10 cm of water column spiked with tested antibiotic. Two growth parameters of *L. minor* were measured: frond number and area. Concurrently, the concentration of tetracyclines in water column was monitored with HPLC-DAD. The main objective of the study was comparing the phytotoxicity of tetracyclines to *Lemna minor* in semifield and microplate assays. Three antibiotics were tested tetracycline, oxytetracycline and chlortetracycline. In the semifield assay the concentration of the antibiotics decreased rapidly, much faster than in the microplate assay. However, in both assays the fronds showed the signs of chlorosis and necrosis. The endpoints did not correlate with the actual concentration, but with initial concentration of the tested drug. It means that even the toxic agent (tetracyclines) has relatively short environmental half-lives, it can cause the negative changes in aquatic plants.

MO 413

Effects of temperature and florfenicol on *Daphnia magna* feeding behaviour

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The aim of the present study was to investigate the influence of temperature variation on the toxicity of the veterinary antibiotic florfenicol to the freshwater grazer *Daphnia magna*. The assays were carried out for 24h at three different temperatures (15, 20 and 25°C), in the presence and absence of light with juveniles and adult females using feeding inhibition as effect criteria. The results indicate that temperature variation changes the effects of florfenicol on *D. magna* feeding behaviour highlighting the need of more research on the combined effects of temperature and pharmaceuticals on freshwater grazers.

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MO 414

Histological changes in fish *Astyanax altiparanae* exposed to 17-Alpha-Methyltestosterone

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The synthetic androgen 17-alpha-methyltestosterone is excreted almost entirely by the gills, urine and feces. This study aims the histological analysis of the effect of this in the kidney, liver and intestines of a Brazilian native fish *Astyanax altiparanae*. Two tanks were kept for 28 days, each with 80 specimens fed diets, one without hormone and other with hormone concentration traditionally used in tilapia fish farming. Temperature was controlled (26 ± 1 °C) and aeration was abundant. At 15, 20 and 28 days of experiment ten individuals were euthanized and organs was subjected to standard processing procedure for light microscopy and stained with hematoxylin and eosin. The control group showed normal histological structure. The group fed with feed to which was added to 17-alpha-methyltestosterone, showed histopathological lesions in the intestine, liver and kidneys. In all organs blood vessels had few blood cells, with reduction of erythrocytes and plasma accumulation. In the intestine, the epithelial lining had groups of hypertrophic cells. In the liver, hepatocytes showed cytoplasmic vacuolation and changes in the architecture of the organ. Kidney ducts had enlarged its lumen, the cuboidal epithelium showed apical vacuoles in the cytoplasm and the space capsule of the glomeruli were dilated. Changes were seen as early as the first sample. The lesions gradually progressed, with worsening of histopathology, culminating in the appearance of necrosis in the epithelium lining the intestinal lumen, the liver parenchyma and renal tubules. During exposure to the hormone, the individuals were fed without much greed, unlike observed in the control group. It was possible to observe the feeding using the light microscope to observe the presence of food in light of the digestive tract. The growth of the exposed group was lower than in the control group, contradicting the anabolic function in the case of lambari. The species was considered 'non-target species', since the cultivation of the same is not made use of 17-alpha-methyltestosterone. However, being a native species and genus widely distributed in inland waters in Brazil, it can be used to monitor water quality for the presence of the hormone, because it proved very sensitive to the presence of the same. The data show, therefore, that the hormone 17-alpha-methyltestosterone induces pathological changes in kidney, liver and intestine related to species *Astyanax altiparanae*. Financial support: FINATEC, CNPq.

MO 415

Use of antibiotics in tilapia cage aquaculture: a case study in the Tha Chin river (Thailand)

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The use of antibiotics to prevent and treat bacterial infestations in aquaculture has raised concerns worldwide because of their potential environmental release and the potential effects on the structure and functioning of surrounding aquatic ecosystems. The majority of the available studies addressing this issue have focused on marine aquaculture produced in temperate and cold regions, whereas the great bulk of the global aquaculture production takes place in tropical and sub-tropical regions of Asia. In the present study, the use of antibiotics in tilapia cage production in the Tha Chin river (Suphanburi Province, Thailand) was investigated. Semi-structured questionnaires were performed in 15 farms between the 6th and 22nd of April of 2011. All the interviewed farmers reported to have used antibiotics during the last year with enrofloxacin (73.3% of farmers used it), oxytetracycline (60.0%) and amoxicillin (40.0%) being the most common ones, followed by sulfadiazine potentiated with trimethoprim (33.3%) and sulfamonomethoxazine (6.7%). The 80% of the interviewed farmers reported to apply antibiotics mixed with feed for about 7 days to prevent fish mortalities at stocking and all throughout the culture cycle when mortalities increased. The antibiotic dosages reported to be applied during the stocking period were found to be more than two-fold the recommended amounts in one third of the interviewed farms. The application of antibiotics was reported to be more frequent during the dry season and during the months with adverse climatologic conditions (i.e., monsoon periods), which corresponded with the periods of lower water quality in the river and higher fish mortalities. The results of this survey suggest that downstream aquatic ecosystems will be chronically exposed (i.e., multiple-pulse exposure in the water phase and prolonged exposure in sediments) to the antibiotics applied in the tilapia cages since a great proportion of the applied compounds will immediately cross the nylon netting structure of the cages and reach the surrounding tropical waters. In this presentation, detailed results of the antibiotic use survey will be presented together with the results of an antibiotic monitoring campaign performed between April and July of 2011 in the Tha Chin river.

MO 416

Tetracycline and Fluoroquinolone concentrations in the Istanbul strait sediments

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Surface sediments from twelve different locations of the Istanbul Strait and Marmara Sea, Turkey were analysed for five antibiotics belonging to two different groups of widely used pharmaceuticals, tetracyclines (TCs) and fluoroquinolones (FQs), by solid-phase extraction and high performance liquid chromatography. These two groups of antibiotics, mainly used to prevent or treat illness for humans as well as for animals, are frequently detected in the effluent of municipal sewage plants, in the aquatic environments and in soils after being spread by liquid manure. The results of analysis revealed that measured concentrations of individual antibiotics were significantly different depending on sampling location. Chlortetracycline (CTC) was not detected in any of the samples. High concentrations were mainly found in urbanized regions of the Strait. The concentrations of the two tetracyclines ranged from not detectable to 27.3 µg kg⁻¹ in freeze dried marine sediments. Comparable results were obtained for the two fluoroquinolones with concentration levels from 1.3 µg kg⁻¹ up 34.1 µg kg⁻¹. This study is the first attempt to show the contamination degree of the Istanbul Strait sediments by emerging contaminants. Particular concern should be given concerning their potential side effects caused by the frequent consumption of mussels and fishes captured in the Istanbul Strait.

MO 417

On-line SPE-LC-MS for ultra trace analysis of antibiotics in river and ground water

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Endocrine disrupting compounds (EDCs) represent a broad range of natural or synthetic chemical substances, mostly of anthropogenic origin. Some typical representatives of EDCs are industrial chemicals, cosmetics, pesticides, sexual hormones, medical products and many common pharmaceuticals. Among the latter group, antibiotics are of a special interest as their presence in the - mostly aquatic - environment is a potential cause for antibiotic resistance of many bacteria. Obviously, the concentrations of these compounds in environmental samples are very low, i.e. in the µg/L scale (ppb to ppt range) which is a real challenge for analysis.

An analytical method for the analysis of selected antibiotics in aqueous matrices like river water is presented. It comprises a sample clean-up and pre-concentration procedure combined with high-performance liquid chromatography (HPLC) for the separation and mass spectrometry (MS) for detection at highest sensitivity and selectivity. The sample preparation step is accomplished with solid phase extraction (SPE). The intended system is fully automated and performed on-line with the HPLC-MS step in an integrated set-up with column switching abilities and under single point software control. The feasibility of the system will be demonstrated by analyzing a selection of representative antibiotics, mostly from the tetracycline and macrocyclic type.

RA04P - Established and emerging footprints – striving towards a valid and comprehensive support for decision-making processes

MO 421

Does 'green power' purchase affect Carbon footprint?

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Which electricity models to be used for Carbon footprint is often debated; e.g. how is “national grid” understood and defined? However, when introducing Guarantee of Origin (GO) and Electricity Disclosure according to the European Renewable Energy Directive (2009/26/EC) and Electricity Market Directive (2009/72/EC), respectively, as well as US Renewable Energy Certificates, the issue gets more complicated.

A GO is defined as a means of proving the origin of electricity, while the objective of the Electricity Disclosure is to provide consumers with relevant information about power generation and to allow for informed consumer choice, not only based on electricity prices. The suppliers of electricity are required to disclose their electricity portfolio with regards to energy source and environmental impacts, specifying the emissions of CO₂ and the amount of radioactive waste. Consumers can make an active choice regarding the environmental performance of the purchased electricity by voluntarily purchase of GO. In US Renewable energy certificates (RECs), represent a similar voluntary product. RECs represent the environmental attributes of the power produced from renewable energy projects and are sold separate from commodity electricity. Several standards and guidelines (e.g. ISO14067 Carbon footprint of products, GHG Protocol) are working on general rules for how such products should be interpreted in Carbon footprint calculations. The aim of this presentation is to show how different ways of dealing with this issue affect the Carbon footprint calculations. There seems to be a need for common guidelines for the interpretation of GOs/RECs in Carbon footprint calculations are needed to be able to make fair comparisons between products and services.

MO 422

Climate impacts of transitioning from coal to natural gas fired electricity generation over time

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Transitioning from coal fired electricity generation towards a greater share of natural gas (NG) is increasingly perceived to substantially mitigate climate change due to lower life cycle greenhouse gas (GHG) emissions. Life cycle assessment (LCA) is an accounting tool that compares technologies based on the GHG emissions over their life cycle. The life cycle GHG balance of a specific technology does not represent actual climate impacts because (1) it is based on a per energy basis, and (2) using GHG emissions as a metric masks the physical processes leading from GHG emissions to climate change and climate impacts. The physical processes include the generation of radiative forcing (RF) and temperature change (ΔT), which are sensitive to the timing and the type of the GHG emissions.

We apply life cycle emissions distributions of coal and NG fired electricity generation to a stochastic climate model to quantify the absolute climate impacts of different transitioning scenarios. We extend current models by explicitly modeling two major sources of uncertainty. First, we bound bottom-up life cycle CH₄ emissions from leakage during NG production using top-down atmospheric measurements. Bounding is important given the current wide distributions of CH₄ leakage in the literature and the resulting controversy regarding the “correct” leakage rate. Second, transient climate sensitivity, which accounts for the magnitude and temporal distribution of the climate response to GHG emissions, is represented stochastically in the climate impact module. Quantifying uncertainty in the climate response is vital for drafting policy recommendations that go beyond GHG emissions by addressing climate change directly.

Results show that substituting coal with NG provides little if any climate benefits despite significantly correcting the CH₄ leakage to lower levels. Assuming that U.S. power generation from NG increases by 25% beyond current projections until 2035, thereby reducing coal power by the same absolute amount, RF will be reduced by only about 1-3% over 25-100 years (0.0005-0.0007 W/m²). Moreover, if reduced coal power generation in the U.S. is used to meet coal demand elsewhere, the coal-NG substitution may actually increase total RF. Current work aims to quantify the influence of climate model uncertainty on these results.

MO 423

Reducing energy and GHG footprints of microalgae biodiesel by the use of co-products from the sugarcane ethanol industry

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Greenhouse gases (GHG) and energy assessments are useful techniques to assess environmental impacts resulting from the distinct stages of an industrial practice. Such stages may include upstream and downstream processes in addition to the main activities carried out during the manufacturing of a specific product. In this study, Life Cycle Energy and GHG Assessment (8, 9, 10) were carried out in order to compare the environmental impact of biodiesel production from microalgae cultivated in open ponds.

Biodiesel from microalgae is being considered today as a superior alternative to land-crops for the production of biofuels. Comparatively, cultivation of microalgae offers a better potential for increasing biodiesel productivity and improving the capacity for CO₂ sequestration (4, 5). This study considered two distinct scenarios in a pilot-scale process using microalgae cultivation for biodiesel production.

Brazil is an important worldwide player in the biodiesel industry with an installed productivity capacity of 5,5 million liters per year (3). The use of biodiesel in Brazil has been favored due to the availability of agricultural land. However, the expansion of oil-seed crops may lead to land use changes which in turn favor the release of CO₂ and nitrogen gases stored in soil (1). The production of biodiesel from microalgae does not show such disadvantages.

The default scenario: (i) biodiesel production based on microalgae biomass outputs, in a system using commercial energy, CO₂ and chemical fertilizers as nutrient sources. In this scenario, the algal cake is also submitted to pyrolysis for the production of oil, gas and biochar; was compared with: (ii) the same conditions as described in the former item (i), but now using CO₂ and biogested vinasse from the sugar cane industry to supply part of the fertilizer demand of the algae, the other part being supplied by the digestate of the anaerobic digestion of algal cake.

Life Cycle Energy and Carbon Footprint assessments allow the identification of possible synergistic aspects of algal biodiesel production and the sugarcane ethanol industry, considering a future integration of the two production system. The preliminary results indicate significant positive effects on the energy and carbon footprints of biodiesel from microalgae.

MO 424

Including urgency in global warming assessment in LCA

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Climate change mitigation is more relevant today than ever, as clearly illustrated by the conclusions in the World Energy Outlook 2011 just published by the IEA, giving us only until 2017 to take action in order to stay below a global warming of 2 degrees Celsius.

However, the issue of assessing climate change impacts of greenhouse gases is complex and still widely discussed. At midpoint level the use of the global warming potential (GWP) is completely dominating, but a major short coming of the GWP is the lack of consideration of short term issues, such as important climatic target times - the points in time where climatic tipping points are expected to be crossed. Several alternative metrics to the GWP have been suggested in recent literature. Some include considerations of short term targets but have problems describing more long term impacts occurring beyond such target times. Addressing both of these issues are essential in the pursuit of mitigating climate change, however they should be addressed separately, as they represent two different aspects of the climate change challenge.

This paper introduces an additional impact category in life cycle assessment (LCA), called the climatic tipping point impact category, addressing the urgency related to prevent the crossing of climatic tipping points. The new suggested impact category will take into account both atmospheric lifetimes of different greenhouse gases and increasing urgency when approaching tipping points. Furthermore, it is illustrated how this addition to existing LCA methodology complements the existing impact category on global warming, ensuring easily interpretable results in both the urgency and the long term category.

Solving the issue of urgency with respect to climate change in LCA further deals with the yet unsolved issue of assessing the climate change mitigation value of temporary carbon storage.

MO 425

Study on the community CO₂ emission based on LCA in Beijing

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This research assessed the size and major components of a community carbon footprint based on the life cycle assessment approach combined with IPCC methods. Data related to CO₂ carbon footprint were collected, which included household energy consumption, public and car transportation, waste production, etc. The result showed that energy consumption including electricity consumption, natural gas and heating accounted for more than 60% of CO₂ emission, with transportation and waste production resulting in a further 24% and 15% of total emissions, respectively. The carbon absorbing effect of public green place was analyzed. As the result showed, the public green place in the community reduced about 25,262kg CO₂ emission every year. According to the status of Beijing, the proposals for reducing the CO₂ emission and the low-carbon community building were put forward.

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MO 426

How valid and comprehensive is our CED practice today?

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Several indicators are under development, while others, such as carbon footprint and cumulative energy demand (CED), are more or less regarded as fully developed and integrated into conventional LCA studies. This paper will, however, focus on methodological gaps and challenges such as double counting and missing characterisation factors used for this ‘fully developed’ environmental impact indicator CED.

These challenges are connected to the following aspects:

- Use of specific data (for example for energy carriers with a heat value different from the pre-defined energy carriers in the database and characterisation methods used).
- Feedstock issues:
- How do you define the raw material used for building a wooden shed?
- Should this definition change somewhere in the life cycle, if you extend the analysis from cradle-to-gate to cradle-to-grave, when using the wooden material as an energy source after dismantling?

The methods for calculating cumulative energy demand depend on the correct input, in the correct form, for calculating comparable indicators. In contrast to calculating carbon footprint, the 'correct' input for calculating CED is heavily dependent on the LCA practitioner being aware of how the LCI indicators are calculated, and the practitioner's knowledge of how to manipulate the pre-defined CED method. In addition, the different CED methods available in LCA software tools are not always implemented with awareness of the challenges connected to feedstock issues. It is also interesting that older CED methods seem to be more accurate than newer ones, due to the inclusion of more substances.

The paper will illustrate these challenges by describing quantitatively the inconsistencies introduced by using specific energy carriers and raw materials which can both be used as feedstock and energy sources. The aim of the paper is to raise awareness of the CED challenges and to improve current practice in order to increase the accuracy of the CED results.

MO 427

Development of water inventory database considering water type and form of water use

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Waterfootprint (WF) is paid attention by water resource depletion and eccentric water. WF can be expressed as total amount of water used at entire life cycle of products.

WF is expected to be effective the following four. First WF can understand the magnitude of the impact of water use. Second risk management strategies for water use. Third products, processes, promote the optimization of water efficiency and water management at the organizational level. Finally Communication and marketing strategies.

WF is discussed for standardization in ISO. According to ISOWD, they require quantity of water used, type of water used, form of water use, geographical location of water withdrawal and return in water inventory. Though these database are not exhaustive, this study developed about 3700 sector water inventory database considering water resource, water type and form of water use with input-output table in Japan.

As characteristics of database, in the input aspects water is classified into rain, river, ground and reused water, and consumption aspects it has three types: rain, river and ground water. Based on the database, annual water use by one average family in Japan and import and export water have been calculated.

MO 428

Conceptual framework for grey water footprint of copper mining production

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Given the concerns about global water resources and the particular situation of aridity and water scarcity in the main mining areas of Chile, a priority for mining industry is to advance towards an effective sustainable resource management of water. The grey water footprint is defined as the volume of freshwater that is required in order to assimilate the load of pollutants discarded a water body taking into consideration the natural concentrations and the existing ambient water quality standards. This work aims to develop a conceptual framework to adequate measure a relevant sustainability impact indicator for the copper mining production through the water footprint method, which is one of the most widely used methods for assessing the consumptive use of water. Therefore, the study focuses on developing a framework for accounting the grey water footprint and establishing the most critical pollutants in the effluents of the copper cathodes production, of both the sulfide and oxide production lines, according to the water footprint method. The methodology of life cycle assessment was applied to establish the system boundaries, the calculation basis and the allocation criteria.

MO 429

Closing methodological gaps of water footprint: scope and aggregation issues

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While for many impact categories such as GHG emissions and fossil resource consumption indicators with long experience exist, for water resources impact assessment the knowledge is still limited and bears the additional problem of high spatial variation. The integration of the different aspects of water impacts into a single water footprint metric is a required for proper and robust communication, also addressing the different levels of uncertainties. Also there is a relevant discussion about what impacts should be included in a water footprint in order to avoid double counting and to have a meaningful footprint. In this project, all impacts related to emissions to or consumption of freshwater water are included.

While for substance emissions to water many sophisticated model exist, there is still a lack of proper assessment of heat releases and anti-fouling detergents that are crucial for thermal power production. These impacts have been addressed and integrated.

In a second step, we developed a scheme to combine the various existing LCIA methods related to water resources on midpoint level of the LCIA factors as well as on endpoint level for ecosystem quality impacts. For a illustrating the issues related to aggregation and communication of such a water footprint, we developed specific inventories for cooling water technologies and state of the art coal and gas power plants, including regional differentiation of the supply chain and corresponding geographies for the impact assessment, creating maps of aggregated water footprints.

More detailed analysis of the results revealed that the aggregation step and hence the final metric is very sensitive to underlying assumptions, aggregation procedures and the coverage of included methods. Although in the ISO 14040 series full aggregation of impacts is not allowed for public reporting, the demand for water footprint labels requires a single number and therefore our results are accounting for and reporting the different uncertainties involved.

MO 430

Evaluation of a range of impact methods for assessing water quantity and water quality for a liquid laundry product

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A large number of impact assessment methods have been proposed for measuring the water quality and water use aspects of products. Twenty methods including USEtox, grey water from the Water Footprint network and midpoint indicators based on Water Stress Index (WSI) were applied to a case study of a European liquid laundry product. The objectives of the study were to: 1) understand the strengths and limitations of each method; 2) evaluate their informational requirements and identify gaps in the data available for the life cycle of a detergent product; 3) contrast potential impacts related to water quantity and water quality at the midpoint and endpoint level and 4) provide some initial insights in understanding the water flows of the product system through a mass balance at the inventory level.

The case study was based on a concentrated liquid detergent used in France. The functional unit was one wash at 40°C. Life cycle inventory data was obtained fromecoinvent and the Quantis Water Database. The life cycle stages taken into consideration were chemical supply, manufacturing, consumer use, and end of life (wastewater treatment and packaging disposal).

The quality of the results is directly linked to the quality of the data used in this evaluation. For example there were data gaps in the primary data collected e.g. country of origin of the raw materials and therefore assumptions were made. Typically the consumer use stage dominates the impact scores for 15 out of 20 impact assessment methods applied.

MO 431

Water assessment of a hand dishwashing product using an LCA approach: opportunities and limitations

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Only since a few years, LCA studies are including metrics to evaluate products from a water use impact perspective. Few methods are ready to use, some are under development. However, the applicability of the existing methods has been primarily demonstrated on agricultural materials or products. For example, water irrigation requirements in food production can be water intensive. In view of an increasing interest on water impact from different products, we ran a study on the water impact a hand dishwashing product. A number of water assessment methods were applied with the purpose of identifying both product improvement opportunities as well as understanding the state of the art both from a database and a method perspective.

The study focused on environmental issues related to water use, looking in depth at inventory methods, midpoint and endpoint methods (14 in total) and covered the entire life cycle of the product. The use of a single product with the same global supply chain, sourced from one location was evaluated in two countries with a different water scarcity conditions.

The study suggests that the consumer use stage is the most important one (99% using the Ridoutt and Pfister method), driven by both direct water use (dishwashing process) as well as indirect water use (electricity to heat the water). Therefore, the largest improvement opportunity is to build consumer awareness on habits and practices in water scarce regions. The study also showed a very different profile if spatially explicit methods are applied on one product used with the same use conditions.

From a methodological approach, a lot more work is necessary to develop good inventory databases (in terms of flows covered and accurate data), which is key for all methods. This will be necessary for the application of water assessment methods in a business context. A number of the applied methods are not regionalized, which was demonstrated to be a critical aspect in comparing the overall water profile of the product. Finally, improvements to some methods are suggested.

MO 432

Science behind biodegradable claims on down the drain consumer products

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This poster provides an overview of various approaches that could be used to evaluate the biodegradability of consumer products. The biodegradability of formulations is increasingly perceived as a key attribute for modern "sustainable" products. Despite biodegradability becoming a common environmental claim on the packages of consumer goods products, there is no standard technical approach to assess the biodegradability potential of formulations (i.e. complex mixtures, preparations, finished product, whole-product formulations). This creates the potential for misleading or even incorrect claims. For example, biodegradation is only relevant for organic constituents and

not for inorganic ingredients (e.g. water). Biodegradability assessments are typically required by law for the hazard and risk environmental assessments of individual organic substances. The existing standard procedures (eg OECD 301 Testing Guidelines) for ready biodegradability are only appropriate for individual organic chemicals or mixtures of similar chemicals (i.e. closely related homologues). The OECD guidance specifically states that these tests are inappropriate for mixtures of dissimilar substances as would exist in a product formulation. Hence, ready tests of whole products are insufficient for establishing the full biodegradability of a product formula. Furthermore, even when one has appropriate biodegradation data on the individual ingredients in a formula, different rules and calculation methods can yield quite different results. For example, the percent biodegradability for a single formulation can vary from <70% to > 90%, depending on whether the percentage is based upon the total number or mass of ingredients as well as if all constituents or only those constituent for which biodegradability is a relevant attribute are included in the calculation. We evaluated the pros and cons of each approach and we conclude that the most scientifically rigorous approach is to calculate percent biodegradability of a formula based upon the mass of only those ingredients for which biodegradation is relevant. Going forward, there is a need for a common scientifically based approach and internationally accepted calculation method to assess the biodegradability of whole product formulations to safeguard the credibility of environmental claims among the general public and consumers organizations.

MO 433

Development of impact assessment for chemical substances covering PRTR substances

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The problems of existing assessment methods for chemical substances in LCIA are the lack of covering chemical substances and that of reliability in model.

The purpose of this research is to develop the evaluation method and characterization factors for chemical substances improving the above problems.

Concretely, the approach of this research are to develop Characterization factors on ecosystem for chemical substances prescribed by PRTR(Pollutant Release and Transfer Register)in Japan and to develop those on ecosystem and human health using USEtox that is consensus model for LCIA developed by UNEP / SETAC Life Cycle Initiative. This model was developed based on the knowledge gained from comparative verification by developers of existing evaluation method. Furthermore, this research will consider developing those corresponds to the evaluation in Asian countries have recently been spreading LCA method. The brief process of this research can be described as follows.

1. Fate model which relates inventory with concentration in each environmental media will be developed. USEtox, one of the most advanced methods will be applied in this research.

2. Exposure analysis will be adopted to estimate the amount of exposure caused by the emission of amount of chemical.

3. Effect analysis will be applied to evaluate impacts on ecosystem and human health. Characterization factors reflecting environmental conditions of Japan and Asian countries will be developed through these analyses.

MO 434

LCIA and fate of metal-oxide engineered nanomaterials in freshwater

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Life cycle assessment (LCA) have in past years proven to be a valuable tool for environmental impact assessment of products and systems, but with the introduction of more new products that contain engineered nanomaterials (ENMs) the tool has experienced an obstacle in terms of performing a holistic life cycle impact assessment (LCIA) of ENMs and the engineered nanoparticles (ENPs) that these materials can release to the ecosystem. Metal-oxide ENMs are often used in consumer nanoproducts where TiO₂ and ZnO are considered industry-popular within this group, along with Ag being a widely used ENM metal in products. Today there is a limited amount of LCA case studies on ENMs published as scientific articles. To be exact these count around 13 studies and in most of those there is a focus on non-toxic impact assessment categories that mainly are related to the raw material and production stages of an ENM product life cycle. Performing a conventional LCIA of an ENM product it can be also concluded that the modelling is rather difficult due to missing inventory data and many assumptions that in the end result in cradle-to-gate processes (usually associated to bulk metal production) dominating the impact.

The metal-oxide and metal ENMs are incorporated into a polymer matrix in the performed LCA study. However, release of ENPs to the environment may still occur through e.g. aging and wear. The fate, exposure and effect of ENM has received much focus but this scientific domain still struggles with a low understanding of many of the processes affecting the fate, exposure and effect. Fate can be considered a central research area that needs better understanding and is the focus of this study. Fate modelling is performed on metal-oxide ENMs with a focus on the two important processes of aggregation and dissolution. By quantifying fate in freshwater through these two processes there is a possibility to apply a simplified model to predict the removal of metal-oxide ENPs due to aggregation followed by sedimentation, and the removal of ENPs through dissolution. Thereby concentration in the freshwater compartment can be estimated and used for improving LCIA in the assessment of particle release by linking the metal ENP concentration in freshwater to potential exposure and effects.

MO 435

Moving forward: sustainability and the U.S. cleaning products industry

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Since the Brundtland Commission of the United Nations in 1987 defined sustainable development, the pursuit of sustainability has been a growing movement for all of society, including industries. While the call for sustainable services, packaging, products and processes has been heard, the answer to how these should be assessed and reported has not been determined. Over recent years, the American Cleaning Institute® (ACI) has worked to define and describe how the Institute and its members are committed to sustainable environmental, health and socio-economic practices. From a foundation of adopted principles on sustainability, ACI has initiated a program to substantiate actions taken by industry to be more sustainable. In order to be as comprehensive as possible, and to include both ACI's ingredient supplier and formulator members, an initial evaluation of sustainability metrics across the membership was conducted. From there, attributes including environmental characteristics were chosen for which member companies reported three consecutive years of data. This information is being used for benchmarking and trend assessment purposes. Cleaning product manufacturers and their ingredient suppliers are being faced with a multitude of different demands and requests for data and information that could support development of sustainability reports up and down the supply chain. ACI's work is creating a program that demonstrates the contributions of the industry to the goals of sustainable development, while engaging in the development of numerous sustainability programs. Engagement in the sustainability programs of other organizations provides the opportunity to have the industry's approaches incorporated into their processes. The United States cleaning products industry is working towards sustainability and the development and refinement of tools to assess the industry's achievements. While the overall picture of approaches for reporting sustainability is still developing, the U.S. cleaning products industry is making substantial progress in reporting major industry-wide trends.

MO 436

Comparing the chemical footprint of hydraulic fracturing fluids used in natural gas extraction

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Hydraulic fracturing, commonly referred to as fracking, is a process by which high pressure fluids are injected into a well to propagate fractures in the rock formation to enable extraction of natural gas and oil from previously untappable sources such as deep shale formations. Although fracking is sometimes used in reference to drilling and extraction practices, it represents only one portion of the life cycle of a well. A variety of chemicals are used in fracking fluid including biocides, breakers, surfactants, scale inhibitors, clay stabilizers, and friction reducers. Concern from the public and the regulatory community regarding the potential contamination of groundwater and surface water bodies by the fracking fluid chemicals has risen to a fever pitch. Recently, some companies have begun to voluntarily disclose the chemicals that they use in their fluids and some regulatory agencies in the United States are now requiring disclosure. Using composition information available through the public database, FracFocus, we have evaluated the chemical footprint of several fluids to describe the overall potential risk posed by each should the chemicals reach human or ecological receptors. The chemical footprint provides an assessment of the environmental performance associated with each fluid and a means for selecting between various ingredients.

MO 437

Application of ERICA index to evaluation of soil ecosystem health according to sustainability threshold for industry impact

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The improved ERICA methodology can be able to show how much an additional stressor can cause or not further risk to the target environment compartment.

The idea behind ERICA, Boriani et al. 2010, was to build a modular and upgradable index. The aim of the improved ERICA is to give an instrument that can be used to measure the effect of new engineered substances (e.g. waste from new technological production, pharmaceuticals) introduced into the environment. This will be of great help for "green" processes and sustainable industries and may help to advertise their product as safe for the environment following impact assessment not derived only from political and economical chosen limit thresholds values.

In this work we have added new theoretical indicators to be used for the soil compartment in assessing sustainable use of new chemicals in the environment.

The fact that a certain new compound added to an existing scenario will not pose additional risk is currently not considered by any legislation.

Thus, industries able to show this will get an extra value for their products. Furthermore this will lead to a awareness by industries of how much their product are impacting the environment in a realistic way as most as possible.

For example degradation is of extreme importance in the soil compartment to understand how the chemical will be transformed and how properties like e.g. persistency and mobility will be different in case of transformation products of the parent compounds.

In a concise methodology ERICA can be used as a prioritization system, while in case a detailed risk assessment is needed, it is possible to check every step of ERICA procedure and obtain the partial results concerning for example different compartment impact or human health risk assessment or environmental specific target risk assessment.

ERICA innovated tool may be used to monitor and document environmental performance and to quantify the sustainability of actual and potential land use. Its main objective will be to develop a new methodology intended to help industry and regulatory bodies to evaluate the positive effects resulting from mitigation actions and to promote eco-efficiency.

1) Boriani E., Mariani A., Baderna D., Moretti C., Lodi M., Benfenati E. ERICA: a multiparametric toxicological risk index for the assessment of environmental healthiness. *Environment International* 2010 36 : 665-674

MO 438

Impacts on carbon pools and biodiversity due to land use and land use changes, exemplified with a case study on Norwegian forestry

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There is a strong need for methods within life cycle assessment (LCA) that enable the inclusion of all complex aspects related to land use and land use change (LULUC). Here a proposal on combining impacts on biodiversity and carbon pools is presented and exemplified with a Norwegian case study on forestry. In the case study the use of one hectare (ha) of forest managed for production of wood for bioenergy production is presented. Impacts from LULUC are measured as a product of time and area requirements, as well as changes in quality. At present, there is no consensus on how to measure quality and quality changes due to LULUC in LCA. A range of methodologies are proposed, but these will often give diverging results and might thus motivate different actions. Here both permanent and temporary changes in biodiversity and carbon pools, limited to above-ground biomass, are included. In the case study different management options and their impact on biodiversity and carbon pools are assessed; transformation from unmanaged to a managed forest, transformation from fallow land to a managed forest, consecutive rotations with Norwegian spruce, and consecutive rotations where Sitka spruce is introduced to the area.

The results from the case study show that the impact on climate change from LULUC is more important than the impact from forestry operations. This clearly demonstrates the need to include LULUC in LCA of forestry products. However, LULUC causes a wide spectrum of impacts. Here this is exemplified by biodiversity and carbon pools, presumably the two areas of protection most affected by LULUC. Still, by only including these two, the case studies show large variability based on what assumptions are made and the results are sensitive to both selection of time horizon and allocation of transformation impacts. Impacts from LULUC can be both positive and negative and the results show that there in some situations will be a trade-off between impacts on biodiversity and carbon pools. This underlines the fact that a mere measure of land used does not provide any meaning in LCAs since it is not possible to know whether the impacts are positive or negative.

MO 439

Impacts of land occupation and water consumption through life cycle of bio-ethanol from rice straw

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Bio-ethanol may contribute to reduce carbon footprint of fuel use and supply renewable energy. In addition, second-generation bio-ethanol made from agricultural residues and wastes is expected to avoid the competition for food and energy supply. However, biomass production as a raw material for bio-ethanol seems to be strongly related to land occupation and water consumption. In this study, the significance of land occupation and water consumption related to bio-ethanol from rice straw was analysed based on life cycle impact assessment. Carbon footprint of bio-ethanol is certainly smaller than that of gasoline. On the other hand, weighted impact assessment result indicates that the impact of land occupation (Net Primary Production loss) for rice straw production is dominant in the case of several countries (27–68% of total impact). The impact of water consumption (health damage due to the increase of infectious diseases and undernourishment) will vary from countries but significant effect can be found in the case of Thailand (58%). Footprint of single issue is very clear but results in misleading. Particularly, bio-products have close connection with land and water use. The result of this analysis shows the significance of both impact categories for assessing the availability of bio-ethanol and implies site-dependent conclusion of bio-ethanol availability.

MO 440

Environmental indicators for sustainable forestry and forest industry (EVERGREEN)

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The aim of the EVERGREEN project (2010-2013) is to increase environmental competitiveness of forest industry products and fibre-based raw material. Scientific-grounded solutions will be developed to evaluate and communicate the most relevant environmental indicators: biogenic carbon balance, water footprint, land use and biodiversity. Carbon footprint methodology is the most established indicator in the forest industry but still lacks the inclusion of biogenic carbon. The aim is to develop and test methods to attribute the forest management related impacts on biogenic carbon stocks for forest products with various life times by using LCA as a tool. Water footprint is under rapid development, but the existing methodologies do not meet the requirements for product specific assessment. This project will evaluate the existing methodologies and participate in method development in ISO 14046. The aim is also to develop a hydrologic model for the 'green water' in Boreal forests and discuss its relevancy.

Land use and biodiversity are gaining more interest but are to be defined more precisely from the forestry basis. The aim is to produce biodiversity indicators that are applicable throughout the supply chains of forest based industry. Focus is on indicators on species diversity and habitat diversity. Land use aspects related to forest biomass use are currently gaining more interest. The methodology is still open and up-to-date scientific views are needed to identify the land use related environmental aspects and to quantify the impacts. Focus is in resource depletion and soil quality impacts of forestry.

MO 441

Life cycle impact assessment (LCIA) method selection: an LCA practitioner's perspective

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LCIA phase in an LCA study aims at assessing the potential environmental impacts based on the life cycle inventory (LCI) and communicating them through a common commensurate for each impact category. There are different LCIA methodologies developed by various researchers leading to difficulty in selection during an LCA study. Moreover, for certain impact categories, these methods use different approaches to derive the characterization factors leading to inconsistency. Selection of an appropriate LCIA method by the LCA practitioner is thus, dependent on multiple factors. The major factors that influence this decision can be -

1. The goal and scope of the LCA Study
2. Type of intended communication - internal communication, business to business communication or business to consumer communication
3. Impact categories covered in the methodology
4. Pollutant emissions obtained from LCI versus the characterization factors available in the LCIA methodology

The paper tries to explore, with examples, the influence of these on decision making of an LCA practitioner. The paper also tries to explore some of the gaps that are usually overlooked in the process.

MO 442

Comprehensive life cycle assessment for cheese and whey products in U.S.

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A comprehensive life cycle assessment (LCA) has been carried out to account for the entire supply chain of cheddar and mozzarella cheese consumption in the United States. This includes specifically product loss at various stages of the supply chain, as well as consumer transport and storage of products. SimaPro 7.3 (Pre Consultants, The Netherlands) was used as the primary modeling software. The EcoInvent database provided information on the "upstream" burdens associated with materials like fuels and refrigerants. Data from the surveys and other U.S. specific information was incorporated into the model to the extent that it was available. Greenhouse gas (GHG) emissions are of significant interest, and on a milk solids (dry) basis the carbon footprint of cheddar and mozzarella are approximately 12.3 and 11.8 metric tons CO₂e per metric ton of cheese consumed, respectively. The 95% confidence band ranges 8.1 to 18.2 metric tons CO₂e per metric ton of cheddar consumed, and 7.9 to 17.2 metric tons of CO₂e per metric ton of mozzarella cheese consumed. For an average moisture content of 35.0% for cheddar as sold at retail, the carbon footprint is 8.0 kg CO₂e per kg cheddar cheese consumed with a confidence band of 5.3 to 11.8 kg CO₂e per kilogram cheddar consumed. On average moisture content of 42.5% for mozzarella as sold at retail, the carbon footprint is 6.8 kg CO₂e per kg mozzarella consumed, with a 95% confidence band of 4.5 to 9.9 kg CO₂e per kg mozzarella consumed.

MO 443

ReCiPe, USEtox, IMPACT 2002+, and TRACI 2 impact assessment methodologies in the context of fluid milk delivery systems LCA

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The purpose of the fluid milk delivery systems Life cycle assessment (LCA) was to provide results and interpretation of the life cycle impact assessment (LCIA) for the U.S. dairy industries that would allow them to engage in more sustainable approaches and reduce their environmental impacts. In order to be able to interpret and communicate results in a transparent way LCIA method sensitivity analysis was performed. The selected methodologies were two midpoint USEtox, and TRACI 2 and two endpoint ReCiPe and IMPACT 2002+ methodologies. The impact category chosen was ecotoxicity or ecosystem. The results have shown differences in cradle-to-grave vs. farm gate-to-grave assessment. For example, in ecotoxicity USEtox midpoint category the largest impact was attributed to raw milk production (almost 100%), but in TRACI 2 only 50% was attributed to raw milk production and 40% to consumption LCA stage. Significant differences were also visible within farm gate-to-grave assessment where LCA stages show different relative contribution in total impact for example, in IMPACT 2002+ ecosystem quality category, 35% of impact is attributed to processing plant, 30% to consumption, 18% to raw milk transportation, 8% to distribution center, 6% to retail and 2% to container construction. Harmonizing LCIA methods will take some time. Meanwhile, sensitivity analysis on LCIA methods and ranking of methodologies by result and relevancy of that information for a studied product targeted audience appeared to be a simple technique in selecting the most appropriate LCIA method, without completely excluding the others. Although all the methodologies provided valuable results, in the context of this project and targeted audience, ReCiPe method has shown advantage by allowing direct identification of the "hotspots" on the level of dairy industry

processes e.g. pasteurization, refrigeration, container material construction etc. Nevertheless, other methods were kept in the framework of the sensitivity analysis. They gave a valuable insight of the drivers that might appear in the results and help in further refining of the interpretation of the result.

MO 444

IMPACT World +: a new global regionalized life cycle impact assessment method

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Most of the impacts modelled in life cycle impact assessment (LCIA) are regional or local. However, LCIA methodologies currently offer generic characterization factors (CFs) that do not account for the spatial variability of impacts. Some LCIA methodologies have partially addressed the issue of regionalization (IMPACT 2002+, ReCiPe, LIME, or LUCAS), but they only cover a specific region of the world. Characterizing life cycle inventories collected within a global economy using for example European CFs implies an underlying assumption that all the emissions occur in Europe or at least under European conditions, which is not necessarily a better assumption than applying global or generic CFs.

IMPACT World+ was developed out of the need to offer a regionalized methodology at a global scale, implementing state-of-the-art characterization modelling approaches developed since the publication of IMPACT 2002+ and LUCAS, and including uncertainty information encompassing both spatial variability and model uncertainty. This not only allows applying more environmentally relevant CFs, but also to regionally assess any geo-referenced emission, and ultimately to determine the uncertainty related to an unknown emission location by associating the corresponding geographical variability to each CF at a given geographical scale.

The novelties introduced in IMPACT World+ are numerous: USEtox model for toxic impacts and water use impacts are included for the first time in an LCIA method with consistent spatialized alternatives, major modelling improvements have been conducted in regards to land use with the inclusion of ecosystem services, acidification with an improved modelling of the atmospheric fate, resource use with the introduction of an extraction-consumption-competition approach, respiratory effects with new epidemiologically derived factors and finally, for eutrophication with a world model at 0.5 x 0.5 degrees.

IMPACT World+ is a new LCIA method which will increase both the relevance and the discriminating power of LCA by allowing to account for uncertainties and spatial variability. Many other improvements have been integrated into the methodology making it more environmentally relevant and scientifically robust.

MO 445

An open source software for Emergy calculation with life cycle inventory databases

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Emergy evaluation is a relatively novel environmental accounting method which has gained credit during the last decade, but is still affected by several drawbacks in its calculation procedures and in its general methodological background, which prevent it from being accepted by a wider community.

Emergy accounting could benefit from the use of existing Life Cycle Inventory (LCI) databases, which comprise hundreds of environmental interventions in thousands of common industrial processes. However, it requires the compliance with a set of algebraic rules that are completely different than those applied in Life Cycle Assessment (LCA) and this has hampered the implementation of emergy calculation through automatic routines in specific software tools.

This work presents an open source software specifically created for allowing the rigorous calculation (respecting all the algebra rules emergy accounting has to comply with) of the Emergy associated to each of the commodities involved in a given product's life cycle along with its related inventoried resources.

We formalized the problem in a matrix based structure which comes directly from the LCA framework and developed a variant of the well known *track summing* algorithm.

Two versions of the calculation routine were implemented: one in Scala (a general purpose programming language integrating features of object oriented and functional languages) and one in C++. The former is a multi-threaded breadth first search (BFS), the latter follows a depth first search (DFS) and is more efficient in terms of memory usage.

The beta version of the software has been tested on different case studies, the most complex of which involved a square matrix of 2154 rows. Besides the emergy of the studied product, the software also allows the calculation of some statistics related to the complexity of the whole network of involved processes.

For a toy problem involving only a 7 by 7 matrix the results were also validated using the software *Emsim*, a free share Emergy simulator able to work with lifecycle systems using a graph instead of a matrix. Differently than our software, *Emsim* does not allow a direct link to automatic calculation routines, since it requires the system's diagram to be drawn by the operator.

The software could be potentially used for a consistent emergy calculation of any product in a LCI database and therefore for a complete combination of LCA and Emergy perspectives in environmental assessment.

MO 446

Complementing ecological footprint with risk analysis for the ecodesign of footwear

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The ecological footprint (EF) was created in the early nineties and has been gaining in popularity ever since. As a pioneering footprint, its applications has expanded and now they are not limited to the regional or national level but include the evaluation of production processes and products. Nevertheless, as a land-based indicator it can hardly account for all of the environmental burdens that may occur during an industrial process or the development of a business activity. Therefore, it is advisable its combined application with complementary environmental evaluation tools.

In this respect, this work proposes the joint application of EF and environmental risk assessment (ERA) to evaluate footwear design proposals from an environmental point of view. On the one hand, the EF component approach based on life cycle inventory was employed to appraise the environmental impact of the shoe-making process. Thus, individual EFs were calculated for each input/output material and energy flow in the inventory data and then aggregated to estimate the total EF of the pair of shoes. On the other hand, the ERA is a standardized process for the estimation of the magnitude, probability and uncertainty of adverse effects on health derived from the exposure to substances present in the environment. It was used to estimate the risk derived from the exposure to certain hazardous substances that raw materials may contain (as some organic compounds or heavy metals) and that would affect the final consumers of the product. Hence, the hazard quotient (HQ) and cancer risk (CR) were determined as additional indicators to EF.

Four models of children shoes were evaluated under the viewpoint of EF and ERA and compared. The synthetic shoes obtained a smaller EF (6.5 gm²) when compared to the leather shoes (11.1 gm²). However, high concentrations of hazardous substances were detected in the former, even making the HQ and CR exceed the recommended safety limits for one of the synthetic models analysed. Risk criteria were prioritized in this case and, consequently, the design proposal was discarded. For the other cases, the perspective provided by the indicators of different nature was balanced to accomplish a fairest evaluation.

The selection of fibres produced under sustainable criteria and the reduction of the materials consumption was recommended, since the area requirements would be minimized and the absence of hazardous compounds would ensure safety conditions during the use stage.

RA11P - Guidance documents and guidelines for environmental risk assessment (ERA): needs, developments and progress

MO 449

Mollusc reproductive toxicity tests - Development and validation of test guidelines

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The Organisation for Economic Cooperation and Development is promoting the development and validation of mollusc toxicity tests within its test guidelines programme, eventually aiming for the standardization of mollusc apical toxicity tests. Through collaborative work between academia, industry and stakeholders, this study aims to develop innovative partial life-cycle tests on the reproduction of the freshwater gastropods *Potamopyrgus antipodarum* and *Lymnaea stagnalis*, which are relevant candidate species for the standardization of mollusc apical toxicity tests assessing reprotoxic effects of chemicals.

Draft standard operating procedures (SOPs) have been designed based upon literature and expert knowledge from project partners. Pre-validation studies have been implemented to validate the proposed test conditions and identify issues in performing the SOPs and analyzing test results. Pre-validation work for *P. antipodarum* consisted in a comparative 28-day study between four laboratories and with two test substances: cadmium (Cd) and bisphenol A (BPA). For *L. stagnalis*, pre-validation consisted in a comparative 56-day study between six laboratories and with two test substances: Cd and tributyltin (TBT). Cumulated fecundity per individual over the test period was

the main test endpoint. Tested animals came from a single laboratory culture (i.e. the University of Frankfurt for *P. antipodarum* and INRA for *L. stagnalis*). Snails were acclimated in partner laboratories and exhibited homogenous age and size at the beginning of the tests. All laboratories found a concentration-dependent decrease of fecundity in both species under Cd exposure. Values of NOECs and LOECs were generally homogenous among laboratories. Results of the pre-validation studies with *P. antipodarum* and *L. stagnalis* exposed to Cd are thus very encouraging. Results for BPA highlighted issues in controlling exposure concentrations, and possibly BPA leaching from test equipment. This study also highlighted that factors, such as e.g., snail origin, temperature and test chemical stability in water, must be controlled in mollusc toxicity tests to avoid data dispersion which may impede the interpretation of effects. Applicability and limitations of the SOP proposed for *L. stagnalis* will be assessed after completion of the on-going pre-validation work. In both cases, optimization of experimental conditions and test procedures is required before the SOPs are ready to be proposed as OECD test guidelines.

MO 450

Temperature effects on reproduction in *Potamopyrgus antipodarum* - optimizing a standard test protocol with molluscs

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Aquatic ecosystems are exposed to a multitude of chemicals via surface runoff, landfill sites or sewage treatment plants. Many of these substances may affect growth, development and/or reproduction of aquatic species and therefore endanger the survival of populations. Standardized and internationally accepted OECD guidelines for the testing of chemicals with invertebrates currently include tests with arthropods and annelids but exclude molluscs, which represent the second largest clade. The German Federal Environment Agency (UBA) and the Department for Environment, Food and Rural Affairs of the United Kingdom have supported the preparation of a *Detailed Review Paper on Molluscs Life-cycle toxicity testing* [1] and a pre-validation round-robin with the parthenogenetic mudsnail *Potamopyrgus antipodarum* as standard test organism. Currently, the project aims to optimize and define test and breeding conditions to minimize variations of test results in preparation of an international validation exercise.

In this study we investigate reproduction variations at the proposed standard temperature (16°C) and two lower temperatures throughout the year. Therefore, we acclimatized *P. antipodarum* at 10, 13 and 16°C and compared reproduction (embryo numbers in the brood pouch). Because test temperature and natural seasonal reproduction patterns can modulate reproductive responses towards a chemical and even mask effects, we assessed sensitivity variations caused by these factors in reproduction tests with the endocrine disruptor octylphenol. To this end *P. antipodarum* was exposed to 1, 3, 10, 30 and 100 µg/L octylphenol at each temperature scenario and at different times of the year and the resulting effect concentrations were compared.

[1] OECD. 2010. Detailed review paper on molluscs life-cycle toxicity testing. ENV/JM/MONO(2010)9, Paris, France.

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MO 451

How breeding conditions influence substance effects - the need for a standard test protocol with molluscs

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Up to now effect studies with molluscs play a minor role in regulatory risk assessment of substances because OECD guidelines for the testing of chemicals have not considered this phylum. The German Federal Environment Agency and the Department for Environment, Food and Rural Affairs of the United Kingdom supported the preparation of a Detailed Review Paper (DRP) on Molluscs Life-cycle Toxicity Testing proposing inter alia the parthenogenetic mud snail *Potamopyrgus antipodarum* as standard test organism [1]. To investigate the impact of breeding and test conditions on the outcome of reproduction tests with *P. antipodarum* we bred specimens in different media and at varying densities or temperatures.

In the "medium" experiment, snails were kept in 800 mL DRP-[1], ISO- or Elendt M4-[2] medium for 12 weeks. Reproduction was assessed after 4, 8 and 12 weeks by the number of embryos in the brood pouch. For the density experiment, reproduction of snails kept in groups of 5, 10, 20, 40, 80 or 160 specimens per 200 mL DRP medium was investigated after 4 weeks. Subsequently, snails from each density group were exposed to 12 µg cadmium/L (CAS: 67-68-5) without further density stress for 4 weeks. In the temperature experiment, snails were acclimated at 7 and 25°C for 4 weeks. Then they were additionally exposed to 5, 10, 20 or 40 µg bisphenol A/L (CAS: 80-5-7) at 7, 16 and 25°C. Reproduction was assessed after 4 weeks.

The use of different breeding media resulted in significantly varying embryo numbers of *P. antipodarum* between all test media after 4 and 8 but not after 12 weeks. Varying snail densities affected reproduction significantly. Furthermore, differing snail densities before the start of the cadmium exposure had a significant impact on the sensitivity towards the heavy metal: the sensitivity increased with density stress in the pre-exposure period. At 7°C and 25°C, a significant increase of reproduction was already observed for BPA exposed snails at 10 µg/L and thus at lower concentrations compared to the standard temperature (16°C) [1] where a significant effect occurred only at 40 µg/L. This confirms temperature as a trigger for reproductive output.

In summary, different breeding and test conditions like snail density or temperature affect the sensitivity towards chemical exposure of *P. antipodarum* in subsequent reproduction tests. To gain reproducible test results with low variability among laboratories, the definition of standard test conditions is essential.

MO 452

A partial life-cycle toxicity test to assess the effects of endocrine disruptors in the great pond snail *Lymnaea stagnalis*

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Molluscs are highly sensitive to endocrine disrupting compounds (EDCs) and therefore are of great interest for environmental hazard and risk assessment. The Organisation for Economic Co-operation and Development (OECD) is thus promoting optimization and validation of mollusc-based toxicity tests. Standardised test methods for assessing effects of prototoxicants, and hence a number of EDCs, are currently under development in various mollusc species, including the great pond snail *Lymnaea stagnalis*. Based upon this on-going work, we designed and performed a partial life-cycle toxicity test in which adults of *L. stagnalis* were exposed to two androgenic compounds (triphenyltin and 17 α -methyltestosterone), and two estrogenic compounds (nonylphenol and 17 α -ethinylestradiol). This test consisted in two parts. In the first part, snails were exposed to the toxicants during 56 days in semi-static conditions, as proposed for the OECD test under development. Survival, growth, and fecundity were regularly monitored. Egg-clutches produced in this part of the test were collected and exposed to clean test water during 35 days, which represented the second part of the test. The biological quality of offspring was assessed through the estimation of the frequency of abnormal eggs in clutches, their mean hatching rates, and the mean shell length and dry weight of hatchlings. Data were analyzed using standard statistical approaches, as recommended by OECD for the analysis of toxicity test data. Preliminary results generated over the first week indicated that exposure of adult snails to the higher tested concentrations of triphenyltin (nominal concentrations of 0.75 and 2.60 µg/L) and nonylphenol (500 µg/L) led to a decrease in food consumption and also to a decrease in growth and reproductive output. This study will allow to further examine whether or not a 56-day partial life-cycle test in adults of *L. stagnalis* is sensitive enough to highlight the reproductive effects of the selected EDCs, or if the monitoring of offspring performances is also required to significantly detect those effects. Obtained results will thus contribute to the process of optimizing the sensitivity of toxicity tests based upon *L. stagnalis* for use in environmental hazard and risk assessment.

MO 453

Environmental risk assessment of a disinfectant for milk extraction systems: comparison of 2 emission scenario documents and EUSES

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The environmental risk assessment of a disinfectant for milk extraction systems was performed with 1) EUSES 2.1 based on local consumption estimates extrapolated from EU/regional tonnage and 2) two Emission Scenario Documents for Biocides (ESD), published in 2002 and 2011, based on local consumption estimates from a dairy farm. The ESD of 2002 describes only emission to manure followed by application to agricultural soil, whereas the ESD of 2011 only describes emission to sewage. Moreover in the ESD of 2011, degradation is not taken into account in Tier 1, whereas in the ESD of 2002 a formula for degradation was already included. Calculations were made for 100% emission to sewage as a worst-case scenario. Scenario 2.5.1 of EUSES (Medical, disinfectants in accommodations) was selected from the exposure scenarios of biocides on a local scale, because there is currently no scenario for PT3-4 biocides evaluation included in EUSES and because scenario 2.5.1 resembled most the PT3-4 use. With the ESD of 2002, the RCRs were all below 1, whereas with the ESD of 2011, the RCR was < 1 for one active substance, but > 1 for the other substance and a refinement of the calculation was required. PEC values obtained with the Tier 1 ESD 2011 calculations were about 10 times higher than those obtained with the ESD of 2002 and about 100 times higher than with EUSES. This can be explained by the fact that the ESD of 2011 does not take into account degradation. An advantage of the ESD of 2011 is that the scenario is more consistent with current practices and that EUSES could be applied in replacement of the ESD. A more accurate environmental risk assessment would be possible with EUSES when a PT3-4 scenario becomes available.

MO 454

Can we assess environmental risks of antifouling paint usage with the prevailing BPD?

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Since many antifouling paints are designed to leach toxic biocides, it is essential to evaluate, prior to use, their effects on aquatic ecosystems, including toxicity to aquatic organisms. This evaluation process, known as environmental risk assessment (ERA), is used during the authorization of anti-fouling coatings and is regulated in the European Union (EU) under the biocidal product directive (BPD). In the ERA, the predicted environmental concentration (PEC) of biocides, due to leaching from the coating, is determined using the modeling tool MAMPEC. In this model, default scenarios representing an EU harbor and an EU marina is incorporated in the software and used in the process of deriving a PEC-value of biocides. However, there may be other sources of biocides to these marine systems, sources that have been largely overlooked in ERA of antifouling formulations. Removal of fouling by pressure hosing usually occurs close to the marina, e.g. at a slipway, when the boat is lifted out of the water after the season. The fouling that is still present on the hull after pressure hosing is typically being scraped off at the boat yard. In this process also antifouling paint particles (APP) will be removed and deposited on the ground. These cleaning activities are not regulated to any great extent, i.e. leisure boat owners are not obligated to collect the waste and the removed APP. Hence, the boat yard may act as a substantial source of contaminants to the recipient (i.e. the marina) as precipitation infiltrates the soil resulting in leaching

and transportation of biocides. The aim of the current study was to determine the leaching of biocides from a boat yard allocated in Stockholm archipelago. We designed the experimental part to quantify the annual leaching of biocides from the contaminated soil to the marina. The data were subsequently used in a full-scale ERA of antifouling paint usage by determining the contribution of different biocidal sources to a marina i.e., from boat hulls, pressure hosing and from the soil at the boat yard. The results showed the biocidal release from pressure hosing and soil to be a substantial source of biocides to the marina. These findings suggest that a PEC-value based on only the biocidal release from boat hulls to be inadequate in predicting environmental risks of antifouling paint usage.

MO 455

Ready biodegradability testing for Insoluble and volatile substances

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Under REACH a PBT/vPvB assessment is required for substances that are manufactured or imported in amounts of > 10 tons per year. The first step of persistency assessments is a screening like the tests on ready and inherent biodegradation provide. Often substances fail the criterion for ready biodegradability or an unequivocal assessment is not possible because the substance is not bioavailable for the degrading bacteria due to its low water solubility and/or volatility. Careful consideration of the physico-chemical properties of a substance is therefore necessary to choose the appropriate test design. As the biodegradation of a substance strongly depends on its mass transfer and the bioavailability for the degrading bacteria, there are several technical issues which need to be addressed while testing the biodegradability of poorly water soluble and/or volatile substances. The OECD guidelines for testing ready biodegradation comprise seven different tests (OECD301A-F, OECD310), which differ mainly in the vessel size, the ratio test solution/headspace, the agitation and aeration during testing and the type of analysis. Based on the substance properties the test type has to be chosen carefully e.g. the use of the aeration or agitation method can result in losses of the substance and underestimated biodegradation. The influence of the test type on the biodegradation of insoluble and volatile substances was investigated and the results will be presented.

MO 456

OECD Guideline 209: investigation of validity criteria

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The revision of the OECD guideline for the Activated Sludge Respiration Inhibition Test (ASRIT) was finalized in 2010. Within the revision the validity criteria were changed significantly and a new criterion was included. The specific oxygen uptake rate of the activated sludge should not be less than 20 mg oxygen per one gram of activated sludge (dry weight of suspended solids) in an hour. As the oxygen uptake rate of active sludge from many municipal treatment plants shows fluctuations and periodically decreases below 20 mg O₂/(g[GREEKX]h), the significance of the validity criterion was examined. It is assumed, that parameter like season, sewage flow and weather conditions influence the oxygen uptake rate. To confirm the assumption, reference tests were conducted over several months and the activated sludge was collected at different days of the week. The dependence of the oxygen uptake rate on the assumed parameter was investigated. Furthermore the correlation between the oxygen uptake rate and the sensitivity of the activated sludge was examined. The results of these investigations will be presented.

MO 457

Development of interim guidance for the inclusion of non-extractable residues (NER) in the risk assessment of chemicals

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Bound residues (BR), including non-extractable residues (NER), are an important factor in PBT (Persistence, bioaccumulation and toxicity) assessment and risk assessment of chemicals. Precautionary risk assessments usually assume 100% bioavailability, i.e. all of the chemical present, is available for degradation or to have potential toxic effects on the biota. This precautionary approach generally overestimates the exposure concentration by the amount that is not available and therefore overestimates the level of risk to biota in the environment. It is also well documented that chemicals that are irreversibly bound to solids are less degradable and less toxic than the total residue would predict. Currently, there is no agreed guidance on how to determine what is available or not, and how it should be considered in the risk assessment.

An ECETOC workshop "Significance of Bound Residues in Environmental Risk Assessment" was held on 14-15 October 2009 in Brussels, and produced a framework outlining a possible decision tree for improving the risk assessment of NER, together with the identification of key research needs to address gaps in the current knowledge base.

Subsequently, an ECETOC Taskforce was set up to i) critically evaluate the proposed risk assessment framework developed following the ECETOC workshop and assess its utility as an interim approach for regulatory assessment of chemicals; ii) develop suitable guidance and trigger values to enable the decision tree to be used and test the utility of the scheme using suitable case studies; and, iii) Provide guidance on study design to provide the appropriate quality of data needed for the risk assessment framework to function within a regulatory decision making system.

The progress of this taskforce will be presented, along with recommendations for addressing Bound/non-extractable residues. An example of a risk assessment using this approach will be presented.

MO 458

Granivorous birds - exposure reduction by de-husking behaviour

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With the publication of the current EFSA Guidance document on bird and mammal risk assessment, exposure of granivorous birds to treated seeds has received increased attention. Seed-eaters are considered no longer as indicator species only for seed treatment uses, but also regarding foliage spray applications resulting in exposure through contaminated weed seeds - here small granivorous bird species need to be evaluated as well.

For granivorous mammals several studies have been conducted and data is published demonstrating that de-husking i.e. removing of the husk (including large portions of residues) prior to ingestion, can significantly reduce the exposure in crop seeds (e.g. Brühl et al. 2010, DEFRA research report 2010). For birds it is long and well known that several behavioral skills and traits are common across different bird families to remove the husk from seeds (e.g. Ziswiler 1965).

One method to quantify the reduction of such exposure routes is to conduct pen studies, i.e. offering treated seeds to caged individuals and measuring the amount of residues (or marker substance) in the remaining seed husks. Such approaches and test designs are feasible for crop and weed seeds.

Since small granivorous birds are often considered as worst-case indicator species (due to their low body weight) their theoretical exposure is relatively high in default risk assessments. Based on the de-husking behaviour of birds (e.g. finches, sparrows and buntings) we will present data and study design proposals on how to use this behavioural trait as refinement tool for de-husking species in higher tier wildlife risk assessments.

MO 459

ECETOC Guidance on identifying endocrine disrupting effects: specificity for environmental species

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The first ECETOC technical report and associated workshop presented a science-based concept on how to identify endocrine disrupting properties of chemicals for both human health and the environment. To be able to discriminate chemicals with endocrine disrupting properties of low concern from those of high concern (for regulatory purposes), it was recognized that the concept needed further refinement, including specificity. For environmental assessments the lead toxic effect can be considered within a study/species or between different studies/species within an environmental compartment.

Within a study an endocrine mediated endpoint can be affected below, at the same, or above the threshold of general toxicity. This should be considered in the first part of the original ECETOC assessment, when linking mechanistic and apical in vivo studies to decide whether a substance is a potential endocrine concern or not.

A substance should only be considered of high concern when the endocrine mediated effect occurs at concentrations lower than those that cause other significant toxicity.

In the case where the endocrine mediated effect is not the lead effect, i.e. the endocrine effect is observed at a higher concentration than that causing other toxicity, then the substance should then proceed with a risk assessment based on the most sensitive (non-endocrine) lead effect.

Once a population-relevant endocrine effect has been confirmed, the next step in the environmental evaluation is to consider the specificity across taxa. Although endocrine effects may be observed as the most sensitive (=lead) effect within one study or organism, this effect may be accounted for in a risk assessment by more sensitive non-endocrine endpoints observed in other taxonomic groups. Risk assessment then allows for a margin of safety that sufficiently covers endocrine specific effects. Any risk management measures based on the lead toxic effect in one taxon will also be protective of other toxic effects (including endocrine effects) in other taxa. Such substances should not be considered as endocrine disruptors, since they do not cause adverse endocrine effects in non target species under realistic conditions of use.

MO 460

Concentration-response relationship for teratogenic effect of 17 β -oestradiol in eelpout *Zoarces viviparus*

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In recent years increased frequencies of malformations among eelpout embryos have been detected in monitoring programs in several Baltic countries (1,2,3). Malformations can be induced in oviparous fish species by exposure to chemicals, including endocrine disrupting substances, in the laboratory. Eelpouts are the only viviparous fish in Northern Europe, which makes them very suitable for investigation of mother-offspring interactions and effects in the offspring upon maternal exposure to various chemicals. The causative agent or agents in underlying the malformations observed in the monitoring programmes are not known, but malformations upon exposure of pregnant eelpout

to octylphenol and 17 β -oestradiol (E2) have been observed (4).

To investigate the concentration-response relationship for malformations in eelpout embryos induced by E2, we investigated the embryonic development upon exposure of pregnant eelpouts to (E2) at nominal concentrations between 12.5 and 500 ng E2/l. The experiment was terminated after 40-44 days of exposure. The various types of abnormal development among the embryos (early death, malformations of eyes and spinal cord) were evaluated and the frequencies were recorded for each experimental group.

Exposure of female eelpouts to environmentally realistic concentrations, 132,7 ng/l, of 17 β -oestradiol early in the pregnancy increases the abundance of malformations in the embryos. The study further showed that delaying the onset of exposure to the highest dose of E2 by approximately three weeks reduced the frequency of abnormal larvae, suggesting that timing of the exposure is crucial.

(1) Gercken J, Forlin L, Andersson J. 2006. Developmental disorders in larvae of eelpout (*Zoarces viviparus*) from German and Swedish Baltic coastal waters. *Mar. Pollut. Bull.* 53, 497-507.

(2) Strand J, Bossi R, Dahllof I, Jensen CA, Simonsen V, Tairova Z, Tomkiewicz J. 2009. Dioxin og biologisk effektmonitoring i ålekvalbe i kystnære danske farvande. Report from the National Environmental Research laboratory.

(3) Hedman JE, Rüdel H, Gercken J, Gergek S, Strand J, Quack M, Appelberg M, Förlin L, Tuvikene A, Bignert A. 2011. Eelpout (*Zoarces viviparus*) in marine environmental monitoring. *Mar. Pollut. Bull.* 62: 2015-2029.

(4) Brande-Lavridsen N, Nielsen RV, Rasmussen TH, Korsgaard B, Bjerregaard P. In prep. A novel type of endocrine disrupting effect: Octylphenol and 17 β -estradiol cause abnormal development in embryos of the viviparous eelpout *Zoarces viviparus*

MO 461

Use of field studies in different regulatory frameworks

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The poster discusses the different roles of field studies in the different regulatory frameworks. In the tiered approach followed in the registration procedure of plant protection products (PPP's), field studies are frequently used in the higher tier. These higher tier studies are used to generate additional information, in order to lower the safety factors of the lower tiers, and to show recovery after an initial effect. For a number of field study types (e.g. mesocosm studies) detailed guidance is available, as well as guidance for the assessment of the studies. In most other regulatory frameworks (e.g. biocides, pharmaceuticals, GMO's, REACH) the role of field studies is less clear. At the most limited guidance is available, that is not very specific. The poster discusses the possibilities and the impossibilities to use the guidance for field studies from one framework for another framework.

In some cases the same active substance is assessed in different frameworks (e.g. PPP's and biocides). In this perspective a number of initiatives have been taken to study whether and how field studies, designed and conducted for PPP registration can be used for biocide registration. As an example the use and assessment of mesocosm studies in PPP and biocide registration is elaborated.

MO 462

Experimental design and statistical analysis of ecotoxicological community studies

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Ecotoxicological field studies may be conceived by industry notifiers in association with external consultants and contract research organisations (CROs). The results may then be interpreted and summarised before inclusion in a regulatory submission in support of a product registration. The submission will be reviewed by representatives from Member State (MS) Competent Authorities, in terms of both study quality and meaning. Not all involved parties may be experts in the conduct and interpretation of such studies. There is a need, therefore, for guidance on both ecotoxicological field study experimental set-up and statistical analysis of ecotoxicological community data - from aquatic mesocosms (invertebrates, algae and/or higher plants), surface/foliar-dwelling non-target arthropod (NTA) field studies and full-field soil mesofauna studies.

Firstly, this project seeks to provide guidance on experimental set up of ecotoxicological community studies from a statistical point of view, such as replication, plot size, study duration, randomisation. Secondly, the project will provide guidance on the statistical analysis of ecotoxicological community effect data. This will include use and interpretation of both univariate and multivariate methods. The project will summarise existing guidance and use examples available in the literature to illustrate key points. This work is funded by the UK Chemicals Regulation Directorate.

MO 463

Development of a framework for toxicological and ecotoxicological risk assessment of fertilisers, soil improvers and related products

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The use of fertilisers, soil improvers and related products may entail toxicological and ecotoxicological risks through the presence of specific chemical substances and/or pathogens. In Belgium, there is currently no real technical guidance on how to conduct a toxicological and ecotoxicological risk assessment of fertilisers, soil improvers and related products. Therefore, an evaluation is made of existing EU methods for toxicological and ecotoxicological risk assessment in similar domains (REACH, plant protection products, biocides, etc[3DOTS]) and of existing national approaches of risk assessment of fertilisers, soil improvers and related products in other EU member states. The application and relevancy of the identified relevant methods is further evaluated and tested in case studies for specific products. The results demonstrate that screening methods, identifying the most critical human health and environmental endpoints based on the hazards of the constituents, improve the usability and applicability of the selected risk assessment methods.

MO 464

Chemical risk assessment under chemical substances control law in Japan

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The purpose of Chemical Substances Control Law (CSCL) is to evaluate, before manufacture or import, whether or not new chemical substances have properties such as persistence, and to implement necessary regulations, in order to prevent environmental pollution caused by chemical substances that pose a risk of impairing human health or interfering with the inhabitation and/or growth of flora and fauna. To minimize the significant adverse effect of chemical substances on human health and environment by 2020 (Agreement in the Environmental

Summit in 2002), CSCL has been amended in 2009 and implemented in 2011. Under amended CSCL, risk assessment will be conducted in step-wise manner for all chemicals including existing chemicals. In this presentation, we will explain chemical risk assessment scheme and approach under amended CSCL and comparing it with other regulatory schemes.

MO 465

Status quo and challenges in the EU scheme of environmental risk assessment for nanomaterials

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Although high potential benefits of engineered nanomaterials (NMs) for overcoming the technological limitations of conventional materials, the unique properties of NMs raise new emergent challenges in understanding, predicting, and managing potential risk to environment. For establishing a standard risk assessment of NMs, different regulatory frameworks have been suggested in recent years in various fields of chemicals, cosmetics, foods, environmental safety and waste in the EU.

As a representative regulation for controlling the potential risk of NMs manufactured or imported in the EU, REACH (Registration, Evaluation, Authorisation, and Restriction of Chemicals) regulation controls the use of NMs. In order to address the specific properties, hazards and risks associated with NMs, additional testing or information is required. Nevertheless, specific REACH guidance for NMs is not readily available. For develop the draft technical guidelines for risk assessment of NMs, the REACH implementation Projects on NMs (RIP-oNs) have recently been conducted by the EU Commission. However, the draft guidelines are still placed on the unsatisfactory level to reach a consensus on the risk assessment scheme of NMs in accordance with regulatory purposes.

The objectives of this study are to analyse and map the status quo and future research challenges in the EU Nano risk assessment scheme based on the RIP-oNs and related recent studies with focusing environmental risk assessment (ERA). The major challenges in regulating NMs are identified as the wide variety of NMs and applications, the limited knowledge on the toxicity of NMs in living system and their transport in living and environmental systems, the lack of harmonized standards or guidance, and the issues related to classification of NMs.

MO 466

Is European chemical legislation suitable for regulating engineered nanoparticles?

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The nanotechnology industry is rapidly growing, and the production and use of engineered nanoparticles (ENP) is already a billion euro industry. ENPs are used in a wide range of applications (e.g. consumer goods and plant protection products) which will inevitably result in exposure to the environment. The current study reviews three major European chemical legislative instruments (REACH, the Water Framework Directive and the Cosmetics Regulation) in order to evaluate whether the risk assessments under these instruments are suitable for ENPs or whether nano-specific adaptations to these frameworks should be recommended. REACH and the Cosmetic Regulation were chosen since they regulate some of the most important uses of ENPs (e.g. cosmetics and industrial applications). The Water Framework Directive aims at protecting aquatic environments in the European Union, and is thus essential in regard to environmental risk assessments. The criteria for this evaluation are based on the substantial amount of experimental data that have been generated in the EU funded NanoReTox project (insert project number here). Within the project we have studied the fate and toxicity, to both humans and the environment, of selected metal ENPs. The empirical approach allows us to evaluate the risk assessment frameworks with regard to very specific criteria and thus gives the analysis another dimension compared to an analysis that would have been based solely on theoretical considerations.

MO 467

PBT-Assessment: the need for an agreed guidance across regulations - assessment of persistence as an example

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The PBT-assessment has been established across the various European substance regulations i.e. plant protection products, biocides, pharmaceuticals and REACH but references between regulations are sometimes lacking. For the three criteria P (persistence), B (bioaccumulation) and T (toxicity), the trigger values are consistent among the mentioned regulations, but the accompanying guidance differs and does not always give sufficiently detailed information. Also, data availability and risk assessment procedures differ across regulations which will influence the outcome of a PBT-assessment. This may be especially problematic for substances registered under more than one regulation, e.g. an active substance that is used both as a plant protection product and a biocide or a veterinary pharmaceutical. Therefore a consolidated guidance across regulations is needed to ensure the identification of PBT-substances on a common basis.

In our experience at the German Federal Environment Agency persistence is the criterion where results of a PBT-Assessment potentially differ most across the different substance regulations, and hence, experts from these regulations across the EU should consult most urgently. In this poster, we present the current situation and introduce our proposal for a harmonized persistence assessment to overcome this situation.

MO 468

Stakeholders' view on ecological risk assessment in the EU: what are we trying to protect and why might we be failing?

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The EC Regulation 1107/2009 states that "A plant protection product ([3DOTS]) shall have no unacceptable effects on the environment, having particular regard to ([3DOTS]) its impact on non-target species, including on the ongoing behaviour of those species". General goals for protection are thus well established in the legislation, whereas specific protection goals are less well defined. Still, accurate protection goals are vital in scientific risk assessment process. Therefore, we asked stakeholders (both practitioners and theorists) involved with ecological risk assessment (ERA) about the challenges they meet in the current ERA scheme and the way they perceive recent changes in guidance documents and legislation.

We conducted 43, semi-structured, confidential interviews with representatives of plant protection product industry, regulatory bodies and academic ecotoxicologists and ecologists. The topics were related to the current protection goals (including introduction of the ecosystem services concept), the strengths and weaknesses of the current ERA and the changes in regulations. We employed the key informant approach in recruiting our participants. They were first identified as key stakeholders in ecological risk assessment of pesticides and then sampled by means of a snowball sampling.

Our preliminary results show that participants, in general, find the current regulations (EC 1107/2009) too vague and the protection goals unclear. They put much hope in the ecosystem services concept, while on the other hand, many participants were concerned that the framework is overcomplicated and adds extra bureaucracy to the procedure instead of making it more practical. There is also much concern about the ecosystem services which cannot be directly quantified, especially from risk managers.

It seems promising that all three stakeholder groups have a similar view on the ERA scheme, although there were some differences with regard to the level of complexity and protection provided by the current ERA. Stakeholders were concerned whether the increasing number of procedures directly translates into a better assessment of risk. Main hopes associated with changes in regulations were to address issues that are not clear at the moment, such as the mixture toxicity problem or implementation of ecological models.

MO 469

Environmental assessment regulations for active pharmaceutical ingredients: a Canadian contrast

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In Canada, the Canadian Environmental Protection Act (CEPA) 1999 requires that all new substances for use in Canada be evaluated for their potential risks to the Canadian environment and human health while the Food and Drugs Act regulates the sale of human and veterinary drugs. Health Canada, in consultation with representatives from industry, non-governmental organizations and consumer groups has developed environmental assessment frameworks for new substances in products regulated by the Food and Drugs Act. One framework is specific for active pharmaceutical ingredients (APIs) in human and veterinary drugs and has been designed to align with the drug development/approval process. When developing this framework, the starting premise was that the regulatory requirements must be science-based and in proportion to potential for risk. Furthermore the approach taken was that any new framework should leverage the work done for environmental assessment guidelines in other jurisdictions internationally and harmonize where feasible. This talk will explore similarities and differences between the proposed Canadian Environmental Assessment Regulations and requirements in other jurisdictions with an emphasis on issues that are distinct to the Canadian context.

MO 470

Mitigation measures for surface and groundwater based on risk assessment of Plant Protection Products in France: from the risk assessment to the stakeholders

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ANSES provides opinions prior to Plant Protection Products (PPP) registration. All opinions are amended with the specific Standard Phrases for safety precautions related to the environment (SPe) in accordance with regulation (EC) No 1107/2009. SPe are based on the risk assessment for the active substance(s) and their metabolite(s). This work focuses on Standard Phrases for safety precautions related to the environment (SPe 1 to 3), that were purposely designed to protect groundwater; and/or aquatic organisms. For the different uses assess all PPP may then be labeled with the following phrases, which should be supplemented with specific recommendation regarding the time period or frequency of application (SPe1); and/or specific soil type or situation (SPe2) and/or specific unsprayed buffer zone (SPe3). Since these Standard Phrases for safety precautions do focus on potential risks accounting for both the exposure and the toxicity for various uses of the PPP they are supposed to be suitable indicators since they summarize the outcome of the environmental fate and/or ecotoxicological risk assessments and thus the main outcome for PPP. The likening between PPP (active substance and metabolites) and specific SPe allow to identify if correlation between a specific waterbody (ie surface water and/or groundwater) and a PPP type (e.g. herbicide, fungicide insecticide) may be set up or not. In addition, SPe data collection at following connections between PPP properties will be used to create a "comprehensive environmental package" reflecting the main conclusions of the risk assessment conclusions to stakeholders like national water agencies which would like to implement specific groundwater and/or surface water monitoring; or national agencies in charge of aquatic environment protection by inspecting use and enforcing regulations for instance.

MO 471

At which level is an additional factor for EPM within the risk assessment of the benthic environment justified?

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For the environmental risk assessment data for aquatic organisms are in most cases available; data for sediment organisms are often lacking. In this situation the PNECsed can be provisionally calculated using the equilibrium partitioning method (EPM), as described in the REACH guidance. The EPM is based on the assumption that both sediment and aquatic organisms are equally sensitive. Only the uptake via the water phase is considered, assuming that substance concentrations in sediment and interstitial water are in a thermodynamic equilibrium. However, notable uptake may also occur via ingestion of sediment or direct contact to sediment. Thus, according to the REACH guidance, the EPM method has to be applied in a modified way for compounds with an octanol/water partitioning coefficient (log Kow) > 5 (or with a corresponding binding behavior, e.g. ionisable substances). In these cases the uptake via ingestion of sediment should be taken into account by increasing the PECsed/PNECsed ratio by a factor of 10.

In this study we gathered information for data on sediment organisms as well as aquatic data and adsorption values from different sources, i.e. EU-RAR, BPD and REACH. From aquatic data and the distribution coefficient, sediment effect values were modeled using the EPM. Hereby we preferred the suspended matter-water partition coefficient Kpsusp as the log Kow is only an indirect descriptor for the adsorption on sediments.

The comparison of the two values revealed that the EPM will not necessarily underestimate the risk, even in cases when no additional factor is used. In most cases NOECsed und NOECsed-EPM are within the general variability of ecotoxicity test results and below the variability between different species. This is also true for surface active compounds with a high adsorption potential.

The results clearly demonstrate that for the risk by oral ingestion, the adsorption potential on sediment, the uptake efficiency in the GI tract has to be taken into account as well. For hydrophobic substances such as PCBs the log Kow > 5 is assumed to be sufficient as trigger, due to the fact that adsorption and uptake efficiency are both correlated to the log Kow. This log Kow > 5 is equivalent to a Kpsusp of > 1410 L/kg. For highly adsorptive, especially ionisable substances, however, a decision based exclusively on the adsorption is questionable.

MO 472

Implications of differences in higher tier studies for deriving threshold concentrations

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Within prospective risk assessment of PPPs, uncertainties are associated to a number of extrapolations needed from test-systems towards the ecosystem. These uncertainties are considered by applying assessment factors to derive a threshold concentration, below which unacceptable effects on natural communities are avoided. The respective procedures are standardized for a lower tier risk assessment, but they are not at the higher tier level because tools and techniques are diverse and substance-specific information need to be included. This can thus lead to differences in terms of endpoints delivered and data interpretation.

To identify magnitude and reasons for variability in endpoints and data interpretation, we review datasets from higher tier aquatic studies to assess if their analyses and interpretation in terms of uncertainties and extrapolations are consistent across the type of study (ex: SSD, micro- mesocosms) and could thus lead to similar acceptable threshold concentration in the field. This analysis will account for the variability between different classes of pesticides (insecticides, herbicides, fungicides), with various modes of action (ex: pyrethroids, neonicotinoids). It will be based on existing datasets and information from the scientific literature and regulatory world. The analysis aims at contributing to the further development of the EU Guidance Document of Aquatic Ecotoxicology.

MO 473

The adoption or adaptation of EU predicted No Effect concentrations for use as Canadian water quality guidelines

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The Canadian Council of Ministers of the Environment (CCME) is a major intergovernmental forum in Canada for discussion and joint action on environmental issues of national concern. The 14 member governments work as partners in developing nationally consistent environmental standards and practices.

The CCME Water Quality Task Group (WQTG) develops Canadian Water Quality Guidelines (CWQGs), which are derived according to nationally approved scientific protocols that describe data requirements and evaluation procedures.

CCME WQTG is considering adopting or adapting Predicted No-Effect Concentrations (PNECs) and Environmental Quality Standards (EQS) for freshwater and marine aquatic life that have been developed in the EU for use as CWQGs as a means of:

- benefiting from work conducted in the EU and avoiding duplication of effort,

- rapidly augmenting the existing suite of CWQGs; and,

- harmonizing CWQGs with international environmental quality benchmarks.

Work was undertaken to develop procedures and guidance for the evaluation of EU PNECs/EQS values as potential candidates for adoption/ adaption as CWQGs, and specifically included:

- i) identification of a candidate list of substances for which EU Risk Assessments under the Existing Substance Regulations and REACH or Water Framework Directive chronic aquatic PNECs or EQS had been derived and for which the detail of the derivation had been reported and was readily available,

- ii) evaluation of the test species used to derive the EU PNEC/EQS and a comparison with the species used to derive CWQGs, including the development of criteria for assessing the suitability of non-Canadian test species as potential surrogates for native Canadian species,

- iii) evaluation of the criteria used to assess the quality and reliability of study data used to derive EU PNEC/EQS, and a comparison with the criteria applied to study data used to derive CWQGs,

- iv) development of a procedure and guidance for evaluating EU PNECs as candidates for adoption as CWQGs.

This presentation will detail the outcomes of the work and describe the guidance and recommendations to CCME WQTG with respect to the evaluation of EU PNEC/EQS for their potential to be adopted as CWQGs.

This work was funded by, and conducted under the auspices of, the CCME WQTG, however, this presentation and the draft guidance report do not necessarily reflect the views of the CCME Water Quality Task Group.

MO 474

Information systems on environmental quality standards (EQS) and quality criteria (QC)

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Environmental Quality Standards for priority substances and national EQS need to be updated on a regular basis. Differences in data, methods and expert judgment have been a major source for a large variety of national EQS-values. Differences in data might be minimized by a targeted data exchange and evaluation. But this needs a targeted initiative by national authorities.

For pragmatic reasons, one first step in evaluation of existing EQS or the prioritization of monitoring data is the IT-search of existing EQS and studies published by other national authorities. Existing web pages from RIVM, *risicos van stoffen* (<http://www.rivm.nl/rvs/>), INERIS Chemical Substance Portal (<http://chimie.ineris.fr/en/index.php>), Swiss Centre for Applied Ecotoxicology, *Qualitätskriterien* (<http://www.oekotoxzentrum.ch/qualitaetskriterien>) or the UBA ETOX database (<http://webetox.uba.de/webETOX>), and others have many similarities, but there are also a lot of differences in handling and content. While OECD has harmonized templates for reporting chemical test summaries (OHT), similar formats does not exist for QC or QS to make data exchange and comparison easier.

Quality Criteria (QC) could be derived under different regulations, for different protected assets, matrixes, monitoring aspects, etc. therefore the values are better to analyse, if this background information is easily available. EQS set under (2008/105/EC) or national EQS normally don't provide this essential background information, which is necessary for a comparison, how the value is derived. A Comparison of EQS of Catchment Basin Specific Pollutants (CBSP) set for the demands of WFD (Arle 2011) showed an extreme difference between EQS for the same substance in a lot of cases. In future differences of EQS for CBSP might be minimized, because the derivation should follow TG-EQS (EC 2010). But reasons for the discrepancy in values can only be explained, if background documents exist. The aim of this study is to identify the necessary information which is needed for a better comparability of QS and QC.

Data bases are run by national authorities for their needs and users, the poster will give an overview of existing systems, and will make a proposal, which basic information's are needed to make values of QC (non-legal binding) and QS (legal binding) derived by different institutions better comparable. A first outline for minimum criteria for reporting and harmonization of exchange formats will be offered.

MO 475

Variability in Environmental Quality Standards - how much is there and what are the causes?

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Environmental Quality Standards (EQSs) define an acceptable concentration of chemical contaminants in waterbodies. Under the Water Framework Directive, EQSs for the most hazardous substances (Priority and Priority Hazardous Substances) are developed at EU level and Member States (MS) must put in place steps to ensure they are not breached. In addition, MS must identify Specific Pollutants that are discharged in significant quantities and set their own national EQSs for these substances.

Many of the Specific Pollutants are common to several MS. Given similar data and protection goals, we would expect different MS to reach similar conclusions about the EQS for the same substance. However, the resulting threshold can vary between MS. In cases where different MS share a river basin, differences in the EQS for the same substance could result in compliance with the EQS in one part of the catchment but failure in another, even if the level of environmental exposure has not changed. This could lead to bias in chemical regulation. Clearly, derivation of EQSs should be harmonised as much as possible.

The completion of detailed Technical Guidance for deriving EQSs in 2011 should help promote consistency in the way EQSs are derived. However, programmes of EQS development for Specific Pollutants are already well advanced in several MS. It is therefore timely to assess the degree of variability between EQSs, the causes of that variability, and to understand whether the new EQS Technical Guidance will help reduce between-MS variability for new EQSs, or revisions of existing EQSs.

Our poster compares EQSs developed independently for the same substances. It describes the variability in EQS, and an analysis of the major reasons for variability, focussing on:

- Underlying assumptions such as the form in which the EQS is expressed (e.g. dissolved vs bioavailable concentrations of metals, free acid vs neutral organic acids)

- Comprehensiveness of the ecotoxicity data on which the EQS is based

- Quality assessment of 'critical' data, including treatment of 'outliers'

- Models used to undertake extrapolation (e.g. deterministic vs probabilistic methods)

- Allowance for uncertainty (size of assessment factors)

We conclude by offering a view on the extent to which the new EQS Technical Guidance will help reduce variability in future, and whether any particular aspects need to be emphasised to promote consistency.

MO 476

Aquatic effect assessment for plant protection products. I. A Dutch proposal addressing the requirements of the Plant Protection Product Regulation and the Water Framework Directive

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Chemical monitoring programs revealed that in a large number of surface waters in the Netherlands, measured concentrations of certain plant protection products (PPP) exceed Dutch quality standards (QS) for water. One reason could be that there have been flaws in the registration procedure or in the derivation of the QS. Another cause

might be that different effect assessment methods are used for registration or standard setting. The responsible ministries decided to update the aquatic effect assessment procedures for plant protection products (PPPs) in the Netherlands, taking into account the requirements laid down in European legislation with respect to PPP authorisation (Regulation 1107/2009/EC) and the derivation of QS under the Water Framework Directive (2000/60/EC).

In this project, decision trees have been developed for the aquatic risk assessment that can be used in the pre-registration authorisation and post-registration monitoring and evaluation. The core of the approach is that risk assessments are performed at two places in the water system, viz. (1) in edge-of-field surface water and (2) further downstream in surface water that has officially been assigned as WFD water bodies. In smaller edge-of-field surface waters (e.g. drainage ditches) the pre-registration criteria of the PPP Regulation apply, whilst in the larger WFD water bodies, the standards derived according to the WFD methodology apply. Post-registration verification of the exposure concentrations in the WFD water bodies against WFD water quality standards will take place using measurements. If results of chemical monitoring programs indicate exceeding of QS for a specific compound which can be attributed to the current "GAP" (good agricultural practice), this may have consequences for its authorisation (post-registration risk assessment procedure) and/or adequate mitigation measures have to be implemented. In this poster, we summarise the methodology for the edge-of-field assessment and the derivation of QS, taking the information in a regular pesticide dossier as a basis. This includes guidance on first tier and higher tier risk assessment, linking of exposure and effects and the incorporation of information on the specific mode of action of PPP in the methodology of QS derivation. In a second poster we present the results of case studies used for the verification of the proposed methodology.

MO 477

Aquatic effect assessment for plant protection products II. Case studies for the verification of a new procedure in the Netherlands

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A proposal has been made to update the aquatic effect assessment procedures for plant protection products (PPPs) in the Netherlands, taking into account the requirements laid down in European legislation with respect to PPP authorisation (Regulation 1107/2009/EC) and the derivation of QS under the Water Framework Directive (2000/60/EC). The core of the approach is that risk assessments are performed at two sites in the water system, viz. (1) in edge-of-field surface water and (2) further downstream in surface water that has officially been assigned as WFD water bodies. In our first poster¹, we summarise the methodology for the edge-of-field assessment and the derivation of QS, taking the information in a regular pesticide dossier as a basis. In this second poster, we present the results of case studies that we performed to verify the proposed procedures. For different types of PPP (two insecticides, a herbicide and a fungicide), we performed first and higher tier risk assessments for the edge-of-field on the basis of dossier data and estimated concentrations in water from model calculations for drainage ditches. In addition, we derived QS according to the methodology of the WFD and compared those with measured concentrations on WFD-monitoring sites. On the basis of the results, recommendations for refining the proposed procedures are made.

1: Aquatic effect assessment for plant protection products. I. A Dutch proposal addressing the requirements of the Plant Protection Product Regulation and the Water Framework Directive

MOPC1 - Soil ecotoxicology

MOPC1-1

Linking environmental availability and bioavailability of metals to snails: holistic approaches using single chemical extractions and DGT applied to field contaminated soils

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Risks of trace metals in soils are currently evaluated on the basis of total concentration although it appears often inappropriate for bioaccumulation or toxicity assessments. Chemical extractions and measurements of metal in the soil solution can provide alternative approaches. As dynamic chemical interactions also matter for bioavailability assessment, the DGT (Diffusive Gradient in Thin films) was proposed as a promising tool in this purpose. Although it has been successfully used to predict metal concentration in plants, its application to soil fauna is still scarce. The objective of the present research was to determine the ability of various chemical methods (conventional extractions and DGT) to predict Cd, Pb and Zn bioavailability to terrestrial snails, focusing on the influence of soil properties and the comparison of flux or concentration to predict bioavailability. Subadult *Cantareus aspersus* snails were therefore exposed for 28 days to fifteen field contaminated soils. We conducted a toxicokinetic experiment allowing to estimate metal bioavailability by mean of assimilation fluxes obtained from one-compartment modelling. Environmental availability was assessed using seven single extraction methods and the deployment of DGT devices. The latter allows to calculate both concentrations and fluxes in the resin that we compared to internal concentrations reached at the end of exposure and assimilation fluxes in snails. For non excreted metals such as Cd, concentrations in snails and DGT were quite well correlated and the addition of soil parameters in regressions did not improve the fit, testifying to the ability of the DGT to properly predict Cd transfer. Considering Cd fluxes, accounting for the influence of CEC allowed to improve the prediction ability of DGT ($r^2=0.59$). For Zn, the consideration of soil pH and Alox allowed to better predict bioavailability to snails ($r^2=0.80$). For Pb, the addition of soil characteristics in regression allowed to greatly improve the prediction (r^2 up to 0.72) although they were not significant. To be a good predictor of bioavailability, a chemical method has to implicitly integrate the physico-chemical and physiological processes underlying bioavailability. In this sense, DGT did not bring a clear added value compared to conventional extraction methods, but, by considering fluxes and modulating effects of soil characteristics, allows to properly predict Cd, Pb and Zn bioavailability to *C. aspersus* snails.

MOPC1-2

Impact of biosolids on soil fauna over a period of several years

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Biosolids derived from municipal sewage treatment plants are applied to agricultural land throughout the world with regulations differing regarding amount and frequency of application as well as regarding limits set to contaminants contained in the applied biosolids and the receiving soil. In the present study, the fate of pharmaceuticals and other substances contained in biosolids was studied after farm-scale application of biosolids from Ottawa city to agricultural land. Together with the fate of conatminants, we followed structural and functional endpoints of soil fauna over a period of several years. Abundance and diversity of enchytraeids and nematodes were selected as structural parameters, while the utilization of carbon by the soil organism community served as a functional parameter and was investigated by means of bait lamina tests. The results of the present study clearly demonstrated an enrichment effect of the application of dewatered biosolids. This effect vanished after two years with regard to structural parameters, but not with regard to functional parameters. No apparent impact on pollutant-sensitive nematode species such as predators and omnivores was detected during the study period. On-going research will verify if long-term effects of biosolids-associated contaminants can also be excluded for the studied parameters and for another relevant organism group, earthworms.

MOPC1-3

Microbial degradation of organic compounds (natural, xenobiotics, and pesticides) and the formation of soil organic matter or biogenic non-extractable residues

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Carbon from any biodegradable organic compound in soil partitions into parent compound, metabolites, non-extractable residues (NER), CO₂, and microbial biomass.

This distribution must be known to assess the fate of the compound in soil. For example, NER from pesticides are considered to consist of adsorbed and sequestered parent compounds or metabolites and thus as hazardous residues. However, they may also partly derive from bacterial biomass, resulting in harmless biogenic residues. In addition, the formation of soil organic matter (SOM) or humic compounds has long been a dominating topic in soil science because the amount and composition of SOM determines soil quality but the processes are still not yet really understood. Microbial biomass residues could be identified as a significant source for SOM. The results indicate that a high percentage of the biomass-derived carbon remains in soil, mainly in the non-living part of SOM after extended incubation. The results provide a simple explanation for the development of the small, nano-scale patchy organic materials observed in soil electron micrographs. These conclusions were confirmed by studies on the biodegradation of isotope labeled pesticides in soil which quantified the contribution of microbial residues to the NER in soil. The amount of label found in biomolecules indicated that virtually all of the NER of the compounds are derived from microbial biomass.

MOPC1-4

Trophic transfer of gold nanoparticles in a soil food chain

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Nanoparticles can be transferred from low trophic level organisms to high trophic level organisms. The present study investigated adverse effects caused by trophic transfer of gold nanoparticles. The low and high trophic level organisms of this study were *Escherichia coli* and soil nematode *Caenorhabditis elegans*. *E. coli* was initially exposed to gold nanoparticles, and *C. elegans* were fed by the exposed cells. The results showed that gold nanoparticles can transfer from *E. coli* to *C. elegans* via food exposure. We observed that the reproduction of *C. elegans* was decreased with increasing concentration of gold nanoparticles exposed to *E. coli*. This study reports the first observation of reproduction inhibition of *C. elegans* by dietary uptake of gold nanoparticles. *This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2011- 0015985).*

MOPC1-5

DNA barcoding tackles the identity crisis in earthworm populations used in ecotoxicology: The case study of South Africa

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DNA barcoding was used to investigate the phylogenetic delimitations of the major earthworm populations used in ecotoxicological research in South Africa. A total of four focal populations (used in published works) and two non-focal populations were assessed. Three of the focal populations (two laboratory based and one field) are referred to as *Eisenia fetida* in the literature. The last focal population (a field based) has been occasionally used as a source of *Dendrodrilus rubidus* specimens. Phylogenetic analyses of the COI gene revealed that all the *Eisenia* populations had been misidentified as all the generated sequences grouped unequivocally with published sequences of *E. andrei*. Moreover, very high sequence divergence (> 25% K2P) found within *E. andrei* could signal the occurrence of hitherto non-described cryptic species. The last focal population was found to be a mix colony harbouring *D. rubidus*, *Microscolex dubius* and at least two other unidentified species. These findings are discussed with an emphasis on their inevitable implications on the current use of earthworms in ecotoxicological bioassays. Left unaddressed, this situation could globally become a considerable setback to ecotoxicological testing as the present study might not be an isolated case.

MOPC1-6

What is the most relevant earthworm species for testing pesticide effects? A meta-analysis

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Human land use, through intensification of agricultural practices and particularly soil pollution by chemical inputs, has often resulted in loss of biodiversity. Earthworms represent the essential of living biomass in soils. They perform important agro-ecological functions since they influence organic matter dynamics and soil structure. They are considered as indicators of soil biodiversity and quality.

Pesticide impacts on earthworms are difficult to measure under field conditions due to confounding effects. Ecotoxicological risks are assessed mainly under laboratory conditions before marketing authorization. The species *Eisenia fetida*, which is not present in cultivated fields, is used as biological models for these tests. It is also often used in laboratory studies thanks to its short generation time and to its easy culturing. However, there is a controversy about the sensitivity of this species towards pesticides, when compared to other species naturally present in agrosystems where chemicals are used.

Using a meta-analysis, we address the following questions: Is *E. fetida* less sensitive to pesticides than other species? Are earthworms belonging to different genus and ecological groups differently influenced by pesticides due to their different behavior? What is the relevance of using only mortality as indicator in such studies and tests? Results will allow us giving recommendations for testing effect of pesticides on earthworms in a relevant way. We will also pinpoint the gaps we have to fill in order to understand how pesticides influence earthworm population dynamics in agricultural fields.

MOPC1-7

Soil threats in the Netherlands

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Considering the EU Soil Thematic Strategy and the proposal for a framework Directive the Dutch government wanted to know what this could mean for the Netherlands in terms of policy and measures or in terms of impact on for instance agriculture and environment. The proposal for the Framework Directive to protect soil mentions the following soil threats: soil sealing, erosion, organic matter decline, salinisation, compaction, landslides and contamination.

Focusing on the soil threats for which risk areas (also called priority areas) should be identified we were asked to try and locate where such areas would possibly be located within the country. For some threats we adopted methods from the ENVASSO-project and for other threats we used traditional land evaluation methods. Our research resulted in maps showing potential risk areas in the Netherlands (Hack-ten Broeke et al., 2009). These maps do not indicate where risks are considered important enough to call for policy measures.

MOPC1-8

Monitoring of the state and development of soil parameters in the Czech Republic

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The quality of agricultural soils in the Czech Republic is monitored by the Central Institute for Supervising and Testing in Agriculture (ÚKZÚZ) via three programmes. These are "Monitoring of the agricultural soils", "Monitoring of inputs into the soil" and an administration of the database "Registry of contaminated sites".

Monitoring of agricultural soils is focused on characterization of the status and the changes of main soil parameters (physical, agrochemical, chemical) at a stable monitoring network. Protection of food chain and preservation of the soil quality is the main reason for monitoring of materials entering the soil. Within the frame of monitoring of inputs into the soils aims at the quality of sewage sludge, lake sediment and fertilizers. The Registry of contaminated sites give information on level of risk elements soil contamination in the Czech Republic.

These programmes and their data compilation enable to obtain information concerning soil contamination effect on agricultural plants, quality of soil after sewage sludge application, etc. Data can be used by other research institutes or universities

MOPC2 - Endocrine disrupting chemicals: recent developments

MOPC2-1

Combined effects of estrogen receptor antagonists on in vitro vitellogenesis

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Some environmental compounds are known to have anti-estrogenic activity and their modes of action (MoA) are believed to include competitive inhibition of 17 β -estradiol (E2) binding to the estrogen receptor (ER) or interference with ER-dependent processes through different pathways including increased metabolism of E2 and receptor crosstalk. Concentrations of single substances in the environment are generally low and effects rarely observed. Presence of multiple compounds having the same MoA may cause concern, however, as their effects may be additive. Additive effects of mixtures can be modelled by the two prediction models; concentration addition (CA) and independent action (IA). The objective of the present study was to determine if the combined effects of mixtures of ER antagonists on the ER-mediated production of the estrogenic biomarker vitellogenin (Vtg) in rainbow trout (*Oncorhynchus mykiss*) hepatocytes could be predicted by the CA and IA prediction models. Model anti-estrogens (4-hydroxy tamoxifen and ZM 189.154) and environmentally relevant compounds (PCBs and PAHs) were tested to ensure inclusion of compounds from different chemical classes with more or less well known mechanisms of anti-estrogenicity. All eleven tested compounds had the ability to inhibit E2-induced production of Vtg. The potency of the tested compounds differed by four orders of magnitude based on the concentrations for 50% inhibition (IC50). The observed order of potency was 2,3,7,8-tetrachlorodibenzo-p-dioxin > 4-hydroxy tamoxifen > 3,3',4,4',5-Pentachlorobiphenyl > benzo(k)fluoranthene > 3,3',4,4'-tetrachlorobiphenyl > Gnaphthoflavone > ZM 189.154 > indeno[1,2,3-cd]pyrene > benzo(b)fluoranthene > benzo(a)pyrene > benzo(a)anthracene. All tested mixtures inhibited E2-induced production of Vtg in a concentration-dependent manner. The mixtures consisting of compounds with similar MoA were well predicted by the models. The mixture consisting of compounds with different MoAs caused deviation from the prediction models consistent with more than additive effects. The results from this study show that CA and IA prediction models can be used to assess combined effects of mixtures of estrogen antagonists with similar MoA. The rationale for the deviations observed with the mixture of compounds with different MoAs warrants additional studies to determine the MoA of the interactions occurring and to assess potential impacts on the health of organisms.

MOPC2-2

Dependence between models and tests in the assessment of the endocrine disruption potential of chemical substances

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MOPC2-3

Testing thyroid disruption: which data are available in different regulations to support an initial suspicion for the environment?

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Identification, assessment and regulation of substances having an impact on the endocrine system of ecotoxicological relevant organisms are of special interest in several legislations in the EU (Chemicals: REACH (EC) No. 1907/2006, Plant protection products: 1107/2009 EEC, Pharmaceuticals: 2001/83/EG (2009) and 2001/82/EG (2009), Biocides: 2009/0076). Especially, since for this group a hazard-based approach is proposed by all of the legislations. However, identifying potential endocrine disruptors (EDs) might be pretty difficult as special tests systems for EDs are not well established in ecotoxicology and still under development. Therefore, the currently requested test systems are not prepared to identify endocrine disrupting properties alone and correlate them to adverse effects. During the last years research effort was mainly focused on sexual endocrine-disrupting chemicals, inter alia identifying effects on fish. Nevertheless, effects on the thyroid system were also investigated but are not part of standard procedures in ecotoxicology, yet. Thyroid hormones play an essential role regarding development processes in mammals, birds, amphibians or fish. Therefore, influences on the thyroid system might result in adverse effects comparable to effects on the sexual endocrine system given that an initial suspicion for effects on the thyroid system can be derived from standard toxicity test data, more advanced and specialized test systems could be requested (special concern has to be proved in most regulations). But when is a substance expected to have an effect on the thyroid system? And based on which data? And what to do if ecotoxicological studies are lacking completely? Do available non-clinical toxicological data and results of in-vitro test help to decide whether a compound affects the thyroid system?

This evaluation aimed at summarizing existing data and investigating the suitability of these data to identify substances which affect the thyroid system. Exemplified data sets of different regulations were compared. Studies and endpoints were investigated and (if available) compared with results of tests on the thyroid system. Additionally,

toxicological data were screened to look for results which indicate an initial suspicion.

MOPC2-4

Non-aromatizable androgen exposure to mosquitofish (*Gambusia spp*): links between molecular, physiological and reproductive system changes

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Mosquitofish (*Gambusia spp*) have the potential to become a bioindicator for EDCs. Male mosquitofish have an extension of the 3rd-5th anal fin rays, and this growth is inducible in females and fry by androgen exposure. Issues with using mosquitofish as a bioindicator include the lack of knowledge of the mechanism of anal fin elongation. Previous studies indicated three genes (sonic hedgehog, muscle segment homeobox C, and fibroblast growth factor 1) as upregulated in juvenile mosquitofish or swordtail anal fins by androgen treatment. The relationship between anal fin growth and reproductive effects also needs to be understood. To address these issues, the objective of this project is to evaluate the effects of androgen exposure on mosquitofish. Two specific aims were developed: 1) Determine the expression patterns of Shh, MsxC, and Fgf1 in the anal fin during androgen exposure, and 2) Evaluate the relationship between anal fin growth and the expression of the egg yolk precursor protein vitellogenin (vtg). Female mosquitofish were exposed to a vehicle control or one of three doses of the androgen 17 β -trenbolone (TB) for 3, 7, 14, or 21 days. Body morphometrics were assessed and livers and anal fins were removed for quantitative polymerase chain reaction (qPCR). In situ hybridization (ISH) was used to evaluate Shh gene expression in the anal fin at days 7, 14, and 21. Results demonstrate that 10 μ g/L TB can induce anal fin growth at early time points while 1 μ g/L TB is significant from the controls at later time points (one-way ANOVA, $p < 0.05$). This may be due to the ability of a higher dose of androgen to stop elongation and promote the formation of advanced structures (hooks and serrae). MsxC was increased in all TB doses at day 7 and Fgf1 was increased at days 3 and 7 by 10 μ g/L TB (one-way ANOVA, $p < 0.05$). Shh was strongly expressed in the distal tip of the anal fin at days 7 and 14. Mosquitofish exposed to a 10 μ g/L TB had reduced hepatic vtg expression (one-way ANOVA, $p < 0.05$). Future experiments will determine how TB dose affects anal fin elongation versus differentiation, evaluating MsxC and Fgf1 expression at additional early time points, and developing an assay to measure fry output during TB exposure to compare these data with vtg gene expression levels. Knowledge gained from these and future experiments will be a starting point for future research towards establishing relationships between biomarkers of androgen exposure and reproductive health.

MOPC2-5

Assessment of Nonylphenol for the environment: do the regulatory conclusions differ if we look at endocrine disruption or 'classical' endpoints?

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Nonylphenol is one of the global high production volume chemicals and the debate of the risk posed by it - especially for surface waters - is a global one too. Levels of nonylphenol in surface waters exceeding the environmental quality standards (EQS) have been found in several waters in the EU although a restriction on the uses of nonylphenol and its ethoxylates under the limitations directive 76/769/EEC (now in Reach Annex XVII, entry 46) have been in force since January 2005. Moreover, its endocrine disrupting properties were assessed in the EU by different organizations. An in depth analysis of its endocrine disrupting properties was performed in 2002 (European Commission, 2002). It substantiated the endocrine disrupting properties of nonylphenol with respect to aquatic organisms and concluded that it may present a risk to aquatic organisms due to adverse effects that may be endocrine mediated.

Nevertheless, there is an ongoing discussion in the scientific and regulatory communities about the severity of the endocrine disrupting properties in comparison with the systemic ecotoxicity of nonylphenol. For the EU risk assessment (RAR 2002) no endocrine disrupting properties were taken into account. The PNECwater was derived by using the algae biomass NOEC of 0.33 μ g/L. Even if we would not consider endocrine disrupting properties in our assessment under REACH algal biomass is no valid endpoint (see ECHA Guidance document: R7b R7.8.5.3) and therefore PNEC derivation would have to change. A different lowest NOEC would have to be used: Mysidopsis bahia NOEC 3.9 μ g/L.

A consideration of the endocrine disrupting properties could lead to NOEC values as low as 1 μ g/L. For example with the following species: Crassostrea gigas NOEC sperm motility <1 μ g/L sowie Arbia lixula NOECdev anomalies < 0.937 μ g/L

This presentation aims at comparing relevant systemic toxicity endpoint with endocrine mediated endpoints and pointing out the regulatory important conclusions.

MOPC2-6

Detection of endocrine effects: changes in the steroid synthesis and reproduction toxicity

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The joint project "Tox-Box - Risk assessment of anthropogenic micropollutants to assure the drinking water supply" aims for a harmonized, hierarchic test strategy to assess the toxicity of drinking water. As one part of this project the Institute for Environmental Research at RWTH Aachen University will analyze and establish endocrine activity as an important toxicological endpoint within the risk assessment of drinking water by means of the GOW-concept developed by the German Federal Environment Agency (UBA). The first step will be to screen drinking water samples and optimize biotest protocols. The samples will be provided by Rheinenergie AG as a water supplier and the Helmholtz Centre for Environmental Research (UFZ), and they will be subjected to a set of different biotests. The ER/AR-Calux®-Test (Estrogen-/Androgen-Responsive Chemical-Activated Luciferase gene expression) will detect receptor-mediated endocrine activity in the T47DLuc human cell line and the H295r-Assay will identify alterations in the steroidogenesis of the human adrenocortical carcinoma cell line (H295r). The third biotest will provide data on the reproduction toxicity of the water samples using the snail Potamopyrgus antipodarum. In the second step of this module endocrine active single substances will be used in the H295r-Assay to investigate the mode of action of the disruptors. The data will be used to assess sublethal/chronic toxicity and to transfer the mechanisms to risks for human health. The third step will include research on drinking water samples by effect-directed analysis (EDA) to identify endocrine active single substances which are currently not being measured. The results from the whole Tox-Box project will be used to establish a new guideline regarding the risk assessment of anthropogenic micropollutants in drinking water.

MOPC2-7

The development of quantitative weighting factors for use in a weight of evidence framework for the evaluation of endocrine activity

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We previously developed a conceptual framework for conducting weight of evidence (WoE) evaluations of endocrine screening and testing data (Borgert et al., 2011). The framework was designed to be broadly applicable, but with an emphasis on data emerging from the US EPA's Endocrine Disruptor Screening Program (EDSP), and specifically tailored to accommodate endpoints measured in the eleven Tier 1 Endocrine Screening Battery (ESB) assays. The framework calls for a transparent process whereby specific hypotheses are formulated and data quality is evaluated with respect to overarching scientific principles, including primary validity of the measurements, reliability of data reporting, and probative capability of the study design to evaluate causation. The framework also calls for assigning quantitative weighting factors to assay endpoints (WREL) that reflect their relevance for deciding specific hypotheses. This is an important step in the WoE framework as it explicitly recognizes the fact that some assays are very specific and sensitive for evaluating certain hypotheses related to endocrine activity but may be irrelevant for evaluating others. For example, an increase in thyroid follicular hyperplasia as assessed in the amphibian metamorphosis assay would be expected to have a very high WREL for evaluating the hypothesis that a chemical has potential thyroid antagonist activity, but would have a very low WREL for evaluating the hypothesis that a substance has potential estrogen or androgen agonist or antagonist activity. The framework recommends that WREL values be derived as numerical weighting factors based on the predictive value of the endpoint for actual endocrine activity in vivo, which would typically be determined in chronic reproductive and developmental toxicity tests, i.e., EDSP Tier 2 tests. This predictive value, however, may be difficult to evaluate until results for a test set of chemicals are available from the Tier 1 ESB and can be compared to results of Tier 2 tests. As an interim step, however, provisional WREL values can be derived based on a rank ordering of relevance for each hypothesis. This rank ordering should also be based on data, but will necessarily involve considerable scientific judgment. An example derivation of rank ordered WREL values for evaluating Tier 1 EDSP hypotheses is presented, with particular emphasis on endpoints measured in the fish screening and frog metamorphosis assays.

MOPC2-8

Modeling mechanisms of nanotoxicity at multiple levels of biological organization through dynamic energy budgets

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Engineered nanomaterials (ENMs) entering the natural environment may have ecological effects by influencing the fluxes of energy and material that regulate the abundance, distribution, and dynamics of organisms. Empirical data alone cannot provide the understanding to guide policy or management action, so theory is essential. Dynamic Energy Budget (DEB) theory offers a conceptual framework for describing the interplay of physico-chemical processes in the environment with suborganismal and organismal processes in living organisms, and thereby linking to population, communities and ecosystems. This has been long recognized in ecotoxicology; indeed some of the earliest work on DEB theory was motivated by the recognition of the limited ecological insight that follows from standardized toxicity tests. DEB theory has the potential to model mechanistically the effects of ENMs on single cells, entire organisms and on populations. The sophistication of the theory that is required varies greatly among applications, and will be illustrated using case studies from the University of California Center for Environmental Implications of Nanotechnology. These include: (i) estimation of toxicity parameters for metal oxide effects on phytoplankton; (ii) detailed modeling of short-term studies of the physiological responses of a marine invertebrate exposed to metal oxide nanoparticles models, and projection of implications for lifetime reproductive output; (iii) modeling the impact of multiple stressors, based on the same data as (ii);

(iv) taking account of sub-organismal information and environmental physico-chemical processes in modeling the influence of CdSe quantum dots on bacterial population dynamics; (v) models to predict the results of experiments on the effects of citrate coated silver nanoparticles on phytoplankton-zooplankton interactions. Our approach complements much recent work on adverse outcome pathways (AOPs) by extending the pathways to include ecological interactions without compromising the critical connections with suborganismal biology.

MOPC3 - Non-target analysis and identification of toxicologically significant emerging pollutants

MOPC3-1

How to evaluate pollution, degradation and metabolism of multi-families of pharmaceuticals and endocrine disruptors in multiple compartments of the surface water media?

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Evaluations like risks or effects assessments of the presence of various xenobiotics in the environment show a continuously growing interest since the past decades. To establish models for degradation of a family of pharmaceuticals and thus evaluate its impact on the environment, scientists need good analytical methods to reinforce databases. While literature focuses on single matrices or single family, this work aims to develop an integrative approach of the surface water media: water, sediments and biota. Those methods are combined to relatively simple and short time-consuming extraction techniques investigated for various matrices for the 20 various pharmaceuticals and 28 Endocrine Disruptor.

NanoLC-MS/MS method has already been applied to a benthic model exposed for eco-toxicological study [1]. All presented LC-MS/MS, automated online-SPE-LC-MS/MS and nanoLC-MS/MS methods have been validated following ICH recommendations. They allow rapid quantification of multiple pharmaceuticals and endocrine disruptors at environmental levels and have been for the study of real ecosystems in French rivers. In parallel thanks to passive samplers calibrated in the laboratory and validated in situ with PRCs, real time weighted average concentration can be determined in surface water. Exposing the benthic model to those concentrations should allow not only the quantification of accumulated and metabolised compounds in biota but also degradation products and coefficient partitioning between water and sediments. Those studies even could correlate fate to effects observed on benthic models. Further investigations with TOF spectrometers should allow non-targeted analysis for identification of new by-products, degradation products or metabolites.

Although this analytical challenge is needed for risk and effects assessments on environment and populations, it is really time-consuming for the analyst. Prioritisation of substances is crucial to select representative pollutants to help scientists building models. This project opens the discussion for assessments of multi-families of various ubiquitous pollutants with a relatively large panel of analysis for different matrices of the same water surface media.

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MOPC3-2

Non-target analysis of emerging contaminants - harnessing antibody selectivity

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The coupling of immunoassays to liquid chromatography is described. Fractions of complex environmental samples obtained by RP-HPLC fractionation have been analyzed with immunoassays for carbamazepine and sulfamethoxazole. Two metabolites, one conjugate and two structurally not so closely related compounds have been identified by this method. Procedures and limitations are discussed in the presentation.

MOPC3-3

Development of a water toxicity sensor based on genetically modified bacteria

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The detection and monitoring of pollutants in surface water is routinely done by chemical analysis, biomonitors using aquatic organisms, or in vitro toxicity assays. Chemical analysis is sensitive and can be fast, but only compounds searched for are detected. Biomonitors with algae, Daphnia and mussels are excellent for the detection of the total toxicity of for example heavy metals or pesticides. However, not all toxicants with relevant effects can be measured, for example genotoxicants or endocrine disruptors. Laboratory based in vitro assays on the other hand, do detect these effects, but take a long time to perform and can not be used on-line in the field.

To be able to fill this gap, a toxicity sensor has been developed, which employs genetically modified Escherichia coli that emit luminescence when they are exposed to compounds with a certain effect. The effect that can be measured depends on the modification of the bacteria. The strain used in the presented sensor at the moment responds to DNA damage. The bacteria are immobilized in a sol-gel on a glass slide and an optical fiber which were continuously exposed to a water flow. The goal is to develop a sensor that can be used on-line in the field with minimal maintenance.

The first experiments with the sensor were performed with Dutch tap water, which does not contain added chlorine. The immobilization in sol-gel did not affect the response of the bacteria. The luminescence of the bacteria increased after exposure to nalidixic acid and mitomycin C. The response started approximately one hour after the start of the addition of the positive control.

The concentration of added growth medium had a strong influence on the response and was thereby the most important parameter. A concentration of 2% Lysogeny broth (LB) was enough to sustain the signal without causing strong fluctuations. Both the background signal and the response of the bacteria declined with time. One possible reason for this might be competition with other microorganisms in the water, but this is still under investigation. As expected, the fiber yielded a signal that was 100-200 times lower than the signal of the plate. This was the result of the much smaller surface area of the fiber tip.

MOPC3-4

Can solar irradiation transform human metabolites of pharmaceuticals back to their parent compound? Insights on the direct photolysis products and pathways of sulfamethoxazole human metabolites

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Sulfamethoxazole (SMX) is a widely used antibiotic, only partially removed by wastewater treatment and therefore often detected in wastewater effluent and in surface waters. However, after human consumption, SMX is partially metabolized in the human body into various metabolites, some of which retain certain biological activity, and which have also been observed in the aquatic environment. Moreover the back transformation of certain metabolites to the parent compound has also been reported in certain environments. Both direct and indirect photodegradation processes have been reported to be important removal mechanisms of sulfamethoxazole in the environment; however no research has been conducted on the photolysis of SMX metabolites.

This study investigates the direct photolysis kinetics, quantum yields and products of a selection of SMX metabolites, namely: acetyl SMX, nitro-SMX, nitroso-SMX and hydroxy-SMX. Photo-experiments were conducted in a merry-go-round photoreactor equipped with 300 nm mercury lamps with variable concentrations (10-100µM) of SMX metabolites, and analysed via HPLC-UV vis and high-resolution mass spectrometry. Results revealed slower direct photolysis kinetics and quantum yields that were 10 to 100-fold smaller than those of the parent compound SMX. The photoproducts generated from direct photolysis of SMX and metabolites were identified. The structural similarity of SMX and acetyl-SMX photoproducts point towards the same photolysis cleavage sites. Moreover, the back-transformation of nitroso-SMX to the parent compound was observed. These results show that pharmaceutical metabolites can be more recalcitrant to photodegradation and even be a source of pharmaceuticals to the environment.

MOPC3-5

Using mutagenic fingerprints for a compound identification

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One of the major challenges in the field of environmental chemistry and ecotoxicology is the identification of compounds or compound mixtures which are responsible for adverse biological effects. One major tool for the elucidation of cause-effect relationships is the effect directed analysis (EDA) which combines the fractionation of a complex sample with biological assays and a subsequent chemical analysis. One drawback of this highly successful approach is its complexity and time demand.

A biological test strategy which directly provides information about the chemical nature of bioactive compounds or compound classes would complement the classical EDA because it facilitates a more target-oriented and thus efficient sample fractionation and/or chemical analysis. In the present study such a methodology is developed for the identification of mutagenic compound classes in environmental samples by the Ames-fluctuation assay.

It is well known that many mutagenic compounds are bio-activated by the metabolism of xenobiotics. In recent years different bacterial strains were generated which overexpress one or more enzymes which are involved in the metabolic toxification and/or detoxification of genotoxins. If a potentially mutagenic compound is bioactive in a given test strain depends on the metabolic competence of the bacteria. Consequently, compound specific mutagenic fingerprints can be generated by making use of a set of genetically modified bacterial strains with different enzymatic equipments. In order to challenge the working hypothesis that such fingerprints allow the identification of compounds or at least compound classes, dose-response-relationships for model compounds were aggregated in a single value, the concentration dependent number of revertants (cdr). The combination of all strain-dependent cdr-values for a model compound which represents a chemical class can be treated as a vector which describes the metabolic activity of a compound class. It is tested, if compound classes can be identified by a comparison of these vectors which is based on the calculation of the cosine similarity.

MOPC3-6

Innovative sample preparation steps for the analysis of pharmaceuticals in soil

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Chemical products are more and more used for agriculture and domestic activities and are responsible for the spread of several substances in the environment, which can be harmful for animals or humans. Among these products, hormonal steroids and pharmaceutical compounds are of growing concern. If several analytical methods are available to determine the content of these compounds in aquatic environment, few methods were described to allow their analysis in solid matrices. However, the few available data concerning the content of hormonal steroids in soil reveal contaminations which can reach hundreds of ng/kg.

Consequently, the aim of this study was to develop an analytical method for the analysis of traces of 11 hormonal steroids, 16 veterinary or human drugs and 11 other well-known human contaminants, used in this study as pollution tag, in soil. An analytical method both selective and sensitive based on liquid chromatography-tandem mass spectrometry was developed. The optimization allowed the determination of the best conditions for the separation by chromatography and for the detection by mass spectrometry.

The analysis of complex matrix such as soil needed a rigorous sample preparation to obtain a repeatable and enough sensitive analysis to achieve the detection limits required. For this purpose, an extraction step using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was set up. This is a relatively new technique, simple, quick and cheap. The use of this method for environmental matrices such as soil is particularly innovative. This technique is based on a liquid-liquid extraction with an organic solvent in presence of salts and buffers. Several parameters were optimized (sample weight, solvent nature, water and solvent volumes, buffer type). A further purification step was necessary to reduce the matrix effects, therefore the SAX (Strong Anion Exchange) cartridge was used in tandem with the Strata-X cartridge for the clean-up.

This analytical procedure allows the determination of the target analytes in the lower ng/g range.

After a validation based on the ICH/2005 norm, this analytical methodology was successfully applied to real samples of soil collected in several areas of France that were treated differently. Some of them were contaminated with manure spreading, others with sludge spreading and others with atmospheric fallouts. Results indicate that all the samples contained several contaminants at level lower than the ng/g.

MOPC3-7

NORMAN MassBank - a community driven open access mass spectral database for the identification of organic environmental contaminants

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The nontarget screening and identification of unknown compounds such as industrial by-products and/or transformation products in complex environmental samples is of emerging concern. Many emerging pollutants are polar in nature and require the use of liquid chromatography coupled to soft ionisation mass spectrometry [1]. Improvements in mass spectrometry, such as accurate mass and high resolution mass analysers, for example time-of-flight (e.g., ToF-MS, QToF-MS) and Orbitrap MS, has been demonstrated to be valuable tools for the identification of new compounds (e.g., [2]). However, a major drawback in the identification of unknowns is the lack of suitable accurate mass and MSⁿ spectral databases as for GC-ESI-MS spectra. Existing LC-MSⁿ databases contain relatively few spectra, especially even less of environmental relevance. The web-based MassBank mass spectral database [3] (<http://www.massbank.jp>) allows storage and sophisticated search (i.e., spectra, substructure and peak search) of any kind of high and low resolution mass spectra including EI-MS, ESI-QToF-MS/MS and ESI-FT-MS. The main objective of our work is the establishment of a community driven MSⁿ database using the MassBank server software for the NORMAN network (<http://www.norman-network.net>) to improve identification of unknowns in environmental samples and to support the prioritisation of emerging compounds. A database containing such data is the starting point for the further development of spectral interpretation algorithms tailored to environmental data for the identification of specific substructures in MSⁿ spectra to assist in candidate selection. Thus, contributions to a community mass spectra database within the NORMAN network (NORMAN MassBank) containing all types of LC-MSⁿ data will help to address the lack of data and improve identification of environmental unknowns. Furthermore, it facilitates the possibility to check instrument comparability and to spend more time on unknowns identification rather than on building of home-made MSⁿ libraries. The database is available at <http://massbank.ufz.de/MassBank> and at the NORMAN website. References: [1] M. Krauss, H. Singer, J. Hollender, Anal Bioana Chem 397 (2010) 943; [2] A.C. Hogenboom, J.A. van Leerdam, P. de Voegt, J Chroma A 1216 (2009) 510; [3] H. Horai, M. et al., J Mass Spec 45 (2010) 703.

MOPC4 - Development in life cycle inventory analysis and modelling

MOPC4-1

Dissemination of LCI data in the context of multiple data platforms: case of the aluminium industry

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As a leading industry association, with a wide global coverage, the International Aluminium Institute (IAI) has collected and published representative Life Cycle Inventory (LCI) data since 1998. However, despite the work expended by the aluminium industry in collecting, analysing and reporting the LCI data, the Life Cycle Assessment (LCA) community as a whole has until now relied on a variety of data sources to conduct LCAs, some of which are outdated and/or reflect a limited part of the global industry. The LCI datasets that IAI publish are built from data collected from both its members and non-member companies, through annual energy and emissions surveys and five yearly life cycle data surveys. Traditionally, the resulting data were assembled in a publically available report. LCA practitioners were hence expected to lift the LCI datasets from the reports for use in their studies. Since the datasets were gate-to-gate, the practitioners were also expected to link the various inputs to compute cradle-to-gate life cycle inventories.

Despite its effort to disseminate the data, the IAI in 2008 realized that many LCA practitioners were not using the most up-to-date data on aluminium production in their studies. It therefore sought a solution that would ensure that the data collected were relevant to the different major LCI databases being used, ensuring widespread use of their data, and also ensuring datasets were disseminated in a way that all databases could integrate the data with minimal effort.

The approach the IAI settled on is to use the ecoinvent database and its new ecoSpold 2 data format as a launching pad for data dissemination.

The approach developed as part of this project aims at facilitating the dissemination of LCI data to as wide an LCA practitioner base as possible and at making data updates, when the IAI acquires relevant data through its various member surveys, an easy and in-house process.

The wide dissemination is ensured by the fact that (1) the chosen seed database (ecoinvent) is widely used and integrated in most LCA software and (2) the data can be translated into other existing formats through pre-existing data translation tools.

The combination of training on the ecoEditor for version 3 software by IAI employees, the possibility to directly submit data to ecoinvent, and the capability to create updated datasets by changing only relevant flows will enable the IAI to create updated versions of the datasets as often as it sees fit. <br type='_moz' />

MOPC4-2

BioEnergieDat: Life Cycle Inventories and IT infrastructure for Bioenergy in Germany

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LCA studies on bioenergy are based on diverse methodological choices and technology information, resulting in discrepancies in data sets of identical technologies or products. The assessment of technologies for decision support calls for further harmonization of methodological procedures, as well as of validation of input data and assumptions on specific processes. In the context of the German energy and climate policy, the need of an adequate database on bioenergy reflecting German framework conditions has been identified. A two year's project for a database on energetic use of biomass for Germany has been launched named 'BioEnergieDat'.

BioEnergieDat is aimed at delivering data sets for selected bioenergy technologies and energy carriers based on German framework conditions for today's state of technology as well as for the projected state of 2020/2030. Data sets shall be usable for different stakeholder needs in the field of bioenergy and for a broad range of applications of process-chain based modelling. To enable this, the common ground of work is the concept of a modular data supply: data sets will be generated and validated for unit modules of the process chain; based on the project's methodological specifications, these unit modules may be combined in a flexible way within different process chains and applications. To provide the technical possibilities for this concept, a web-based Open source IT infrastructure is developed, including data storage for the unit modules and a calculation tool for LCA modelling and for conducting case studies.

The presentation will outline projects outcomes which have been achieved so far: Concerning data sets 39 process chains for bioenergy were elaborated by combining biomass feedstocks and technologies presently encountered in Germany. The work on methodology is based on considering 'perspectives' in inventory modeling. The work on IT infrastructure includes data storage for the unit modules and a calculation tool for LCA modelling.

Due to its modular approach and the related development of IT, BioEnergieDat implies a broader outreach: it shall support methodological development as to specific questions of bioenergy like substitution effects or land use, it shall support data quality procedures, and it shall provide novel IT solutions for efficient, reliable and fast exchange of data and information between different tools, methods and user environments, supporting not only national but also international networking.

MOPC4-3

Improving regional specific life cycle assessment of energy crops in Germany

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Ambitious goals have been set to reduce by 40% the German greenhouse gas (GHG) emissions by the year 2020 (compare to 1990 levels). One of the strategies followed by Germany to achieve this target has been the development, use and promotion of renewable energies. Biogas production is a type of renewable energy that has been growing significantly in Germany over the past decades, being the country with the major biogas production in the world [1].

The contributions of agriculture to GHG- emissions and ammonia volatilization are currently under controversial discussion [2]. Frequently, the estimations of agricultural GHG- emissions within a Life Cycle Assessment (LCA) are based on generic emission factors (e.g. IPCC factors) only, leaving behind all other site specific conditions [3-4]. Another ongoing discussion refers to the potential reduction of such emissions if digestate (residue after biogas production) is returned to the field.

To address these questions and support decision makers, our project aims to reduce specific emissions factors for ammonia, nitrous oxide and methane that can be included in regional-specific LCA studies for energy crops. These emission factors will be derived from direct field measurements of these gases after the application of digestate as well as mineral fertiliser.

Furthermore, the project strives to develop precise but also generalisable statements about ammonia volatilisation and alteration of the soil carbon content due to returning digestate from biogas plants to the field. The outcomes of the joint research project should lead to an improved environmental assessment of energy crop production including additional aspects such as fertiliser use efficiency and soil fertility. The project results should provide approaches - not just - for the sustainable production of energy crops but also for a more efficient cultivation of other renewables.

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MOPC4-4

Validation of PestLCI 2.0, an updated and expanded model to estimate pesticide emissions for use in LCI

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As the effects that pesticides have on non-target organisms are directly proportional to the amount of pesticide emitted to the environment, it is important to accurately quantify pesticide emissions to the environment in the Life Cycle Inventory (LCI) phase of Life Cycle Assessment.

PestLCI 2.0 is an LCI model to calculate pesticide emissions from agricultural fields to 3 environmental compartments (air, surface water and ground water). After calculation of the primary distribution of pesticides, i.e. the distribution between crop and soil, different fate modules are used to model the fate of the pesticides after deposition on the crops and in the soil, thereby determining the compartment specific emission pattern of the pesticide.

PestLCI 2.0 is an updated and expanded version of the PestLCI model (Birkved&Hauschild, 2006). The model has been expanded with a fate module for macropore flow, the effect of tillage on macropore flow has been added while the algorithm sets of other modules have been updated. The applicability of the model is no longer limited to Denmark: the climate database has been expanded to include sets of climate data for 25 different locations in Europe, and the soil database now contains 7 European soils. The database of pesticides has been expanded to 90 active ingredients.

To validate PestLCI 2.0, pesticide emissions calculated by PestLCI 2.0 were compared to the emissions calculated by risk assessment (RA) models used in the FOCUS project. For surface water emissions, the MACRO model was used for drainage emissions; PRZM was used to determine the emissions caused by runoff. Emissions to ground water were calculated with PEARL 4.4. During validation the input parameters of PestLCI 2.0 were set to resemble the scenarios of the RA models as closely as possible.

The results indicate that the RA models and the LCI model generally are in good agreement. Surface water emissions calculated by PestLCI 2.0 are less than 2 orders of magnitude lower than those calculated by MACRO and PRZM, reflecting the difference between the realistic worst case approach of RA, as opposed to the average scenario approach of LCA. The emissions to ground water calculated by PestLCI 2.0 are higher than those calculated by PEARL 4.4. This is a consequence of the inclusion of macropore flow into PestLCI 2.0.

Overall, the results show that PestLCI 2.0 is considered well-suited to estimate pesticide emissions for use in LCI.

MOPC4-5

SewageLCI 1.0, an inventory model to estimate chemical specific emissions via sewage system

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Wide Dispersive Use (WDU) of chemicals results in considerable quantities of chemicals being emitted to the environment directly or via Sewage System (SS) effluents.

Emissions to SSs are in product system modeling handled by inclusion of Sewage Treatment (ST), assuming emission of an average ST effluent with a ST plant class specific effluent composition. It is thus assumed that the specific chemical is 100% removed and that an effluent with an average (chemical) composition is emitted. Performing Life Cycle Assessment (LCA) on unique chemicals with WDU, among other issues, necessitates correct representation of chemical-specific emission patterns via SSs as well as quantification of chemical-specific potential impacts instead of the potential impacts of an average effluent.

This poster presents a novel approach for quantification of chemical-specific emissions via SSs. SS configurations and the fraction of a chemical with WDU being emitted via SS effluents are nation specific. In order to reflect the national specificity of SSs configurations, statistical data on the SS configurations in all EU countries were compiled from Eurostat. Combining these data with chemical removal efficiencies of biological treatment from the STPINWIN model allows for estimation of removal fractions in the most common sewage treatment systems (primary, secondary and tertiary treatment as well as direct emissions and independent waste water treatment). Emissions from sewage systems not only occur to aquatic recipients. Besides estimation of the fractions emitted to aquatic recipients, SewageLCI therefore also allows for estimation of the fractions of a chemical emitted to air and the fraction emitted to soil in the case of sludge amendment to soil.

The approach of the model is tested on two common chemical groups used in large volumes in detergents; an enzyme (a protease) and an alcohol ethoxylate. Both chemical groups are modeled with and without sludge amendment to soil. In the scenarios with sludge amendment to soil, the fractions emitted to the environment from the fields receiving the sludge are quantified applying PestLCI 2.0.

The results obtained indicate the fractions of the chemicals emitted to the aquatic recipients varies more than an order of magnitude between different countries due to large differences in the national SS configurations. Further the approach reveals the benefits of a modular inventory modeling approach through the SewageLCI and PestLCI combination.

MOPC4-6

Interpretation phase in life cycle assessment software programs: a case study for Brazilian biodiesel

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Software programs to perform Life Cycle Assessment (LCA) have the function of reducing the processing time for inventory and impact assessment. What is not clear is whether LCA software programs are able of generating enough outcome to elaborate reports based on interpretation phase requirements. A set of check tools in interpretation phases defined by ISO 14040/44 and the European Platform on Life-Cycle Assessment's International Reference Life Cycle Data System (ILCD) handbook. According to these references, mainly when a report - conclusions and recommendations to a third-party for comparative studies - is needed, three quality checks have to be performed:

(a) completeness, (b) sensitivity, and (c) consistency check. A case study was reproduced in three available LCA software programs (OpenLCA, Umberto, GaBi). The first three LCA phases were identical in each program. The outcome provided, which differentiates in availability of data to perform the three quality checks. As result, the expectation of the evaluation of three checks inside the interpretation phase was not completely achieved. The completeness check was the only verification tool, which could be performed with the support from the three LCA software programs, although restrictions were reported. The sensitivity analysis by scenario variations could be carried out in general, since new propositions had been manually included. The OpenLCA did not have however a sensitivity analysis. The consistency check was not possible to be executed entirely inside any software program. Arguing that the iterative LCA approach and limitations of the study are attributed by user and not by limitations of program leads to a direct relation of expert judgement and report quality. This means that the information reported and the quality checks are only influenced by the practitioner and all informations needed can be extracted from the data provided. However, to perform a consistency check, for example, information about the differences in data sources, data accuracy and data coverage are necessary and they cannot be obtained by generating data in any LCA software program. The lack of information given by LCA software programs leads to a weaker third-party report. In spite of the fact that is possible to access and view most of the data needed in order to carry out the checks, the use of software program do not exclude further data process and the critical review by specialists.

MOPC4-7

Methodological approach for validation of LCI modules of Italian life cycle inventory database (I-LCA v. 3.0)

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ISPRA is involved in research activities to build and to implement a national, on-line and public database for LCA information (I-LCA), as priority action to support the development of competitiveness for enterprises according sustainable paradigms. The starting point was the database I-LCA version 2.0 developed by ANPA in 2000. Now

ISPRA intends to update and develop that project in a way to be in compliance with the European Platform on LCA (EPLCA). In this context, the objective of this paper is to illustrate the appropriate methodological approach on the conformity of the data set through criteria (quality, method, nomenclature, etc ...) in order to be in compliance with the format defined by European Platform on LCA. Furthermore, ISPRA is now planning out a research design aimed at developing of new specific-site LCI (Life Cycle Inventory) modules concerning the national transport sector based on primary data already available. Through the acquisition of a methodological approach has been possible to identify the criteria that give the data inventory characteristics of validity and conformity to standards set by the European Platform of LCA, namely quality, consistency and robustness of data. This methodological approach was applied to the modules contained in the database I-LCA v 2.0. This LCI database, currently under revision and updated enclosing about 400 manufacturing processes and services, is divided into four main areas (materials and processes, energy, transport and end of life). The verification of conformity was carried out on some modules (related to the energy sector) that previous were been transcoded for I-LCA v.3.0 according the format developed by the European Platform on LCA. This first internal review has showed that some of the modules are in compliance with the reference standard, while other modules should be implemented in order to achieve the same degree of completeness. The creation of a public database LCA, with quality and consistent data, methods and assessments, it is extremely important not only for the crucial help in implementing LCA studies, but also in order to promote a different orientation patterns in consumption and production. In this context, ISPRA intends to plan out a research design aimed at developing experimental application to a specific transport sector based on primary data already available.

MOPC4-8

Setting up collaborative research on LCA and related topics

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EDF, GDF SUEZ, Renault, Saint-Gobain, Total and Véolia, with the support of RECORD and of ADEME, are the co-founders of a new non-profit Corporation, SCORE LCA, created to financially support collaborative research on LCA and related topics. SCORE-LCA will drive collaborative research thanks to the annual funding of each member, and the potential complement of public funding.

The research topics will be defined and selected by SCORE LCA members, on an annual basis. An "Experts committee" (from University, Research Centers and Consultancy) and a "Scientific advisory panel" will assist the members in this selection of topics. Each research project will be done by a selected organism (or group of organisms) such as research institutes or consulting company. They will be selected by SCORE LCA members through a call for tenders. Each project will be supervised by a specific panel formed by members and selected experts from the "Experts committee". The set up of SCORE LCA will be done before end of 2011.

The research topics that will be covered by SCORE LCA studies encompass the following points:

- LCA systems modeling, and inventories calculation,
- Calculation algorithms and LCA tools,
- Indicators and impacts,
- Analysis and interpretation methods,
- Combination of LCA and other methods to assess products and services,
- Surveys.

The co-founders are willing to see SCORE LCA growing thanks to new memberships from other companies. SCORE LCA is opened to any entity, and has the will to be useful to the public: most of the outputs will be disseminated, and representative of SCORE LCA will bring their contribution to other public or private organisms at European level. The interest to belong to SCORE LCA is to be able to participate in the definition of the research topics and to be able to monitor the studies.

SCORE LCA is currently preparing call for tenders on two first topics. Contractors will be selected among the candidates at the beginning of 2012, and the work will start.

SCORE LCA has a European vision and expects that researchers from all Europe will collaborate to fulfill these research projects.

The goal of the poster is to present the activities of SCORE LCA, including the two research topics that will be covered first. Some representative will be there to answer the questions from the attendees to the conference, including from the Universities or Research Laboratories which would like to be candidates to the first call for tenders.

MOPC5 - Established and emerging footprints – striving towards a valid and comprehensive support for decision-making processes

MOPC5-1

Development of a framework for assessing the chemical footprint

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In the last few years, environmental footprint concept has obtained an increasing interest by both the scientific and political communities. The chemical footprint evaluation aims at assessing at which extent actual emission of chemicals harm the ecosystems above their capability to recover (the so-called carrying capacity of the system).

The evaluation of the chemical footprint, bringing together approaches and results from various disciplines (from ecotoxicology to ecology, from ecological/human risk assessment to life cycle thinking and related tools), may support the future development of policy both for environmental protection and green economy.

A review of existing approaches and methodologies able to be integrated in a chemical footprint framework will be presented and assessed. The following issues are the key elements:

- Assessing the intensity of chemical pressure, in terms of release in the environment by class of chemicals, economic sector of use, typology of release (point source or diffuse) and potential harm to the environment in a life cycle perspective

- Linking the release in the environment with the carrying capacity of the system (vulnerability analysis, exposed ecosystem assessment, Eco epidemiology)

MOPC5-2

The footprint family: differentiation, classification, and integration

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In the past decades, a variety of footprint concepts has entered the sustainability community under intensive debate. In this paper, we first review relevant literature. The idea of footprint originates from the ecological footprint described as a biophysical assessment tool that measures the human impacts of energy and materials consumption. After that the water and carbon footprint concepts were introduced analogous to the ecological footprint, representing the freshwater required or GWP-weighted GHG emissions. Obviously there is no single indicator able to represent the full spectrum of environmental impacts, but footprint indicators rather need to be applied jointly.

Attempt has been made to develop an integrated footprint assessment. The Global Footprint Network recently defined the "Footprint Family" as an aggregation of the ecological, water and carbon footprint to track human pressure. However, the definition was not yet clear and comprehensive, exclusion indicators like the chemical footprint being discussed by the SETAC, and failure to capture the rationale of Footprint. From a methodological point of view, the Footprint is a method rather than an indicator. Thereby, we provide for the first time a definition of the "Footprint" as an approach to convert the environmental impacts of human activities into a physical quantity (area, volume, weight, etc.). In this sense, the "Footprint Family" would rephrase as a suite of indicators able to measure the environmental impacts from different angles, by representing how many resources for each kind human have appropriated.

According to the ecosystem compartments where resources flow and stock, we classify the footprint indicators into four patterns, the biologic, the atmospheric, the aquatic and the geologic. For example, the ecological footprint belongs to the first pattern, the carbon footprint to the second, the water footprint to the third, and the energy footprint to the last. As for the chemical footprint, it depends on the exact pollutant pathways. A description of the boundary, function and characteristics for each pattern is given. The remainder of the paper is to identify the similarities and differences among different patterns to show how these indicators overlap, interact and complement each other. We hope this paper will serve as a reference for the footprint research, as well as a support for policy makers, as it brings clarity to the concern about what the footprint actually is.

MOPC5-3

Comparison of life cycle-based methods to assess the consumption of Luxembourg

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A plethora of decision-making tools and sustainability indicators is available today for supporting national stakeholders and public authorities in the process of implementing resource management regulations or environmental impact mitigation strategies. The reliability of these methods, as well as the requirement for data collection and impact assessment methods is widely debated, especially for policy making support purposes. In this poster presentation, we intend to discuss several methodological approaches to identify a reliable framework to support reduction strategies (of emissions and resource exploitation) at national level. More specifically, in this poster we present the assessment of a country's net consumption (where Consumption=Production+Imports-Exports) using different life-cycle assessment (LCA)-based methods (i.e. Ecological Footprint-EF, ReCiPe and Solar Energy Demand-SED) along with different environmentally extended input-output tables (EEIOTs) of EU-27, USA and The Netherlands. The case study of Luxembourg is used to evaluate this methodological framework. By using EF, SED, and ReCiPe we cover the main environmental impacts generated by a country's consumption. However, results highlight the need for higher consistency of EEIOTs. Indeed, there is a high uncertainty related to the type of environmental extensions (EE) coupled to each economic sector. This aspect plays a major role when applying different assessment methods, since results show important changes according to the set of resources and emissions included in the EE. However, the increase of scenarios with different EEs does not substantially influence the results at the level of economic sector's contribution. Whatever the scenario considered and the method applied, the financial and banking sector relies to more than 45% to the consumption of Luxembourg. Direct and indirect contribution analyses by sector allow outlining strategies to reduce impacts. The strengths and weaknesses of the approaches adopted are discussed with special emphasis on the benefits for potential applications in policy support.

MOPC5-4

Improving the relevance of carbon footprint calculations: temporal issues and global warming indicators

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Carbon footprint calculations are widely used to compare the impact of different products or projects on global warming. An increasing number of standards and guidelines based on life cycle assessment (LCA) are developed to provide a framework for those calculations. There is a broad consensus to multiply each greenhouse gas (GHG) emission by its GWP (Global Warming Potential) value, calculated for a chosen time horizon, and to add them up to get a carbon footprint expressed in kg CO₂-eq. However, this approach implies some assumptions and simplifications that can bias the results and the conclusions drawn from them.

Recent papers have shown that the use of GWPs calculated for a given time horizon can lead to inconsistent analysis, and that the global warming impact can be very sensitive to different metrics and time-related issues. The use of a time horizon is a value-laden choice that can have significant impacts on the decisions taken from carbon footprint results. There is also an increasing tendency to consider some time-related phenomena such as storing carbon in biomass or delaying GHG emissions. Some researchers have shown that temporary carbon storage can have a beneficial impact on climate by decreasing cumulative radiative forcing over a given time horizon, but can cause an increase in temperature right after the end of the storage period.

In this paper, the dynamic LCA approach is used on a fictitious case study comparing the impact on global warming of two objects with a 50-year lifetime: one made of wood, and a second made of a non biomass-based material. The dynamic LCA approach is applied to determine the time-dependent cumulative radiative forcing for each product. A new dynamic characterization factor is also developed to calculate the instantaneous increase in temperature caused by the life cycle emissions. This characterization factor is based on the concept of GTP (Global Temperature Potential), an analytical expression to calculate the temperature increase at any time following a GHG pulse-emission.

The results show that the choice of a time horizon for the analysis or the use of an alternative metrics can completely change the conclusions of a comparative carbon footprint study. Carbon footprint calculations are often used to guide policies, consumer choices and corporate decisions. To improve decision-making, these aspects should be considered for cases where GHG emissions and sequestrations take place over a long period of time.

MOPC5-5

Ecological footprint of the Port of Vigo (NW Spain)

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Sustainability of the fisheries sector is nowadays a key issue, since current practices of this activity are depleting marine resources and endangering biodiversity. Besides, indirect impacts, like those originated from related activities and services must also be addressed. For assessing the global impacts that this sector may have on the environment, the indicator Ecological Footprint (EF) can be used. This tool considers the energy and raw materials fluxes to and from any defined system or economy, transforming them into areas of land or water required by nature for generating and/or absorbing these fluxes. One of the main advantages of EF is its ability to inform general public about the impact an activity has on the world's biocapacity. EF analysis has been applied to nations, regions and to a wide variety of sectors and activities. Pressure of nations on marine ecosystems has also been assessed by this methodology.

Galicia is the main fishing region in Spain and one of the largest in the European Union. In fact, the Port of Vigo (SW Galicia) is the biggest fishing port of the world. Therefore, a representative part of the fishing extractive sector relies on the port activities. However, there are other important activities within the port which also requires resources consumption and thus, need to be evaluated. In this study, the environmental impact of the global activity (fishing and transportation) of the Port of Vigo was assessed by EF. The results obtained will provide information to the Port Authority on the main impact categories, in order to take the necessary measures to improve its environmental management strategy.

MOPC5-6

Ore grade decrease as an indicator to address mineral resource scarcity in life cycle assessment

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Resource use is an increasingly important topic on the political agenda. Mineral resources have dissipative use meaning that they are available on Earth but often degraded or dispersed. This way, mineral resources extraction will invariably continue and so will the decline of ore grades for all minerals worldwide, given no new discoveries (Mudd, 2009). Consequently, in order to extract the same amount of mineral, higher volumes will be mined. Ore grade is an inherent property of mineral resources and yet it also expresses the scarcity of a resource. In Life Cycle Assessment, however, ore grade decrease has not been used as an indicator to address resource scarcity up to now. The goal of this paper is to develop a meaningful life cycle impact assessment method to assess mineral resource scarcity and to calculate characterization factors for ten minerals.

The method here proposed expresses the decrease in ore grade as response to an increase in production. Characterization factors for mineral resource use were defined as the marginal change in average ore grade of mineral x (fraction) as response to the additional amount extracted of this mineral (kg). We calculated for the following ten element(-group)s characterization factors: cobalt (Co), copper (Cu), diamond (C), gold (Au), iron ore (Fe), lead (Pb), molybdenum (Mo), nickel (Ni), silver (Ag), zinc (Zn) and platinum-group metals. Production volumes and ore grade data per mine between 1998 and 2010 were retrieved from the Raw Materials Database developed by the Raw Materials Group. Results are presented in fraction of ore grade decrease per mass of mineral extracted. A life cycle study will be included to illustrate the applicability of the method proposed.

MOPC5-7

Applicability of land use impact assessment methods in LCIA - findings from practitioners' perspective

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Inclusion of land-use related environmental aspects into LCA has been under active development in recent years. Many indicators have been developed and proposed for different aspects of land use but have, as yet, not been tested and compared in LCA applications. Aim of this study was to test the applicability of the general framework and several land use LCIA indicators in an illustrative case study from practitioners' perspective.

Nine different indicators were selected to represent three different impact endpoints of land use: resource depletion, soil quality and biodiversity. The case study covered a beer production system with all the life-cycle stages from barley cultivation to the serving of beer at restaurant. Three optional system expansions were studied to estimate the possible impacts of substituting feed protein (soybean, rapeseed and silage) with mash co-product from brewing. A comparison with wine production was also made for illustrative purposes.

All tested land-use indicators were applicable in LCIA. Some indicators were found to be highly sensitive to assumptions on land transformation, which sets high requirements for LCI data quality. Challenges were faced in obtaining reliable data in the inventory and impact assessment phase. The majority of the land-use impacts occurred in the cultivation phase, but significant impacts were also found far down the supply chain. Scarcity of land-use LCI data sources limits validation and cross-comparison. Interpretation of indicator results is complicated due to the limited understanding of the environmental impact pathways of land use. Most of the land-use indicators led to results that were consistent with each other.

None of the tested indicators describes the full range of environmental impacts caused by land use, nor fulfils all the criteria for an effective ecological indicator. Many well-known general LCIA method sets include an impact category 'land use', but fail to cover all three impact endpoints. We recommend presenting land occupation and transformation LCI results, the ecological footprint and at least one of the biodiversity indicators. Regarding soil quality, the lack of reliable regional data currently limits application of the proposed methods. The criteria of effective ecological indicators should be reflected in further work in indicator development. Development of regionalized characterization factors is of key importance to include land use in LCA.

MOPC5-8

Development of biodiversity and resources indicators assessing potential impacts throughout the life cycle of products, processes and services

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While carbon and water footprint indicators begin to be fully integrated in the decision making process of industrial stakeholders, the question is still effective for other potential environmental impacts since environmental burdens could be shifted to other environmental stressors such as biodiversity and resources. Within Life Cycle Assessment (LCA), existing impact assessment methods do not fully address this issue when communicating to non-LCA practitioners, because of complex units and only partly integrated issues (e.g. arable land as a resource, not integrated in impact assessment methods so far).

In this context, an operational methodological framework has been established to assess the environmental performances of products, processes or services using two indicators: resources and biodiversity. The biodiversity indicator contains aquatic ecotoxicity (USEtox), terrestrial ecotoxicity, aquatic eutrophication, aquatic acidification, terrestrial acidification/nitrification (IMPACT 2002+), impacts of turbined water, cooling water, and a new framework for land occupation and transformation. The resources indicator includes the impact on the stock of the extraction of minerals, fossil fuels, peat, and overexploitation of wood and arable land.

Developments of new characterization factors for land occupation and transformation are based on the work of Koellner et al. (2008). The CML approach and country-specific data have been combined to obtain characterization factors for the resources indicator, developed in line with the substance flows of the ecoinvent database. The potential impacts of the 4000 ecoinvent processes have been calculated and statistically analysed to point out any inadequacy in the methodology regarding the operationalization. Case studies have been conducted within Veolia Environment's services and processes, showing the strengths and potential for future development of the indicators created. Finally, the potential benefits in terms of helping decision making process when combined with other environmental indicators have been identified. The main advantage of this new approach is to propose a transparent method, easy adaptable in the future, and which covers impacts usually not included in LCA, such as regionalized arable land depletion, wood use and water. To provide a comprehensive picture of the system assessed, it is recommended to combine those indicators with both the water and carbon footprint indicators already existing.

MOPC6-1**A Setac pellston workshop on the risks from Pesticides to pollinators: feedback and basis for the future**A.O. Alix¹, P.D. Delorme², T. Steeger³, T. Moriarty⁴, D. Fischer⁵, M. Coulson⁶, J. Frazier⁶, C. Lee Steere⁷, F. Streissl⁸, J. Pettis⁹, J. Pflugfelder¹⁰, M. Vaughan¹¹, J. Wisk¹²¹Dow AgroSciences, Abingdon, United Kingdom²Pest Management Regulatory Agency, Ottawa, Canada³US Environmental Protection Agency, Washington, United States of America⁴BayerCropscience, Washington, United States of America⁵Syngenta Ltd, Bracknell, United Kingdom⁶Pennsylvania University, Pennsylvania, United States of America⁷Australian Environment Agency, Barton, Australia⁸EFSA, Parma, Italy⁹USDA, Baltimore, United States of America¹⁰Swiss Bee Research Center, Switzerland, Switzerland¹¹Xerces Society for Invertebrate Conservation, Portland, United States of America¹²BASF, U.S., United States of America

There have been reports, in recent years, of declines in native and managed pollinators in several regions of the world. Modern crop management practices, progressively implemented in order to allow the extension of cropped areas and ensure a proper control of pest populations and diseases have often been pointed as a factor responsible of this decline. Plant Protection Products (pesticides) are part of these management practices. Thus regulatory texts have been adopted all around the world, requiring an assessment of the impact of these products on the agricultural environment, and among others on arthropod and pollinating species.

Concern regarding the extent to which the use of pesticides in crop protection affects pollinators varies among countries. The perception we have on pollinating species appears to results in first instance of our knowledge on their implication in crop pollination and related consequences of a temporary decline on the crops of concern. With regard to evaluating the potential impact of pesticides on pollinating species, these efforts have progressively focused on key indicator species, like the honey bee (*Apis mellifera*), primarily since the test guidelines for assessing the effects of pesticides on non-target terrestrial invertebrates have been developed mainly on this species, for practical reasons related to rearing facilities [8]. In Europe as in North America, the risk assessment for pollinators has focused on the honey bee; however, the extent to which these studies are used in risk assessment varies, which thus appears as the only species for which a dedicated risk assessment is performed, the other non target organisms being assessed at the level of trophic groups (i.e. fish, aquatic invertebrates, algae, aquatic plants, birds etc). A global Pellston SETAC workshop on estimating the potential risks of plant protection products to insect pollinators was organized in January 2011, to bring together the best available science regarding exposure and effects assessment methodologies for Apis and non-Apis species, and harmonize further the risk assessment approaches among North and South America, Europe, Asia and Africa for a global improvement of the protection of insect pollinators in cropped area.

The proceedings of this workshop will be finalized in the coming weeks and this presentation aims at offering the audience an outline of these proceedings.

MOPC6-2**Activities of the OECD expert group on pesticide effects on insect pollinators in the context of the risk assessment for pesticides**A.O. Alix¹, A.P. Pease², P.D. Delorme³, C.H. Hart³, S.P. Poret⁴¹Dow AgroSciences, Abingdon, United Kingdom²US Environmental Protection Agency, Washington, United States of America³Pest Management Regulatory Agency, Ottawa, Canada⁴Organisation for Economic Cooperation and Development, Paris, France

There have been reports, in recent years, of declines in native and managed pollinators in several regions of the world. Modern crop management practices, progressively implemented in order to allow the extension of cropped areas and ensure a proper control of pest populations and diseases have often been pointed as a factor responsible of this decline. Agricultural pesticides (plant protection products) are part of these management practices. Regulatory texts have been adopted all around the world, requiring an assessment of the impact of these products on the agricultural environment, and among others on arthropod and pollinating species. As a complement to this assessment, appropriate feedback from the field as an indicator of possible side-effects of pesticides on pollinating species and of conditions of pesticide use that ensure limited exposure is essential to evaluate the effectiveness of risk management measures. Finally, early alerts and clear communication of key research findings related to pollinators are also needed to allow fast and effective decisions.

In this context, the creation of a dedicated working group was approved by the OECD Working Group on Pesticides (WGP) in May 2010. The objective given to this

"Pesticides Effects on Insect Pollinators" (PEIP) Expert Group is to develop a toolbox for risk assessment, risk management, incident reporting, and research about pollinators, focusing on honey bees and non-Apis bees and on the specific case of pesticides as potential factor of stress.

MOPC6-3**EFSA Opinion on the science behind the development of a risk assessment guidance of plant protection products on bees (*Apis mellifera*, *Bombus* spp. and solitary bees)**F. Streissl¹, R.L. Luttik², C.S. Szentesi¹, D. Auteri¹¹EFSA, Parma, Italy²RIVM, Bilthoven, Nederland

A decline in pollinators was reported from several different regions of the world. Pollination is a very important ecosystem service for food production and maintenance of biodiversity. Pesticides are one of the factors that may contribute to the decline of pollinators observed worldwide. This has led to concerns that the current risk assessment for pesticides need revision. As a response to this regulatory challenge the European Commission tasked EFSA to develop an updated Guidance for pesticide risk assessment and bees. The opinion on the science behind the risk assessment for bees analysis the existing test protocols and risk assessment schemes. It gives recommendations on how to assess cumulative and synergistic effects and how to assess the risk of substances which are highly toxic to bees such as neonicotinoids. The approach of definition of specific protection goals is used the first time in the context of developing pesticide risk assessment. The present opinion of the PPR panel provides the scientific basis for the final Guidance Document of EFSA.

MOPC6-4**Activities of ICPBR in the context of the risk assessment for pesticides to bees**A.O. Alix¹, H.M. Thompson², G. Lewis³, J. Pistorius⁴, G. Smagghe⁵, K. Wallner⁶, J. Wassenberg⁷¹Dow AgroSciences, Abingdon, United Kingdom²The Food and Environment Research Agency, York, United Kingdom³JSC, Harrogate, United Kingdom⁴Julius Kuhn Institute, Braunschweig, Germany⁵Ghent University, Ghent, Belgium⁶Universität Hohenheim, Stuttgart, Germany⁷CTBG, Wageningen, Nederland

The International Commission for Plant-Bee Relationships (ICP-BR) organizes working sessions in Europe on the harmonization of methods for testing the toxicity of pesticides to bees for over 30 years. ICP-BR develops the scientific process underlying decisions from European administrative authorities, EPPO (European and Mediterranean Organization for Plant Protection) and OECD (Organization for Economic Cooperation and Development). ICP-BR Bee Protection Group symposia have acquired considerable authority in the area of legislation and regulation concerning bee protection related to the use of plant protection products, bringing together the European expertise of national authorities, industry, research and beekeepers. The Bee Protection Group held its first meeting in Wageningen in 1980 and over the subsequent 32 years has become the established expert forum for addressing the risk of pesticides to bees. It has operated by reaching consensus amongst a wide range of experts active in this field drawn from industry, regulatory authorities and research institutes across the European Union (EU). Operating through the EPPO honey bee sub-group, it has produced the testing methodology and risk assessment guidance currently used under Directive 91/414/EEC and Regulation 1107/2009/EC. Returning to Wageningen in November 2011, this meeting was the most recent event where research in the area of pesticide effects on bees was presented and where implications for risk assessment were discussed and taken on board in working group activities. This presentation aims at giving an overview of ICP-BR activities with an emphasis on each of the seven working groups (testing effects on bee brood; semi-field and field testing; acceptability of effects in testing; statistics; risks related to sowing dusts; risks of guttation and monitoring studies) activities.

MOPC6-5**Review of higher tier methods for assessment of the risk of pesticides to honey bees**

J. Miles, A. Alix, V. Kramer

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Risk assessment procedures have been developed for domesticated honeybees (*Apis mellifera*) potentially exposed to residues of insecticides. After initial tier I screening risk assessments further studies may be conducting for those substances for which a potential risk cannot be excluded. Higher tier studies are often performed in cages or tunnels containing colonies of honey bees which can forage on treated crops. Cage test are typically smaller in size than tunnels but allow for the testing of more treatments often with more replication than can be offered by the larger scale tunnel tests. Field tests can be conducted to establish the effects of the product under more realistic conditions but due to use of free flying colonies there is less control than in the tunnel and cage studies. Finally, post-registration monitoring can offer additional information on specified uses of the product under commercial conditions and can be used to give feedback on the outcome of the risk assessment and the effectiveness of any risk management practices put in place to protect bees. This paper will review the various higher tier methods with respect to their ability to accurately predict the potential effects of pesticides to honey bees and how they can be employed in a robust risk assessment. The range of parameters investigated typically include; mortality, foraging, behavior, brood and colony

development. The ability of higher tier studies to predict potential adverse effects will be evaluated and how these parameters can be employed in a risk assessment with clear decision making linked to honey bee and pollinator protection goals.

MOPC6-6

Assessing the comparative risk of plant protection products to honey bees, non-target arthropods and non-apix bees

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Risk assessments are conducted for plant protection products (PPP) with respect to potential impacts on non-target species. These include pollinators such as the honey bee but also to other non-target arthropods (NTA). In common with other areas of ecotoxicological risk assessment sentinel species are employed aiming at ensuring a high level of protection/conservatism. Tier I screening risk assessments are intended to rapidly exclude those substances which pose a low risk to non-target organisms and to focus resources on those for which a potential risk cannot be excluded and further studies may be undertaken to characterize the conditions and occurrence of risks. In the European Union a Hazard Quotient (HQ) approach is used to assess the risk to both honey bees non-target arthropods at Tier I. This is calculated by dividing application rate by the LR50 (Lethal Rate 50). In other regions such as North America, a contact toxicity trigger of 11 µg active substance/bee is currently employed.

At the Pellston workshop it was suggested that for a risk assessment for sprayed products the honey bee could be a suitable surrogate species. However, to account for potential differences in the sensitivity between the honey bee as a test organism and other non-Apis bees a safety factor of 10 (for interspecies differences) was suggested. On the basis of ecotoxicological data of the European data sets for honey bees and NTA, the relative risk of PPP to NTA, honey bees and non Apis bees, as depicted by HQ values is compared and where possible data on NTA pollinators (e.g. Syrphids) and non-Apis bees (e.g. *Bombus* sp.) are also included. At tier I using HQ trigger of 50 for honey bees, 2 for NTA, in accordance with the current EU legislation, and 5 (i.e. 50 divided by the extra factor of 10 abovementioned) for non-Apis bees, the NTA scheme identified more compounds and uses to move forward for further evaluation. The suggest non-Apis HQ trigger of 5 gave a similar pass / fail rate to the NTA scheme but was slightly confounded by a lack of determined end points. However, even taking this into consideration the most restrictive tier I assessment was that for NTAs. This poster examines the potential for a tier I risk assessment to cover NTA, honey bees, non-Apis bees and other arthropod pollinators as part of a tiered risk assessment scheme and compares the empirical HQ approach with methods using more specific species exposure estimates.

ET03P - Animal Alternatives: methods, endpoints, and testing strategies

TH 001

Cartilage and bone malformations in the head of 6 day old zebrafish embryos after exposure to hydrazides and hydrazines

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In terms of alternatives to animal testing, the zebrafish embryo test (ZFET) has received much attention as a refinement or even a replacement for the acute fish toxicity test (OECD TG 203). Using the ZFET, not only acute mortality can be investigated, but also specific sublethal alterations can easily be observed. Hydrazides and hydrazines are well known for their diverse biological effects. The toxicological and medical potential of both chemical classes on animals and humans was already identified at the beginning of the 20th century. Fertilized zebrafish embryos were exposed semi-statically to isoniazid, benzhydrazide, ben-zylhydrazine and phenylhydrazine for 6 days, fixed in paraformaldehyde and stained as whole mounts. The range of toxicity is very broad; after 144 h, the LC50 of isoniazid is about 4 g/L, whereas those of benzhydrazide, benzylyhydrazine and phenylhydrazine are about 50, 10 and 1 mg/L, respectively. All four chemicals caused wave-like deformations of the notochord and cartilage malformations at sublethal concentrations. Even at the lowest test concentrations, exposure to all test chemicals resulted in notochord breaks, whereas the amount of breaks diminished with increasing concentrations, and the rate of undulations of the notochord became more pronounced. Despite the different variable extent of acute toxicity, the dose-dependent malformation of cartilage and decrease of ossification were comparable between all test substances. The cartilages of the neurocranium, e.g. the ethmoid and basal plate, proved to be more stable than cartilages of the pharyngeal skeleton such as, e.g., Meckel's cartilage and the ceratohyal. Ossifications are much more susceptible to the test compounds than cartilages, with reduction of bone mass as the most prominent alteration.

TH 002

Use of 'moribund' stage in the OECD fish acute toxicity test and its effects on suffering and LC50-values

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In the acute fish toxicity test according to OECD (Organisation for Economic Cooperation and Development) Guideline 203, LC50 is assessed in terms of the concentration of a test substance at which 50% of the fish die within an exposure period of 96h. The criterion for death is defined as (§ 19): 'no visible movement, no response when touching the tail.' By using the criterion of 'moribund' to replace the 'death' criterion, the duration of suffering could be reduced. Fish with severe sublethal effects might be declared 'moribund' (there is no official definition for "moribund" stage in fish), and are removed from the test as soon as this occurs (premature discontinuation of experiment). The criterion has already been introduced to ecotoxicological testing in the UK, and is being used by some laboratories in Germany and Switzerland. "Moribund" fish affect main study outcomes as the LC50 derived on fish declared as "moribund", the LC50 moribund, may be lower than the conventional LC50. This was evaluated by a retrospective analysis of 328 fish acute toxicity tests of an industry laboratory based on five different definitions of 'moribund', and of 111 tests from ten other laboratories from Europe and the US. Using the criterion of 'moribund' affected up to 79% of the studies. The LC50 moribund was lower than the conventional LC50 in up to 52% of the studies depending on the definitions of "moribund". Based on the results of the present study, a successful inclusion of the 'moribund' criterion in an updated OECD guideline for the acute fish toxicity test would reduce the period of suffering by up to 92h and lower the value of the main toxicity endpoint generally by a factor of about 2; maximal by a factor of about 16.

TH 003

Screening of the estrogenic activity of environmental samples using transgenic cyp19a1b-GFP zebrafish embryos

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Due to the potential environmental hazards on reproduction and population development in the aquatic ecosystem by estrogenic substances, their hazard assessment play a paramount role for regulation of chemicals, biocides, pesticides and pharmaceuticals in the EU and worldwide. Furthermore, they are also highly relevant for biomonitoring within the EU Water Framework Directive. For the assessment of their potential adverse effects on the environment, biological test systems play an essential role. The identification of endocrine disrupters with the chemical analysis is limited due to the low but still biologically relevant concentration in the surface waters. Besides, assessing the environmental hazard of mixtures of endocrine disrupters through classical monitoring is not only expensive but also not easily predictable. For this reason, effect-directed analysis using bioassays can be an excellent supplement of the classical monitoring of aquatic ecosystems.

Transgenic models appear to be a powerful tool for high- throughput screening of changes in the gene expression induced by estrogenic chemicals. Using the zebrafish cyp19a1b-GFP transgenic embryo, which expresses GFP under the control of the zebrafish cyp19a1b promoter, adds the complexity of a full organism to the simplicity and reproducibility of cellular reporter gene assays.

In this study we present the first results using this transgenic line for effect-directed analysis of a contaminated sediment sample. In this sample from the Czech river Bilina a previous analysis using the yeast screening assay has identified fractions containing estrogenic compounds. We were able to confirm this study by means of the transgenic cyp19a1b-GFP zebrafish strain. The results were also validated by artificial mixtures of compounds, which were identified by the effect-directed analysis, resembling the compositions of the fractions. Our study also includes data on the validation of the transgenic zebrafish strain with known estrogenic substances and its confirmation using molecular and an in-vitro method. The transgenic cyp19a1b-GFP in-vivo assay proved to be an excellent screening method with high sensitivity and reproducibility for environmental samples.

TH 004

Micronucleus and alkaline comet assays in Japanese Medaka larvae. Integrative methods for sensitive detection of genotoxins in aquatic ecosystems

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Pollutants accumulated in aquatic compartment can induce several adverse effects on living organisms. Among these, some are known to be genotoxic and able to induce mutations and tumour development. To assess and monitor aquatic pollutants and their effects, fish embryos and larvae are suitable models due to their chemical sensitivity and easiness to breed. Micronucleus and alkaline comet assays are two genotoxicity assays allowing DNA damage analysis at different steps of mutagenesis. Comet assay detects repairable DNA damage as single/double DNA strand breaks and alkali labile sites. Micronucleus assays (MN) detects non-repairable DNA damage such as aneugenic and clastogenic lesions. This work develops an integrative and sensitive micronucleus assay in Japanese medaka at larval stage. MN responses were compared to alkaline comet assay responses.

Japanese medaka, *Oryzias latipes* embryos were exposed by immersion during all embryonic development to different concentrations of three model genotoxic compounds: cyclophosphamide, benzo(a)pyrene and cadmium chloride. Twelve 2dph-larvae per replicate were used to prepare cell suspension using enzymatic and mechanic dissociation. Both assays were performed on the same cell suspension. Comet assay was processed according to a previously validated protocol on this species. MN assay protocol was optimized to reduce number of larvae required and improve cell isolation, viability and concentration. MN slides were stained with acridine orange.

Compared to most of the MN assays performed on erythrocytes, this method is integrative and takes into account the whole organism response to genotoxic compounds. It enables an overview of genetic damages accumulated throughout the all embryonic development and in the all body. Moreover, as ethical future of animal health and welfare, number of individual required for experiment is small and assay is performed at sac-fry stage (not consider by EU animal experimentation legislation). This assay can be easily included in the Japanese medaka embryo-larval assay MELA.

TH 005

Effects of propylparaben during medaka (*Oryzias latipes*) embryo development using in vivo morphometrical/quantitative approaches

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Parabens are antimicrobial preservatives widely used in personal care and food products. Among them, the most used are generally methyl and propylparaben (PB) which in turn seem to be the most detected in aquatic ecosystems. Besides the numerous reports about the in vitro and in vivo estrogenic activity of PB and other parabens, relatively few studies have been conducted that determine sublethal effects to aquatic organisms. In this sense, the use of fish embryos and cleutheroembryos are promising alternatives to existing ecotoxicity tests without reducing the validity of the risk assessment. These stages also provide the application of in vivo methods for the analysis and quantitation of biomarkers indicative of exposure/toxicity. Hence, we propose a battery of non-invasive morphometrical tools to examine physiological and anatomical features in embryos and cleutheroembryos of medaka (*Oryzias latipes*) so the effects of waterborne exposure to PB (40-4000 µg/L) can be investigated. Preliminary findings show PB ≥ 400 µg/L resulting in significant quantitative differences, although these exposure effects may not necessarily compromise fish normal development. Further studies will be needed to ascertain whether these effects may result in long term toxicity. This work is being made possible thanks to Spanish Government Grants RTA2010-00004-C02 and CTM2010 19779-C02.

TH 006

Evaluation of genotoxicity of sediment samples using zebrafish embryo and comet assay

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Genotoxic hazard estimation of sediment-bound anthropogenic pollutants is still a challenge since many genotoxicants tend to adsorb onto particulate matter. Testing of extracts usually overestimate risk because bioavailability of the compounds is not considered and enrichment processes like Soxhlet extraction might lead to false positive results due to artifact formation. For genotoxic risk assessment often biomonitoring experiments with in the field caught fish are performed. The in vitro zebrafish embryo model represents an attractive alternative since it offers the possibility to simulate in situ exposure scenarios and is considered as a replacement to animal experiments according to current legislation.

In this work the novel method for determination of genotoxicity induced by particle-bound pollutants is presented. Therefore zebrafish embryos were exposed to whole sediments and DNA damage was assessed by means of the comet assay in whole body cell suspensions (Kosmehl 2006).

Within the joint research project DanTox, funded by the German Federal Ministry of Education and Research (<http://www.bio5.rwth-aachen.de/DanTox>, 2009 - 2012), freeze dried sediment samples from the Rhine River and outer Vering Canal in Hamburg were investigated. Various exposure scenarios were applied and varied from 0 to 72 hours post fertilization.

Preliminary results showed a high intra-assay variability of DNA damage since a heterogeneous cell mixture from homogenized tissues was obtained. Nevertheless, the in-vitro fishembryo model turned out to be a sensitive tool for detecting total genotoxic hazardous potential of sediments.

Even though it should be considered that the egg chorion represents a selective barrier for toxic compounds. As well, the metabolic competence of the embryos, which is closely related to the stage of development, demands further investigations by varying different exposure windows.

TH 007

Modeling concentration-response relationships of quantitative gene expression in zebrafish embryo and its application to predict mixture effects

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Chemicals in the environment are rarely found as single compounds but rather as part of (complex) mixtures. Mixtures may provoke effects which are difficult to describe based on the knowledge of effects for single compounds. Over the last decades several models (concentration addition, independent action) have been tested and applied to predict mixtures using apical endpoints like lethality or growth inhibition. However, the effects of mixtures at a molecular level are still poorly understood.

Using a modeling approach we aim to investigate mixture effects on gene expression patterns in zebrafish embryos. Initially we analyzed effects of single substances on the gene expression of specific genes to model concentration-response relationships. Based on these results we will conduct mixture experiments investigating whether or not existing models for apical endpoints can be applied for molecular responses as well. Finally, the modeling approach will be extended to the entire transcriptome level by analyzing the effect of mixtures using microarrays. The overall goal is to apply the findings for a better interpretation of gene expression patterns provoked by exposure to environmental samples.

First results clearly show that gene expression follows monotonous concentration-response relationships. However, at higher concentrations (above LC10) we noticed an inhibitory effect and/or highly variable responses. We compared classical toxicological models such as the sigmoidal Hill-slope to peak and other alternative models. To date we have completed the detailed analyses of 4 different chemicals that have been identified by a previous microarray analysis as sensitive and specific markers for exposure to chemicals with different modes of action.

TH 008

An evaluation of the zebrafish embryo model for estrogenicity testing of wastewater treatment plant effluents

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Wastewater treatment plants are known sources of water pollution which may cause endocrine disruption in fish and other aquatic biota. Wastewater treatment plant effluents contain complex mixtures of compounds with various estrogens, xenoestrogens, antiandrogens, and other compounds which may potentiate each other. Therefore to access the overall potential estrogenic effects in fish, it is necessary to test the whole mixture (rather than assessing individual chemicals alone). To measure estrogenic effects in the effluents, we have combined zebrafish embryo model with quantitative RT-PCR. Modulations of three estrogen responsive genes have been evaluated including specifically aromatase B, vitellogenin 1, and estrogen receptor α . The effects have been tested with series of waste water treatment plant effluent samples collected during the pan-European monitoring campaign (coordinated by the JRC Ispra, Italy), and the results obtained with the zebrafish bioassay have been compared to comprehensive analytical data of various contaminants. The present work contributes to the characterization of the zebrafish model as a tool for investigation of in vivo estrogenicity in complex contaminated samples. [The work has been supported by Brno PhD Talent Financial Aid to Adam Jonáš and the projects INCHEMBIOL (MSM0021622412) and CETOCOEN (CZ.1.05/2.1.00/01.0001)].

TH 009

The normal development of the fathead minnow

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The fathead minnow (*Pimephales promelas*), the zebrafish (*Danio rerio*) and the Japanese medaka (*Oryzias latipes*) range among the most commonly used fish species in aquatic toxicity testing. However, acute fish tests (OECD TG 203) require the use of large numbers of test animals. In order to reduce the number of experimental animals, attempts are underway to establish the zebrafish embryo test (ZFET) as an alternative to conventional acute fish toxicity testing. Whereas the ZFET is currently undergoing OECD validation, fathead minnow embryos have only scarcely been used for embryo toxicity testing. Prior to the use of the fathead minnow in a standardized embryo test, it is important to clearly identify those stages during development from fertilization to hatching and beyond which are of toxicological relevance. Based on fathead minnow-specific developmental data, the embryo toxicity test was adapted and refined for the fathead minnow embryo. Fathead minnow staging was performed in petri dishes containing dilution water (prepared according to OECD TG 203 Annex 2, 1992) at 25 ± 1 °C. Pictures were taken every 5 minutes between 0 - 3 hpf and every 30 minutes from 3 to 12 hpf. Pictures of embryos older than 12 hpf were taken every 60 minutes. After initiation of spontaneous movements (approx. 26 hours post-fertilization (hpf)), the embryos were dechorionated using 2 mg/ml pronase solution and anaesthetized with 0.016 % tricaine solution. In fact, it turned out that most of experimental parameters could be kept identical to those used in the ZFET. Using the fathead minnow embryo test, LC50 data were generated for cadmium chloride, a substituted aniline (3,4-dichloroaniline, which is currently used as the positive control in the ZFET) and a substituted phenol (2,4-dichlorophenol). Fathead minnow embryos proved to be slightly less sensitive for cadmium chloride and definitely less sensitive for 3,4-DCA than adult fathead minnow (TG 203), whereas for 2,4-DCP no difference in sensitivity could be detected. However, the comparison with the ZFET showed that zebrafish embryos are significantly more sensitive. For all three substances, toxicity increased after hatching (≥ 96 and 120hpf). Differences in toxicity the fathead minnow and the zebrafish embryo tests might be due to differences in the chorion (thickness, structure, quantity and size of pores).

TH 010

A modified approach for the determination of bioconcentration factors (BCF) in fish

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The method for the determination of bioconcentration factors (BCF) given in OECD guideline 305 builds on the exposure of adult fish to a dilute aqueous solution of chemicals at a constant concentration. Thus, numerous samples of water and fish tissue have to be analyzed to calculate the BCF. The number of fish killed during the test is high, the test can take several weeks and is expensive. Due to the high number of fish used for BCF determination, many alternative methods have been developed with the aim to facilitate the screening for BCF, e.g. with fish larvae or with fish eggs and 14C-labeled compounds.

In the ProDarT project funded by the German Federal Ministry of Education and Research (BMBF, project no. 315399), the Helmholtz Centre for Environmental Research (UFZ) and the laboratories of the Federal Environment Agency (UBA) made a further attempt to determine BCF and to further reduce both effort and the total amount of test animals. Moreover, a method of sample preparation prior to gas chromatography-mass spectrometry analysis (GC-MS) was developed. So far the approach has been tested

with phenanthrene, chlorpyrifos and methyl triclosan.

Aqueous stock solutions of the chemicals were prepared in pure water. An aliquot of each stock solution was diluted in well aerated artificial freshwater according to DIN EN ISO 7346-3. Fertilized eggs of the zebrafish (*Danio rerio*) were exposed in the dilute solutions at 26°C. At the start of exposure and also 1, 6, 24, 30, and 48 hours later the fish embryos were investigated for lethal and sublethal toxicological endpoints. Furthermore, samples of the aqueous medium were taken. The compounds were extracted by solid phase extraction (SPE), and analyzed with GC-MS. Samples of fish eggs were taken at 24 and 48 hours after the start of exposure. The eggs were smashed in ethanol and treated in an ultrasonic bath. These extracts were concentrated and analyzed with GC-MS. Concentrations in the aqueous medium were compared with the residues detected in fish eggs and degradation curves were calculated.

TH 011

Exploring animal alternatives: seeking a replacement for whole effluent toxicity testing in fish

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In the United States (US), any facility discharging effluent directly to surface waters must conduct periodic evaluations of whole effluent toxicity (WET). One method commonly used to obtain WET data is the 7 day larval growth and survival test (LGS) in which newly hatched fathead minnows (FHM), *Pimephales promelas* are exposed to effluents for 7 days with mortality as an endpoint. Given the importance of animal welfare in toxicity testing, the current study aimed to identify a test method that is able to fulfill the regulatory requirements for WET data, while minimizing the use of protected organisms or life stages. One possible alternative is the fish embryo toxicity test (FET). The FET has already been sanctioned for WET testing in the European Union (EU) using embryonic zebrafish (ZF, *Danio rerio*); however, for the FET to be adopted in the United States, it must be applicable to species commonly used in contract laboratories (i.e., FHM) and demonstrate comparable sensitivity to the LGS. Thus, the main objectives of this study were 1) to develop test methods that harmonize the FHM-FET with the ZF-FET and the ZF-LGS with the FHM-LGS and 2) to compare the sensitivity of the FET and the LGS using both FHM and ZF. To accomplish the first objective, tests using existing protocols for the FET and LGS were carried out with both species under different temperature and feeding regimes in order to determine optimal test conditions. The existing protocols were then appropriately modified and applied to the remaining experiments. To address the second objective, lethal (e.g., NOEC, LOEC, EC50) and sublethal (e.g., length, weight, deformity frequency, molecular) endpoints were evaluated following FET and LGS testing of each species using the reference toxicant 3,4-dichloroaniline. These results provide evidence for the ability of the FHM-FET to serve as an alternative to the FHM-LGS for WET testing carried out in the US. The adoption of FET testing for this purpose would result in the use of an earlier life stage to obtain sub-chronic toxicity data, representing a refinement of current test methods in the spirit of the three R's.

TH 012

Final results of the OECD validation study on the transferability, intra- and inter-laboratory reproducibility of the Zebrafish Embryo Toxicity Test

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The OECD Acute Fish Toxicity Test Guideline (TG 203) is an integral component in the environmental safety assessment of industrial chemicals, agrochemicals, pharmaceuticals, feed stuffs, and biocides. One of the most promising alternative approaches to the acute fish toxicity test is based on the use of zebrafish embryos. In 2005, the German Federal Environment Agency submitted the draft TG on 'Fish Embryo Toxicity test (FET)' to the OECD Test Guideline Program and a supportive Draft Detailed Review Paper. Subsequently, the OECD established the ad hoc Expert Group on the Fish Embryo Toxicity Test. Based on the outcome of expert meetings, OECD decided to perform a validation study (coordinated by ECVAM and steered by a validation management group). The validation study aims at evaluating the transferability, and the intra-/interlaboratory reproducibility of the Zebrafish FET (ZFET). Newly fertilised zebrafish eggs (20/concentration and control) are exposed for up to 96h to chemicals. Four apical endpoints are recorded daily as indicators of acute lethality in fish: coagulation of the egg, lack of somite formation, non-detachment of the tail bud from the yolk sac and lack of heart-beat. LC50 values are calculated for 48h and 96h exposure. During the validation, 20 chemicals were tested at 5 different concentrations in 3 independent runs in at least 3 laboratories with appropriate controls. Stock solutions and test concentrations were analytically confirmed for 11 chemicals. The poster will give an overview on the validation study design, the results and the correlation of the ZFET with acute fish LC50 data.

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TH 013

Genotoxic effects of sediment extracts and native sediments from Hamburg harbor and the Rhine River in *Danio rerio*-embryos

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The European Water Framework Directive (EWD) aims to achieve a good ecological and chemical status in the surface water of European rivers. However, since particulate matters and sediments are highly relevant as secondary sources for environmental pollutants, applied sediment toxicology plays an important role to ensure the successful implementation of the goals of the EWD. The present study is part of the joint research project DanTox, which - among other specific endpoints - investigates genotoxic effects of sediment extracts in zebrafish (*Danio rerio*) embryos. The comet assay is a widely used bioassay gaining increasing popularity as a screening test for genotoxicity. However, since the ecological significance of the comet assay results is not clear yet further correlations with biotests with definite endpoints such as the micronucleus assay are recommended. Within this study both biotests will be compared, in order to gain a reliable genotoxic assessment of environmental samples.

Sediment samples were taken from the Rhine River (Ehrenbreitstein and Altrip) and Hamburg Harbor (outer Vering Canal). After Soxhlet extraction of freeze dried sediments embryos were exposed to the samples for 24, 48 and 72 hours post fertilization (hpf) as well as for a time-window between 72 and 96 hpf according to the standard fish embryo test DIN 38415-6. Single cells were isolated from zebrafish embryos using a glass/glass tissue grinder and used in the comet assay as well as in the micronucleus assay. Maximum test concentrations causing no lethality were derived before using the fish embryo toxicity test.

The results of the comet assay showed a significant increase of DNA-damage for both sediment extracts compared to the negative control after 72 hpf exposure. At present it was neither possible to determine a time-depending difference nor to measure a concentration-depending reaction. The results of the micronucleus assay will be available at the SETAC world-meeting in Berlin.

To achieve the goals of the European Framework Directive it is of particular interest to establish a combined vertebrate-based sediment contact assay with different biological endpoints (e.g. embryotoxicity, genotoxicity and AhR-mediated toxicity) to determine the ecotoxicological effects of sediment-bound pollutants. This part of the DanTox project will help to identify and characterize genotoxicity of sediment extracts from the Rhine River and Hamburg Harbor.

TH 014

The threshold approach for acute fish toxicity testing of formulated plant protection products: a proposed framework

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The OECD has recently published guidance on the use of the threshold approach for fish acute toxicity testing. Essentially the approach uses a limit test at a single threshold concentration determined by the results of *Daphnia* and algae tests. If no mortality is observed in the limit test the fish acute value can be expressed as greater than the threshold value. However, if mortality is observed a full concentration-response test is triggered. To assess applicability to plant protection formulated products, Syngenta constructed a database of 185 products (fish, *Daphnia* and algae endpoints) and retrospectively applied the threshold approach. This representative dataset demonstrates that a reduction of approximately 38% of the fish might be achieved. Furthermore, the approach also provides a refinement as in 76% of threshold tests no mortality would be observed and in 64% of cases there would be no toxic effects at all, including sub-lethal symptoms. However, there is a need for regulatory acceptance of this method particularly in relation to the use of the data in risk assessment, where a fish specific assessment is required and approaches may require a better defined estimate of fish acute toxicity. Consequently, the UK NC3Rs held a workshop in December 2010, bringing together industry and a number of European regulators to discuss the applicability of the approach. The workshop discussed a potential framework for applying a threshold type approach, incorporating all the available information including relative fish sensitivity, data on the component active ingredients, formulated product information (*Daphnia* and algae) and potentially the Predicted Environment Concentrations. Such an approach may be used to better define a fish threshold approach that is workable within the EU regulatory framework for Plant Protection Products.

TH 015

Different test conditions for hydrophobic and fugitive substances in the Fish Embryo Toxicity Test for a reliable risk assessment

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Background: This study is part of the DanTox project, which aims to identify specific toxicity and molecular modes of action of sediment-bound pollutants and selected primary pollutants using zebrafish embryos. Ecotoxicological investigations of volatile and hydrophobic chemicals in tests with aquatic organisms are difficult to conduct, since they evaporate from the water and/or adsorb to plastic surfaces. This could lead to substance loss during exposure, which can cause an underestimation of their ecotoxicological hazard potential. In order to optimize test conditions for such chemicals, the aim of this study was to compare embryotoxic effects caused by selected chemicals under static and semi-static test conditions in glass and plastic vessels.

Material and methods: The embryotoxicity of three selected substances (benzofurane, quinoline and chlorpyrifos) were tested in plastic and glass vessels under static and semi-static conditions using the fish embryo toxicity test with zebrafish (*Danio rerio*). For semi-static tests, the solutions were exchanged every 24 h. Embryotoxicity were observed after 24, 48, 72 and 96 h post fertilization (hpf).

Results and discussion: Benzofurane showed in glass vessels a significant higher mortality under static than under semi-static conditions after 72 hpf. In contrast, tests in plastic vessels had a slightly higher toxicity under semi-static conditions, which was not expected. Quinoline showed in glass vessels a slightly higher toxicity under static than under semi-static conditions and a slightly higher toxicity under semi-static conditions in tests conducted in plastic vessels. In contrast to benzofurane, quinoline showed minor differences in toxicity with increasing exposure time. Differences in toxicity between quinoline and benzofurane are probably based to different vapour pressure as well as Henry's law constant. Chlorpyrifos showed only a low mortality (< 30 %) under all test conditions.

Conclusion: Only some significant differences, but mostly just tendencies between the different test conditions were recorded. Thus, no precise recommendation can be given for a reliable risk assessment for selected volatile and adsorptive heterocycles. However, for further investigations chemical analyses are required to determine the difference between nominal and measured concentrations under all test conditions.

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TH 016

A quantitative and mechanism specific toxicity assessment for the fish embryo test, using fluorescent zebrafish

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The zebrafish embryo toxicity test zFET is currently the most advocated animal alternative test in ecotoxicology. To date, the application of the zebrafish embryo toxicity test zFET has focused on acute toxicity assessment, where only lethal morphological effects are accounted for. An application of the zFET beyond acute toxicity, however, necessitates the establishment of more refined and quantifiable toxicological endpoints. A valuable tool in this context is the use of gene expression-dependent fluorescent markers that can even be measured in-vivo. The fluorescence visualises specific cells or morphological structures, which can help elucidating the underlying toxicological mechanisms and enhance the detection of adverse effects and the sensitivity and specificity of the conventional zFET.

This project investigates the benefits of using fluorescent marker applications on zebrafish to study chemical toxicity on vascular, myotomal and neuronal development, thereby improving the zFET.

The transgenic line Tg(fli1:EGFP)y1 expresses enhanced GFP in the entire vasculature under the control of the fli1 promoter, and thus enables the visualisation of vascular defects in live zebrafish embryos. We assess the fli1 driven EGFP-expression qualitatively and quantitatively, and found an exposure concentration related increase in vascular damage for chemicals like triclosan, cartap and genistein. The effects are now being confirmed by QPCR. We additionally explored whole-mount immunofluorescence to visualise myotomal defects, using a monoclonal antibody staining specifically myosin in the embryonic muscle fibres. These investigations showed a chemical concentration depended change in muscle morphology for cartap and genistein, what also could be quantified and declared as a robust parameter. Presently, another transgenic zebrafish line is being evaluated, expressing GFP coupled to the glial fibrillary acidic protein (GFAP) gene. The Tg(gfap:GFP) embryos promise the identification of neurotoxic chemicals in the zFET in-vivo, and first data will be presented.

The use of fluorescent transgenic lines and antibody staining enables the detection of chemically induced vascular, myotomal and neuronal malformations in the embryos, which were otherwise not visible in bright field assessment. The fluorescent signal based methods allow a sensitive and quantitative effect assessment, which will broaden the scope and minimize the data variability of the zFET.

TH 017

Toxicity of chlorine-produced oxidants to Arabian killifish embryos for acute developmental stage exposures

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Fish embryos are excellent models for studies aimed at the understanding of toxic mechanisms and the indication of possible acute and chronic effects. Therefore, we present the use of Arabian killifish (*Aphanius dispar*) embryos, an indigenous species to the Arabian Gulf, to study the effect of chlorine-produced oxidants to marine organisms in the Qatari Coastal Area. The objective of this study is to develop chlorine toxicity data for the marine Fish Embryo Toxicity (mFET) test. The test is designed as a means to replace or refine the use of marine juvenile and/or adult fish in standard approaches evaluating toxicity of chemicals and effluents. Embryos were collected from a breeding stock of sexually mature Arabian killifish. Testing was initiated as soon as possible after fertilization of the eggs with exposure to aqueous concentrations of calcium hypochlorite for up to 240 hours. The investigated endpoints included; coagulated eggs, somite development, heartbeat, tail detachment, hatchability and post hatch mortality. The results showed a developmental stage-dependent response to chlorine. During earlier developmental stages, chlorine had reduced effects on the embryos and the survival rate and hatchability were high, even at relatively high concentrations. In later developmental stages (pre to post hatch, eleutheroembryo), the embryos were significantly more sensitive to chlorine than in the early stages of development. The chorion, (membrane surrounding the egg), is believed to provide a barrier against chlorine in early stages of embryo development. Taking into account the findings presented here, Killifish embryos exhibit the ability to be an indicator organism for environmental risk assessments of the Qatari Coastal Area. Benefits include, animal alternative, ease of fish breeding, clarity of the embryos, reduced sample size, reduced waste generation and shorter study duration.

TH 018

Sub lethal effects of methyltestosterone in adult zebrafish (*Danio rerio*)

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Methyltestosterone (MT) is a synthetic hormone used in fish-farming to induce male monosex in *Oreochromis niloticus*. In Brazil, the commercial hormone is illegally used as it is not regulated by national authorities for these purposes. In this study, we aim to evaluate the sub lethal toxicity of commercial MT (cMT) and its active ingredient 17 α -methyltestosterone (aMT) to adult zebrafish. Comparison between the two compounds (cMT and aMT) is relevant for the regulation of MT in Brazilian aquaculture. For this purpose, micronucleus and other nuclear abnormalities were chosen as genotoxicity markers and measured on peripheral erythrocytes cells of zebrafish and cholinesterase-ChE, lactate dehydrogenase- LDH and glutathione-S-transferase- GST were chosen as biochemical markers and measured in different fish organs. Fishes were exposed during 4 days at concentrations between 4 and 5000 μ g/L of cMT or aMT.

Results showed that the most responsive biomarker tested was GST with a dose-dependent inhibition verified in several organs. GST, as a family of enzymes involved in the general process of detoxification of xenobiotics with electrophilic centers, is expected to be induced under situations of stress; however inhibition, as verified in the present study, is also described in literature for several types of compounds. Differences between males and females observed concern not only the type of tissue that was responsive but also the sensitivity (in general males were more sensitive than females). Regarding MT types, cMT seems to elicit a response at lower doses. This trend was also observed for head ChE for which an inhibition was observed (indicating a possible disruption of nervous system by MT) although a dose dependent pattern was not observed. LDH response presented high variability and thus, no conclusions can be drawn on the effects of MT in the anaerobic pathway of energy production.

Overall, results indicate that important effects of MT exposure are observed at sub lethal level that should be assessed in more detail in a perspective of risk assessment of MT use to non-target organisms. Moreover, differences between males and females responses should be taken into account, since different physiological mechanisms of response may be occurring in aquatic organisms affected by MT effluents. Finally, the apparent higher effectiveness of cMT should be carefully assessed so that proper legislation can be established for MT use in aquacultures.

TH 019

Comparative sensitivity of different early life stages of zebrafish to copper and NaCl

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Aquatic contamination is a major constrain to the health of fish in freshwater ecosystems. Especially metals, such as copper, can be extremely toxic even at low concentrations. Additionally, due to climate change, future environmental alterations are expected as it is the case of increasing salinity derived from sea level rise, which can also affect aquatic ecosystems equilibrium. Furthermore, during their early development fish suffer several morphological and physiological modifications and thus, it is crucial to make a proper evaluation to investigate how these stress factors can affect the different life stages. Accordingly, this work aimed to assess the sensitivities of different early life-stages of *Danio rerio* to copper and to NaCl (to simulate increased salinity). For this, embryos (2h hpf) and larvae (96h hpf) of zebrafish (*D. rerio*) were exposed to copper sulphate and NaCl and several morphological and functional endpoints were monitored during a 96h period. In the first experiment, embryos were exposed to copper (4.78 - 39.88 μ M) and NaCl (1.85 - 26.27g/L); in the second experiment, larvae were exposed to the following range concentrations of copper (0.45 - 4.7 μ M) and NaCl (5 - 8.75g/L). Dose dependent mortality was observed for both stages under exposure to copper and NaCl, however, the larval stage was more sensitive to copper and to NaCl than embryos. Moreover, in the embryo assay with NaCl, it was only observed significant mortality after the 96h. The protective enclosure of embryos could be one of the major factors contributing for their highest resistance. Even so, both copper and NaCl were responsible for other significant sublethal effects on embryos. Copper had an inhibitory effect on hatching and caused a reduction on body length and on the other hand, NaCl caused several tail deformities and edemas. These adverse effects on embryos can, as mortality, compromise the long term survival of these organisms. It is important to note that copper was the stress factor where the differences between sensitivity of embryos and larvae were more pronounced. Obtained results suggest that toxicity of copper and NaCl can be very different depending on the life stage. Also, several sublethal effects must be taken into account to better evaluated the real toxicity of these compounds to zebrafish.

TH 020

Arabian killifish: An indicator model for laboratory environmental risk assessments of the Qatari coastal area

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Fish embryo toxicity (FET) studies can provide an alternative to traditional fish acute toxicity testing. FET methods offer many benefits; they are considered animal alternatives, they require relatively small sample size and reduced time to determine toxicity endpoints. The use of Arabian killifish (*Aphanius dispar*) embryos is proposed as an alternative to using juvenile or adult fish for environmental risk studies in the state of Qatar. The Arabian killifish are small fish about (5 -6 cm) total length, from the family cyprinodontidae. Given the lack of data regarding Arabian killifish embryology and breeding behavior, research was initiated in early 2010 to establish a laboratory fish culture capable of producing high quality embryos in sufficient quantity for toxicity studies. Another important objective was to identify the developmental stages for the Arabian killifish and to understand their relative sensitivity to more established FET species. The standard FET approach that uses Zebrafish (*Danio rerio*) or Medaka was adapted to the Arabian killifish. Behavioral patterns, feeding optimization, diurnal cycle, male to female ratio as well as other factors were studied in the laboratory. Embryo development was observed and documented from fertilization to hatch. Reference toxicant studies were performed and the results compared to other species. A reproducing laboratory culture was established. Embryo development was studied and the key development stages were identified. The results of the reference toxicant testing confirmed similar sensitivity with other species. We have been successful in the production of quality embryos for testing, both in terms of viability (fertilization) and quantity released. The developmental stages of the Arabian killifish have been identified to provide an array of potential endpoints for ecotoxicity studies. Organism sensitivity appears to be within the desired range for vertebrate species. We conclude that the Arabian killifish exhibits the traits needed for successful FET testing and that they have utility as a vertebrate indicator species.

TH 021

Exploring the relationship between initial test medium concentrations and accumulation of organic chemicals in cells/tissues in *in vitro* toxicity experiments using an equilibrium partitioning model

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Reliable toxicity data are a crucial requirement for conducting hazard/risk assessment of contaminants in the environment. However, data are limited or non-existent for a large number of contaminants of potential concern (1). Due to the high cost, time requirements and ethical considerations surrounding *in vivo* animal experiments, it is quite likely that *in vitro* toxicity data will play an increasingly prominent role in risk assessment and setting of exposure guidelines in the future (2,3). While *in vitro* data can almost always provide important mechanistic insight (e.g. mode of action), direct use of such data for assessing the toxicological implications of measured contaminant levels in humans and wildlife requires that cellular concentrations be characterized (as opposed to the initial test medium concentrations, which are typically reported). Several *in vitro* studies have noted that measured cellular concentrations of hydrophobic chemicals are substantially higher than the initial test medium concentrations and stress the importance of relating external to internal levels (4-7). These findings can be rationalized in the context of the phenomenon of bioconcentration, whereby organic chemicals partition preferentially into biological phases (such as lipids) at the expense of the aqueous phase. Other partitioning behaviours of potential relevance in the context of an *in vitro* toxicity test include sorption to the vessel walls, volatilization into headspace and interactions with serum constituents (e.g. proteins) or other dissolved organic matter. To explore the behaviour of organic chemicals in such test systems, these sorption relationships were integrated into a mass balance equilibrium partitioning model which was then applied to a generic test system for chemicals covering a range of physical-chemical property values. Key model outputs include mass distribution, cellular concentrations, aqueous phase concentrations (in comparison to water solubility limit) and external:internal concentration enhancement factors as a function of hydrophobicity. The influence of assumptions regarding the affinity of cells for chemicals (i.e. sorption capacity), and mass of cells:volume of aqueous phase were also explored. While the results are illustrative in nature (i.e. cannot be directly extrapolated to any particular study), they provide useful insights into experimental design (e.g. how to avoid super-saturating the aqueous phase) and interpretation of results.

TH 022

Quantification of chemical concentration in multiwell plate-based assays: requirements, possibilities and challenges

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The use of animals for experimental studies, e.g. toxicity testing of chemicals, gives rise to serious ethical concerns. The development of new alternative procedures and models is therefore timely. Among the most promising alternative methods are cell line- and fish embryo-based assays. Both approaches are commonly conducted using multi-well plates, making them amenable to high throughput screening. Many replicates and very little testing material is required. However, these formats also come with caveats. Because of the small volume, even minute manipulations within culture wells, such as the addition of a test chemical, can be expected to have a significant impact on the dynamics within the well. The high surface to volume ratio, combined with the open nature of the systems, favor losses of test chemicals due to sorption and/or evaporation, leading to a reduced bioavailability of test chemicals. Until today, confirmation of chemical concentrations in culture wells has rarely been done. Although, e.g. the OECD draft guideline for the fish embryo toxicity test requires to check that stable chemical concentrations are maintained during the whole exposure duration. Therefore, we set out to design protocols for sampling, sample preparation and chemical analysis applicable to the small volumes present in multi-well plates for cell line- and embryo-based toxicity assays. Chemical concentrations were determined for stock solutions, dosing mixtures and the exposure medium at the beginning and at the end of exposure taken directly out of the wells. In general, a volume of 500 µL was required for chemical analysis and 24-well plates have been found very suitable to comply to the requirement of sufficient exposure medium. Analytical protocols were developed for 30 industrial organic chemicals. For 20% of the tested compounds the samples could be subjected directly to quantification based on high-pressure liquid chromatography (HPLC) without any further treatment required. For the remaining compounds, simple liquid-liquid extraction of the aqueous sample with cyclohexane was performed prior to analysis by either gas chromatography, HPLC or mass spectrometry. For acute exposures of cells or embryos, accounting for measured chemical concentrations helped to correct for the reduced chemical bioavailability. This resulted in an improved absolute relationship between cell- and embryo-based assays versus acute fish toxicity.

TH 023

Characterising the bioavailability, toxicity and metabolites of benzo[a]pyrene using animal alternatives bioassay

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In vitro methods is anticipated to provide alternatives to animal testing by implementing the 3R's (reduction, refinement and replacement). Several *in vitro* methods have proved to be versatile tools for toxicity assessment and have shown a potential for high throughput screening of chemicals. Primary hepatocytes isolated from livers are commonly used in toxicity testing and are being used for assessment of the bioaccumulation properties of chemicals due to retaining innate cellular properties such as a toxicity targets and biotransformation capability. Although the cells may represent alternatives to use of whole animals in some respects, culturing and exposure of cells in high-throughput formats devoid of the natural toxicokinetics and partition behaviour may introduce experimental artefacts giving rise to deviations from *in vivo* conditions. This applies in particular to compounds that are volatile, hydrophobic and require metabolic activation (bioactivation) which today represent challenges to *in vitro* toxicity assessment. The aim of the present work was to assess the role of bioassay conditions on the bioavailability and toxicity of benzo[a]pyrene (BaP) in a primary culture of rainbow trout (*Onchorhynchus mykiss*) hepatocytes. Assessment of cytotoxicity and classical biomarkers such as EROD induction and CYP1A gene and protein expression was used to characterise the responses to this hydrophobic pro-mutagen and carcinogen under different bioassay conditions. ¹⁴C-labelled BaP was used to determine the partition behaviour and truly bioavailable fraction of BaP under the different bioassay conditions used.

TH 024

Cytotoxic, mutagenic and genotoxic effects of sediments from Czech river basin

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This study summarizes various effects of 14 sediments collected from rivers in Southern Bohemia (Czech Republic). After Soxhlet extraction, chemical analyses of sediment extracts was performed and extracts were further used in toxicity experiments. Fish hepatoma cell line RTL-W1 was used for neutral red cytotoxicity assay and for micronucleus test of genotoxicity. Mutagenicity of tested samples was studied by Ames fluctuation assay.

Most of studied sediment extracts was cytotoxic for RTL-W1 cells, however, only one of them was significantly genotoxic in micronucleus test. Some of tested sediment extracts were mutagenic in Ames fluctuation assay with added S9 fraction. No or only minor effects were seen in this test without S9 addition. Results from toxicological experiments will be further compared with chemical data of sediments and then full results of this work will be presented.

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TH 025

Development and characterization of a piscine intestinal barrier model using the fish cell line RTgutGC

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Background. At present, fish in large number serve as experimental animals for the testing of chemicals and water samples. According to the 3R principles (replace, reduce, refine), our aim is to develop *in vitro* systems which are based on continuous cell lines from rainbow trout (*Onchorhynchus mykiss*). For the present study, the intestinal cell line, RTgutGC, was used to develop an intestinal barrier model for fish. Therefore, cells were grown on filter membrane inserts such that they face two compartments. The compartmentalization allows to create asymmetric exposure conditions as found *in vivo* to provide an environment in favor of cell polarization and differentiation into mature enterocytes. Enterocytes is the main cell type of the intestinal epithelium and responsible for the absorption of nutrients. The cells are characterized by the expression of a brush border membrane and tight junctions *in vivo*. Tight junctions are proteins which join adjacent cells and thus create tight epithelia which present a limiting factor for intercellular toxicant transport.

Methods. The tightness of the developed epithelium was judged by means of transepithelial electrical resistance measurement (TEER). The presence of tight junction proteins was analyzed by immunostaining. Electron microscopy served to prove the state of differentiation of the cells cultured according to different protocols.

Results. Cells were found to build up a multilayer when seeded at high densities (446429 cells/cm²) while seeding of low densities (62500 cells/cm²) led to the desired

monolayer formation. TEER values were shown to depend on cell density. For seeding of high densities, values were in a range between 30 to 70 Ω *cm². For seeding of low densities, TEER measurement resulted in values between 10 to 30 Ω *cm². Immunofluorescence labeling of ZO-1, a protein of the tight junction protein complex, was successful. Cell differentiation, as judged by morphological features, was however not achieved by culturing the cells under asymmetric conditions and by providing them with a selection of substances described to be involved in intestinal cell differentiation.

Outlook. Future studies will focus on strategies to induce cellular differentiation in RTgutGC. The starting-point will be the development of a fish cell specific culture medium, because the currently used culture medium is based on mammalian serum which might inhibit piscine cell differentiation.

TH 026

DNA repair capacities in fish cell lines RTL-W1 and RTGill-W1 and application of a base excision repair comet assay

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In vitro models such as fish cell lines are possible alternative to in vivo fish testing, and new toxicity endpoints such as genotoxicity deserve to be considered in these models.

However fish cell lines are suspected to possess low DNA repair activities, which could influence their sensitivity in genotoxicity testing.

The aim of this study was to evaluate in RTL-W1 and RTGill-W1 cell lines, the repair kinetics of alkylation damage following exposure to methyl methane sulfonate and recovery with or without 3-aminobenzamide (3AB), a BER inhibitor. Both standard and Fpg-modified comet assay have been used. In a second step, a Base Excision Repair comet assay (BERc) has been applied and validated in those cell lines. Its principle is to evaluate the BER activity of cell extract by applying them after the lysis step of the comet assay on a substrate DNA containing a high amount of BER specific lesions. The ability of the extract to generate additional breaks in the DNA at damaged sites is a measurement of the BER activity in the cell extracts.

A significant difference in the level of residual damage in cells recovering with or without 3AB demonstrated BER capacities in both cell lines. The time-course of SSB and DSB repair was similar in the two cell lines and showed a return to the background DNA damage level within 24 hours. The repair of the Fpg-specific lesion follows a similar time-course.

Preliminary experiments allowed us to establish optimum conditions for the BERc assay (density of cell extract, digestion step duration, adequate freezing and storing conditions). The application of the established protocol to both cell lines revealed the efficiency of the initial BER step in those cell lines without significant difference. Exposure to 3AB generated a significant decrease in the incision capacities of cell extracts confirming the specificity of the assay regarding BER activity measurement. Results show that RTL-W1 and RTG-W1 exhibit measurable BER capacities in the same range, and that repair kinetics followed by standard and Fpg-modified comet assay as well as BERc assay are appropriate methods to measure DNA repair in fish cell lines. The BERc assay is however much easier to perform, although it allows to measure only the initial step of DNA repair. Complementary work is in progress in order to validate the use of the BERc as a new genotoxicity biomarker allowing the assessment of the impact of pollutant exposure on DNA repair capacities.

TH 027

Application of PLHC-1 as in vitro bioassay for Ah Receptor active compounds in environmental matrices

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The present study investigates CYP1A response in Poeciliopsis lucida hepatoma cell line (PLHC-1) exposed to soils extracts by looking at gene transcription and EROD activity. The aim is to evaluate the potential application of this fish cell lines as in vitro model for the toxicological screening of environmental samples. Soil samples were collected in two sites (A, B) close to an industrial incinerator. Sample were extracted two fractions (Non Purified and Purified) were collected. GC/MS analysis was performed to detect levels of priority pollutants and TEQs were calculated. Modulation of cyp1a gene transcription was measured in cells exposed for 24 h to a model CYP1A inducer as TCDD (1,10,50 pM) and to two dilution of tested extracts (1,10 mg/ml for P and 0.5, 5 mg/ml for NP). EROD activities were measured in cells exposed for 24 h to a range of dilution of TCDD (0-10 nM) and to tested extracts (0-50 mg/ml for P and 0-10 mg/ml for NP) dissolved in DMSO (max 1%). EROD TEQ values were also calculated. TCDD determined a dose-dependent up-regulation of cyp1a gene transcription 15-folds respect to controls at 10pM and 109-folds respect to control at 50pM, thus confirming the sensitivity of the adopted model. A significant up-regulation of cyp1a was observed only at the highest concentration for the purified fraction of both samples A and B (2.1 and 1.8 folds respectively). The non purified fraction of both samples determined a significant dose dependent up-regulation of cyp1a from 2.4 to 15-folds up-regulation for sample A and from 2.1 to 13-folds for sample B. Concerning EROD activity the EC50 measured for TCDD was 17.96 pM. A similar tend of EROD induction has been observed for A NP and B NP, while no modulation of EROD by A P was observed and only a slight induction was observed for B P (maximum of 7.4 pmol/min/mg prot at 50 mg/ml). A higher EROD TEQ values was calculated for A NP compared to B NP. Overall results confirm PLHC-1 cells as highly sensitive model to AHR active compounds as significant effects were observed even at low levels of contaminants. Results from bioassays does not agree with TEQ values calculated based on chemical analysis suggesting that compounds other than PCDDs/PCDFs heavily contribute to CYP1A induction at both gene and activity levels. At low contaminants concentrations EROD activity resulted more sensitive than cyp1a gene.

TH 028

Use of AhR superinducers for potential improvement in the micro-EROD assay

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The micro-EROD bioassay allows the determination of dioxin-like compounds in samples of diverse origin by induction of the cytochrome CYP1A1 activated through the Aryl hydrocarbon receptor (AhR). Superinducers are compounds that are not able to bind to the AhR, but increase their response magnifying the expression of cytochrome P4501A1 by inducing the AhR to higher activity. In this work, three compounds known as superinducers; cycloheximide (CHX), MG132, and Dexamethasone (DEX) are studied in the EROD bioassay. The enzymatic activity of the cytochrome P4501A1 is used to detect the activation of the AhR in response to these compounds, potential improvers of the sensitivity in the micro-EROD bioassay. The bioassay is carried out with hepatic cells able to express the CYP1A1 and different incubation and pre-incubation concentrations of superinducers before following standard procedures. Additionally, RT-PCR is performed for cells incubated with CHX in order to quantify the potential CHX superactivation. The results indicate that under the studied conditions, the CHX and MG132 "superinducers" did not improve the performance of the bioassay. Nonetheless, DEX showed to be a promising compound promoting the CYP1A1 expression in the EROD bioassay. Further studies in the signalling mechanism of DEX in the AhR response and its possible improvement in the micro-EROD bioassay sensitivity are now remaining to be performed.

TH 029

Evaluation of epigenetic DNA methylation in a zebrafish (Danio rerio) cell line model

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Genetic imprinting has been shown to be an early molecular event with strong correlation to adverse disease, thereby emphasizing the potential of DNA methylation as a potential environmental biomarker. However, the study of multigenerational epigenetic DNA modifications is a novel approach in ecotoxicology, and experiments can be both time and labour consuming. Therefore the potential of a high throughput in vitro screening method was assessed by exposing a zebrafish liver cell line ZF-L to various non-mutagenic model substances. Methylation of CpG islands of selected genes were monitored by bisulphite conversion and high resolution melt analysis after short term exposure to sub-lethal concentrations. The basal methylation level in unexposed ZF-L cells was close to the 100 % standard for some genes and close to 0 % for others. Exposure of ZF-L cells to 0.5 μ M 5'-Azacitidine lead to hypomethylation of genes with both low and high basal methylation, indicating similarity to mechanism in mammals. The genes selected for analyses were genes with putative importance in different cancers, and the significantly hypomethylated genes were in insulin like growth factor binding protein 1b, angiopoietin-like 3, glutathione S-transferase P1 and cyclin-dependent kinase inhibitor 2A. The sensitivity of the HRM methylation analysis was shown to detect methylation differences down to one CpG, based on clear separation of 0 % and 10 % methylation in an amplicon containing 10 CpG sites. Validation of the HRM assay will be shown by comparing to sequencing of the amplicons from bisulfite treated templates. It is concluded that cell line exposure in combination with HRM may provide an initial screening assay for CpG methylation with high throughput capacity.

TH 030

Development of 3D fish hepatocyte spheroid cultures as an in vitro tool for environmental chemical toxicity

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Three-dimensional organotypic cell culture models (spheroids) provide significant advantages in pre-clinical and toxicological research. Specifically in the fields of pharmacotoxicology and carcinogenesis, the model offers key aspects of the in vivo environment that are superior to standard monolayer models. While spheroid technology is now established for mammalian toxicity studies, to date it has not been considered for environmental applications in model aquatic species. Currently, whole organisms (fish) are a mainstay of environmental toxicity monitoring. While fish primary cells and cell lines are relevant to the assessment of chemical toxicity and the evaluation of environmental contaminants, monolayer cultures of these cells have significant limitations such as short culture periods and loss of functionality. The present study describes the development and application of 3D fish hepatocyte spheroid cultures for the assessment of toxic environmental contaminants. The liver-specific functions and toxicity endpoints of this model suggest that fish spheroids could be suitable as a new organotypic in vitro screening tool for use in environmental toxicological monitoring.

TH 031

In vitro kinetics of chlorpromazine in cytotoxicity assays with Caco-2, Balb/c 3T3 and HepaRG cells

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Different in vitro alternatives are available to test the toxicity of compounds. For these assays, different cell types can be used. Cell viability is usually determined after the cells have been exposed for 24-72 hours. However, the different cell types need different culture and assay conditions, including the addition of serum to the medium. The

aim of this project, part of the European project Predict-IV, was to study the biokinetics of the pharmaceutical chlorpromazine (CPZ) in three different cell assays. The cells used for the assays were i) the human intestinal cell line Caco-2 cells, ii) mouse fibroblast Balb/c 3T3 cells and iii) the human liver cell line HepaRG cells. The Balb/c 3T3 cells were cultured for 24 hours and the Caco-2 cells for 1 week. The HepaRG cells were cultured for 4 weeks, with 2% DMSO in the medium during the last 2 weeks. The cells were exposed to different concentrations of CPZ (98.8% ionized at a physiological pH) for 48 hours. After different time points, samples were taken from the medium, cells and well plastic. In addition, the viability of the cells was measured by the Alamar Blue method. The freely available CPZ concentration was measured by nd-SPME and all samples were measured by HPLC-UV. In the medium samples, a decrease in the amount of CPZ was seen over time; the most pronounced decrease was seen in the metabolically competent HepaRG cells. Plastic binding of CPZ was negligible in all three cell systems. A complete recovery of CPZ was found in the Caco-2 and Balb/c 3T3 cells. In the HepaRG cell assay, less parent compound was recovered. The Alamar Blue assay showed that the Balb/c 3T3 cells were the most sensitive to CPZ based on the nominal concentration. However, protein binding influences these results; therefore, the free concentration is an important factor. In conclusion, chlorpromazine showed differences in biokinetics in the three cell assays. Thus, this study shows that it is important to take the freely available concentration of the compound and its biokinetics into account for a reliable interpretation of cytotoxicity results.

TH 032

The luminescent bacteria toxicity assay: 20 years of interlaboratory round-robins

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The luminescent bacteria toxicity assay was developed in the early 1980s, to provide a reliable system able to confirm compliance of wastewater discharges.

Since the early days, numerous studies were done and have been published to demonstrate correlation of traditional aquatic toxicity results with results from this assay. The assay has been used to establish a coherent database of comparable toxicity data that has been published, proved of practical use, and also used in Quantitative Structure-Toxicity Relationships worldwide.

The assay is based on the inhibition of bacterial luminescence caused by the presence of contaminants in a water sample. Turbidity and color may interfere in the luminescence detection, and water samples must be clear, colorless and transparent. In spite of this disadvantages, the assay is being used worldwide for water quality monitoring.

As in other toxicity bioassays, in this case different types of contaminants affect differently the enzymatic inhibition resulting in the reduction of light emitted by the bacteria.

It has been observed that some considered chemical contaminants may potentiate the bacterial thus resulting in a "negative toxicity". The observed light reductions may be the result of several different biological mechanisms depending on the chemical nature of the contaminant.

In view of all this questions we started in 1993, the organization of a yearly interlaboratory comparison exercise linked to an open forum, to improve the existing knowledge on the advantages and restrictions of this assay, and its response when used to determine the aquatic toxicity of different types of contaminants. An output from these interlaboratory round-robins has been the study of the inter- and intra- laboratory variability of the results obtained, proving the reliability of the assay, and contributing to the quality assurance practices of the luminescent bacteria assay users.

In the course of these round-robins the toxicity of several reference substances was determined using the luminescence bacteria assay and in this communication we present a summary of the results collected. Several hundreds of values from toxicity determinations confirm that this assay provides reliable results in a simple, quick and inexpensive way.

TH 033

The on-line water toxicity monitoring system based on the luminescent bacteria toxicity assay

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Water quality monitoring has been a topic of many studies and discussions regarding the virtues and disadvantages of analytical chemistry and biological tests. Every day smaller amounts of more and more substances are being detected in water samples, providing increasing numbers of analytical data, whereas aquatic toxicity bioassays simply determine the combined biological effect that water contaminants may cause to laboratory test organisms. Although continuous physicochemical analysis is nowadays possible using commercial equipment, water monitoring results are usually based on discrete or continuous samples taken periodically to the testing laboratory.

In this work we present the results of the study on the performance and reliability of an on-line, continuous biomonitoring water system based on the bacterial luminescence toxicity assay.

The Microtox Toxicity Analyzer® uses, since the 1980s, luminescent bacteria as test organism and has proven a simple, reliable, and quick method to evaluate the toxicity of aqueous samples.

The TOXcontrol biomonitoring system® (developed by microloan BV in 2006) uses also luminescent bacteria as the test organism to evaluate, on-line, the toxicity of a flowing water supply. The system examines every 30 minutes a sample of the flowing water and collects data on its toxic effect to the bacteria. The software can also measure toxicity of a reference sample collecting EC50 values of chemical contaminants, in a similar way as the Microtox Toxicity Analyzer.

Experimental studies were performed to verify and validate the results produced by the TOXcontrol system, in comparison with results from the Microtox Toxicity Analyzer, using samples of several reference substances and also water from the intake of a drinking water treatment plant (DWTP) and also the effluent from a wastewater treatment plant.

As expected, water samples showed low toxicity to luminescent bacteria in both systems, but the TOXcontrol® online system can detect peaks of unwanted levels of toxicity as a warning system in both cases. It can successfully prevent low quality water from entering the DWTP, and the discharge of treated wastewater to receiving water when not in compliance with current regulations. The work done with several reference substances, and comparison with the results from the Microtox Toxicity Analyzer, confirms the reliability and acceptable variability of the toxicity data collected by the TOXcontrol system.

TH 034

Estimation of pectins to be used as sorbents towards bacterial biosensor

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Natural polymeric material like chitosan, humics and pectin have been gaining interest as potential adsorbents of heavy metals because of their nature-abundant and biodegradable (1-3). Moreover, due to the polyfunctionality they can be used in the chelation remediation in the treatment of metal intoxication. Owing to the presence of free carboxyl groups in the monomeric fragments of the polymer backbone, the pectin polysaccharides react with metals to form stable low-dissociating compounds, chelates. But, pectin to be used as a chelating/binding agent in the treatment of metals should be beforehand estimated on own toxic side effects. This study is aimed to estimate the toxicity for two samples of pectins - beet- and apple-derived - in the bioassay test towards luminescence bacteria.

Luminescence bacteria bioassay is a rapid, inexpensive (in contrary of the test on living animals), reliable method to identify toxic. Toxicity analysis of the pectin samples were conducted following the standardized Ecolum protocol (analog of Microtox®) in which genetically modified *E. coli* cells were exposed to serial dilutions of pectin solutions.

The luminescence inhibition after 30 min. was measured by the Biotox-10M analyzer (NERA, Moscow, RF). Toxicity indices (T) were calculated with the formula $T = (I_0 - I) / I_0 \times 100$, where I_0 -bioluminescence in control probe, I - in the testing probe.

The data obtained was used to calculate the EC_{50} - the median pectin concentration that caused a 50% reduction in bioluminescence, and NOEL (no observed effect level)- the pectin concentration that caused a reduction bioluminescence up to $T < 20$.

Both the studied pectin preparations in concentrations $> 0.05\%$ decreased bioluminescence intensity. At concentrations 0.05% (ADS mass wt.) for apple-derived pectin and 0.005% (ADS mass. wt) beet-derived pectin no toxicity were registered. Among samples examined, beet-derived pectin showed the higher toxifying ability. NOEL value of beet-derived pectin as 0.0086% was lower than NOEL value of the apple-derived pectin as 0.17% . This fact was seemingly connected with content of functional groups, esterification degree and molecular mass of the used preparations.

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TH 035

A resin buffered nutrient solution for controlling metal speciation in the algal bottle assay

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The metal speciation in solution is uncontrolled during algal growth in the traditional algal bottle assay. The metal speciation will vary during the test as a result of the uptake of the metal by the green algae. A resin buffered nutrient solution was used to address the issue. This method was applied to test the effect of complexes on cadmium (Cd) uptake by the green algae *Pseudokirchneriella subcapitata*. Standard nutrient solution was enriched with the anorganic ligand chloride (40 mM) or with synthetic ligands. A reference nutrient solution with the same ionic strength and the same basic composition was used. The chloride treatments were prepared with the cation exchange resins Amberlite-CG120 (Rohm & Haas comp., 4.4 mmolc g⁻¹) to contain equal Cd²⁺ (pCd=10; pCd=-log(Cd²⁺)) but varying dissolved Cd. The synthetic ligand treatments were prepared in the presence of Chelex®-100 (Bio-rad, sodium form, 0.62 mmolc (g wet weight)⁻¹) to obtain equal free Cd²⁺ activities (pCd=8.2) in solution. The nutrient solutions and its references were subsequently used in an algal assay in 100 mL beakers containing solution with a cation exchange resin (+R) as a deposit at the bottom of the beaker. Beakers containing solution only (-R) were taken for the chloride treatments. The resin had been equilibrated with the solution prior to the assay. *Pseudokirchneriella subcapitata* was grown for 72h in stagnant solution and shaken three times a day. Growth was unaffected by the presence of the resin (p>0.05). The Cd

concentration in solution of the -R devices decreased 50-58 % of initial values. The Cd uptake by the green algae was two times higher in the chloride -R treatment than in its reference solution. The metal uptake in the cells in the +R treatments was unaffected, despite a larger total Cd concentration in solution in the ligand containing solutions. This confirms that Cd²⁺ is the preferred ion and that Cd complexes do not contribute. In contrast, Cd uptake in the -R devices was two-fold larger in the NaCl treatment than that in the NaNO₃ treatment ($p < 0.001$), suggesting that CdCl₂-n complexes are bioavailable. The bioavailability of the complexes in the traditional algal assay is apparent only because the complexes act as a buffer of the free metal ion in solution. Resin buffered solutions may be advocated to better identify the role of metal complexes on bioavailability to primary producers because free metal ion activity is buffered by the resin.

TH 036

Effects of heavy metals on free-living nematodes: a multifaceted approach using growth, reproduction and behavioral assays

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Toxicity tests, using single species bioassays, are a promising way to understand the biological effects of toxicants. The aims of this study are: (a) to assess sublethal (0.01 to 1mg/L) effect concentrations of lead, zinc and nickel for the free-living nematode *Caenorhabditis elegans* using a liquid assay focusing on growth and reproduction as endpoints. And (b) to test a behavioral assay with taxis to food as an endpoint, focusing on the same metals (lethal and sublethal concentrations). The liquid assays with *C. elegans* showed that only the highest tested concentrations of Pb, Zn and Ni caused a significant negative effect on growth and reproduction. For the behavioral assays, we compared an approach in which the pollutant was only present in the food spots with one where the pollutant was homogeneously distributed in the agar medium. The results at lethal metal doses differed according to the metal as well as to the type of assay. For instance, *C. elegans* almost completely avoided Zn-contaminated food patches, and less nematodes reached the attractant spots on the polluted agar experiment. Sublethal concentrations of Ni resulted in a preference for contaminated food-spots than to "clean" *E. coli*. Our results show that behavioral assays may provide important insights complementary to those of survival, growth and reproduction tests.

TH 037

Screening of soils using an avoidance assay with springtails

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Soil contamination is of increasing concern due to the continued use of chemicals. Moreover, current regulations require that the potential for soil contamination must be reported for registration of chemical substances. On the other hand, classical toxicity tests require exposition of the suspected soil to soil-dwelling organisms for a period of time to assess the biological impact of contaminants. Reproduction and mortality have been the effects studied on earthworms, springtails and other organisms as indicators of the soil health. Most of the standard assays call for an exposure time of several days (from 48 hours to 21 days), but regulators require quick tests that quickly assess the quality of soils allegedly contaminated enabling them to take correct decisions.

The behavior of soil organisms exposed to chemical contaminants in soil is used as the end point in the avoidance test. Soil organisms move away from contaminated soil and the degree of avoidance is related to the amount of pollution in the soil.

Since several years we have studied the avoidance effect caused by chemical contaminants in soil to earthworms and springtails, with the aim of developing a quick and practical assay to screen large numbers of potentially contaminated soil samples capable to support decision taking on soil quality and remediation practices. The avoidance test can provide toxicity results based on the behavior of test organisms after exposure times from 2 to 48 hours. Recently, the avoidance test with collembolans has been standardized (ISO 17512-2:2011). This endpoint does not imply mortality or reproduction inhibition, and collembolans are organisms widely present in all territories therefore providing a good indicator of soil health.

In this communication we present the results of the collembolans avoidance test in the evaluation of the environmental quality of soil samples from known contaminated sites in Catalonia and of soil samples artificially contaminated with recognized soil pollutants. The work done confirms that results from this assay are in good correlation with results obtained with other soil toxicity tests and show also a good relationship with analytical chemistry results. Since the test provides reliable and quick results, it can be widely applied to the evaluation of the soil environmental quality in areas such as contaminated sites, remediation processes, application of wastes to agricultural soils, sediments, monitoring studies.

TH 038

Behaviour response of the nematode, *Caenorhabditis elegans* after the treatments of Benzene

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Behaviour change has been considered important indicator of chemical toxicity, as it reflects integrated physiological alteration due to chemical exposure and thus is directly related to important ecotoxicological parameters such as reproduction. However due to difficulty of monitoring, it has not been widely used. In this study, response behaviour of *Caenorhabditis elegans* was observed after benzene was treated at 0.5 ppm under air exposure conditions in wildtype and three stress response mutants (*cyp35a2*, *pmk-1*, *cep-1*). The movement patterns were analyzed with different parameters before (3 hours) and after (3 hours) the treatments. The wild type (N2) showed decrease in speed and acceleration, while stop duration and turning rate increased after the treatments. Stop number and turning rate appeared to increase in mutants, but different mutant strains were also characteristic in showing different parameters in response to the treatments. The *cyp35a2* mutant was contrasting to N2, showing low level of stop duration and high level of stop number. The *pmk-1* mutant was differentiated from *cyp35a2* with increase in stop duration and turning rate after the treatments. The *cep-1* mutant showed a similar trend to N2, but stop number was higher and stop duration was shorter than the parameters obtained with N2 after the treatments. This study suggest *C. elegans* behavior changes to chemical exposure have a potential of being used as toxicity indicator and the response of different *C. elegans* mutant can provide an insight of the relationship between toxic mechanism and higher level effect.

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TH 039

Differences in the number of micronucleated red blood cells and other nuclear abnormalities in Ciconiiformes: potential bioindicators of genotoxicity in birds

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Over the past decades, the presence of micronucleated blood cells has been used as a widespread tool to detect genotoxic effects of xenobiotics in mammals, fish, amphibians and birds. In order to: (i) investigate the usefulness of the micronucleus test in Ciconiiformes and (ii) monitor the occurrence of genotoxic effects in Portuguese populations of Ciconiiformes, the frequency of micronucleus (MN) and other nuclear abnormalities (lobed nuclei, segmented nuclei, kidney shaped nuclei and notched nuclei) was assessed in wild individuals of *Ciconia ciconia*, *Ardea cinerea* and *Ardea purpurea*. About 80 blood smears, collected between 2007 and 2011 in birds from different geographic origins, were analysed and statistical tests were performed to determine eventual temporal and geographical differences in the frequency of MN and other nuclear abnormalities. Results showed elevated levels of MN and other nuclear abnormalities, and the sum of frequencies of all other abnormalities was generally higher than MN on the samples studied. Significant differences in the frequency of total nuclear abnormalities were observed also between different years. These results suggest that the assessment of total nuclear abnormalities, rather than only MN frequencies, might be a useful tool to complement the study of induced genotoxicity in birds.

TH 040

Impact of thiol reactivity on the excess toxicity of α,β -Unsaturated Ketones, Esters, and Aldehydes

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In aquatic toxicology, the minimum (narcosis-level) toxicity of an organic compound can be well estimated applying hydrophobicity-based quantitative-structure activity relationships (QSARs), addressing its' membrane affinity. By contrast, electrophilic compounds may undergo chemical reactions with nucleophilic target sites (e.g. 'SH and 'NHR) of glutathione (GSH), proteins and DNA, leading to enhanced toxicity (quantified by the toxicity enhancement T_e) as compared to *iso*-hydrophobic narcotics. For α,β -unsaturated carbonyls as one class of electrophiles, Michael-type addition forms a prominent route to cause excess toxicity toward aquatic organisms. Here, the kinetic glutathione chemoassay, employing GSH as a surrogate for thiol groups *in vivo*, allows one to quantify chemical reactivity in terms of the second-order rate constants k_{GSH} and thus may form a non-animal component of integrated testing strategies (ITS) for REACH and the new European cosmetic guideline. In this communication, the kinetic GSH chemoassay is employed for assessing the electrophilic reactivity in terms of k_{GSH} of 52 α,β -unsaturated ketones, esters, and aldehydes. Comparison with toxicity data in terms of 48-h 50 % growth inhibition of *Tetrahymena pyriformis* resulted in structural alerts for the predictive identification of excess-toxic compounds. Moreover, regression models for predicting both log EC₅₀ and log T_e were derived, using log k_{GSH} and log K_{ow} as parameters.

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TH 041

Excess toxicity of organic electrophiles in the *Tetrahymena pyriformis* bioassay informs about reactive modes of action

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In recent years, the concept of integrated testing strategies (ITS) has been developed as a practical approach toward the 3R goals of reducing, refining and replacing animal testing for the toxicological and ecotoxicological assessment of industrial chemicals under the European Directive REACH. The goal is to combine complementary non-animal (in chemico, in vitro, omics) and non-test (in silico) methods with a strong emphasis on mechanisms of action, thus compensating for the reduction in adverse outcome pathways associated with every individual alternative method. In this context, organic electrophiles represent a prominent group of particularly toxic contaminants that cover various classes of chemical substances, and may also be generated in vivo through metabolic processes. Their high intrinsic toxicological potential results from their ability to deplete the anti-oxidant glutathione, and to undergo chemical reactions with nucleophilic sites of proteins and the DNA. In this study, a simple in vitro approach is presented that enables a targeted analysis of electrophilic modes of action of organic compounds. To this end, 50% growth inhibition (EC_{50}) of the unicellular ciliates *Tetrahymena pyriformis* of a given compound is determined and compared with the corresponding narcosis-level toxicity of an isohydrophobic counterpart. The resultant toxicity enhancement T_e , derived as ratio of narcosis-level over actual EC_{50} , informs about the lack ($T_e < 10$) or presence ($T_e \geq 10$) of reactive mechanisms as major determinant of the observed in vitro toxicity, and can be used to derive respective structural alerts for a predictive toxicity assessment. Moreover, different mechanisms of reactive toxicity may translate into different level ranges of the toxicity enhancement, thus providing pertinent information in the context of ITS schemes. The approach is illustrated with results for quinones and hydroquinones as two compound classes that may generate reactive oxygen species (ROS) through redox cycling, and - without (quinone) or after metabolic oxidation (from hydroquinone to quinone) - chemically modify endogenous nucleophiles through Michael-acceptor addition reactions. Financial support from the EU project OSIRIS (Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Testing Information), contract no.: 037017, is gratefully acknowledged.

TH 042

Predictive assessment of the aquatic toxicity of Michael acceptors using a kinetic chemoassay with 4-nitrobenzenethiol (NBT)

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According to the European Directive REACH, the hazard and risk assessment of chemical substances should be performed with as little animal use as possible, following the 3R principle and its goal to reduce, refine and replace animal testing in the interest of animal welfare. In this context, the concept of integrated testing strategies (ITS) has been developed, exploiting different types of non-animal data such as in vitro, in chemico and in silico information.

In aquatic toxicity, the well-accepted narcosis paradigm states that every chemical substance exerts a minimum toxicity proportional to its hydrophobicity. Accordingly, excess toxicity - identified through a toxicity enhancement over baseline narcosis - is expected for those compounds where reactive or specific modes of action govern the adverse effect. Here, organic electrophiles form an important group of potentially excess-toxic contaminants because of their chemical reactivity toward nucleophilic sites of glutathione as well as endogenous macromolecules (protein, DNA).

An efficient non-animal approach for screening the excess toxicity of organic electrophiles is to measure their reactivity toward model nucleophiles, employing so-called chemoassays (in chemico). In this work, 4-nitrobenzenethiol (NBT) is taken as a model for endogenous thiol groups. It has the advantage to be directly detectable by UV-VIS absorption, enabling a fast and low-cost quantification of the toxicologically relevant reactivity. Using this approach, second-order reaction rate constants of reaction with NBT, k_{NBT} , have been determined for a number of α,β -unsaturated carbonyls (Michael acceptors). These electrophiles are able to react irreversibly with nucleophilic groups through a Michael-type addition mechanism, and therefore have the potential to exert substantial excess toxicities. Our results demonstrate a good correlation between $\log k_{NBT}$ and the extent of the excess toxicity (the latter of which was derived from in vitro results of the growth inhibition bioassay with the ciliates *Tetrahymena pyriformis*). Thus, the NBT chemoassay is found to be a simple and promising tool for the predictive assessment of the aquatic toxicity of organic electrophiles.

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TH 043

Linking high-throughput In Vitro Screens with EDSP assays: the causal relationship challenge

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One step in complying with Test Orders under the US EPA's Endocrine Disruptor Screening Program was compilation of Other Scientifically Relevant Information (OSRI). The extent and quality of OSRI was evaluated to determine what requirements were met by existing data, and hence, which of the 11 Tier 1 Endocrine Screening Battery (ESB) assays were needed. Many of the List 1 chemicals under ESB Test Orders were analyzed in the EPA's ToxCast Program, hence ToxCast data were submitted as OSRI in lieu of conducting many similar ESB in vitro assays, and to help support waivers for some in vivo assays. ToxCast seeks to predict human toxicity by measuring the activity of chemicals in a suite of some 500 cellular, biochemical and molecular assays conducted in high-throughput mode. The concept presumes that these assays can identify a chemical's ability to activate so-called 'toxicity pathways.' In evaluating acceptability of OSRI, EPA rejected ToxCast results claiming that the reliability, responsiveness and relevance of ToxCast were undetermined and that ToxCast does not evaluate all known endocrine targets. In rejecting ToxCast data, EPA claimed its validation status was inferior to that of the individual ESB assays. However, even the predictive value of the in vitro ESB assays for in vivo endocrine activity is still unclear at this time. Although ToxCast data was rejected as OSRI for the ESB, ToxCast data was used to assess and deem acceptable the risks of oil dispersants used for the Deep Water Horizon spill. These decisions have significant implications for the use of screening assays in regulatory programs, yet inconsistencies in application confound what those implications might be. As both the ESB and ToxCast programs appear to rely on correlative analyses rather than dispositive evidence that assays individually or in combination measure precursor events or frank toxicity, the use of high-throughput assays, like ToxCast, to screen chemicals for further testing may be no less probative than some of the ESB battery. However, despite potential advantages offered by high-throughput methods to quickly screen for possible modes of action, it will be necessary to elucidate aspects of biological and physiological function as well as causal relationships between biochemical events and adverse effects before these data can offer improvements in the assessment of endocrine-mediated toxicity.

TH 044

Study of key characteristics using multicriteria analysis for development of chronic inherited toxicity test

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The multicriteria analysis is a useful tool for comparing different conditions and the results are normally oriented to the operational decisions. The development of methods that identify environmental risks and detection of compounds that cause deleterious effects to living organisms in an area at low cost, reproducibility, ease of implementation and speed of analysis are relevance to the promotion of activities that assist the sustainable development and minimize impacts to the ecosystems of developing countries. This study is aimed at developing a multicriteria analysis to define key characteristics to design a method for chronic toxicological evaluation, considering aspects of the complete life cycle of organisms, as well as the inherited effects in tropical countries. A multicriteria matrix was prepared based on Quality Function Deployment model where each key characteristic was evaluated taking into consideration the interrelationships between them. In order to establish the criteria to be used in the matrix factors for assessing the response time, technical difficulty, cost, reproductivity and number of citations in the literature were defined. The total score was calculated by the sum of scores, multiplied by the corresponding weights. The criteria technical difficulty is of great importance in this study as being proposed for use in developing countries, where technological development is generally behind. In line with results, the key features with the highest score and also considered for the development of a chronic toxicity test were reproduction, mortality, time to first reproduction and total size of the organisms.

ET06P - Ecotoxicology of amphibian and reptiles. Novel approaches for linking contaminant effects with population declines

TH 049

Relevant lizard species for the risk assessment of plant protection products in Northern and Central Europe

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According to the new data requirements under the EU regulation 1107/2009, reptiles come into question regarding risk assessments of plant protection products (PPP). Although no specific data requirements on reptiles are stipulated in the respective EU documents (e.g. SANCO 11802/2010/rev July 2010) some toxicity data are available in the open literature. These data are intended to be used in the risk assessment. One potential approach for reptile risk assessment may be the way how it is successfully established for birds and mammals. The major routes of exposure in that case are diet and drinking water. In order to provide a scientific basis for reptile risk assessment, a detailed literature survey on habitat use and the biology of lizards occurring in North and Central Europe (geographical zones according to SANCO/6896/2009 rev 1) was conducted. The study focused on the habitat use of the different species, but also evaluates the different diets as a source of exposure. The data obtained may allow the determination of relevant species at risk and how they can be linked to potential exposure in agricultural practice.

TH 050

Relevant snakes and turtle species for the risk assessment of plant protection products in Northern and Central Europe

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According to the new data requirements under the EU regulation 1107/2009, reptiles come into question regarding risk assessments of plant protection products (PPP). Although no specific data requirements on reptiles are stipulated in the respective EU documents (e.g. SANCO 11802/2010/rev July 2010) some toxicity data are available in the open literature. These data are intended to be used in the risk assessment. One potential approach for reptile risk assessment may be the way how it is successfully established for birds and mammals. The major routes of exposure in that case are diet and drinking water. In order to provide a scientific basis for reptile risk assessment, a detailed literature survey on habitat use and the biology of snakes and turtles occurring in North and Central Europe (geographical zones according to SANCO/6896/2009

rev 1) was conducted. The study focused on the habitat use of the different species, but also evaluates the different diets as a source of exposure. The data obtained may allow the determination of relevant species at risk and how they can be linked to potential exposure in agricultural practice.

TH 051

Relevant lizard species for the risk assessment of plant protection products in Southern Europe

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According to the new data requirements under the EU regulation 1107/2009, reptiles come into question regarding risk assessments of plant protection products (PPP). Although no specific data requirements on reptiles are stipulated in the respective EU documents (e.g. SANCO 11802/2010/rev July 2010) some toxicity data are available in the open literature. These data are intended to be used in the risk assessment. One potential approach for reptile risk assessment may be the way how it is successfully established for birds and mammals. The major routes of exposure in that case are diet and drinking water. In order to provide a scientific basis for reptile risk assessment, a detailed literature survey on habitat use and the biology of lizards occurring in South Europe (geographical zones according to SANCO/6896/2009 rev 1) was conducted. The study focused on the habitat use of the different species, but also evaluates the different diets as a source of exposure. The data obtained may allow the determination of relevant species at risk and how they can be linked to potential exposure in agricultural practice.

TH 052

Are the Mediterranean chameleon species possible focal species for risk assessments of PPPs in Europe?

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According to the new data requirements under the EU regulation 1107/2009 reptiles and amphibians have to be addressed in the risk assessment of plant protection products (PPP). One necessary background for such an addressing are reliable data on the exposure of PPP. For reptiles generic data recorded in agricultural landscapes are nearly completely missing. In a five months field study about the distribution and ecology of the chameleons of Egypt it was proved that both European chameleons are species which thrive in areas developed by man and are candidates as focal species for risk assessments for PPP in fruit orchards and olive grooves in their distribution areas in Europe. In 83 night-searches with torches of at least 2 hours duration each chameleons could be detected in 18 of 28 vegetated locations, but only two of them were natural vegetated. No chameleons were found in mixed vegetation (urban, natural and agricultural habitats in close environment). In the agricultural habitats half of the *Chamaeleo africanus* were observed inside the crop and 40% of the *Ch. chamaeleon*, respectively. All other specimens used the direct surroundings, like hedges or isolated trees between fields. These findings in Egypt are supported by first field data on habitat selection of chameleons in Europe (Greece, Spain and Portugal). A higher percentage of the adults were found inside the crop than in the direct surrounding vegetation, but the majority of juveniles were found in the surrounding vegetation.

TH 053

Relevant snake species for the risk assessment of plant protection products in Southern Europe

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According to the new data requirements under the EU regulation 1107/2009, reptiles come into question regarding risk assessments of plant protection products (PPP). Although no specific data requirements on reptiles are stipulated in the respective EU documents (e.g. SANCO 11802/2010/rev July 2010) some toxicity data are available in the open literature. These data are intended to be used in the risk assessment. One potential approach for reptile risk assessment may be the way how it is successfully established for birds and mammals. The major routes of exposure in that case are diet and drinking water. In order to provide a scientific basis for reptile risk assessment, a detailed literature survey on habitat use and the biology of snakes occurring in South Europe (geographical zones according to SANCO/6896/2009 rev 1) was conducted. The study focused on the habitat use of the different species, but also evaluates the different diets as a source of exposure. The data obtained may allow the determination of relevant species at risk and how they can be linked to potential exposure in agricultural practice.

TH 054

Plant protection products and juvenile amphibians: no matter of concern or unexpected risk?

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The current risk assessment of plant protection products requires a diversity of tests with vertebrate species, especially fish and the African Clawed Frog (*Xenopus laevis*) from aquatic environments, and birds and mammals from terrestrial environments. Concerning amphibians, many studies are available concerning the influence of plant protection products on survival and growth of aquatic larval stages, but the knowledge on toxicology and ecotoxicology of adult frogs is scarce. This is surprising since juvenile and adult frogs are supposed to be at risk of pesticide uptake due to their highly permeable skin which is only a weak barrier against plant protection products. Additionally, many frog species are exposed to a variety of plant protection products, since they migrate through agricultural fields either from their larval aquatic habitats to over-wintering sites in autumn or back in spring for egg-laying.

In order to fill the data gap of the potential risk of plant protection products on the terrestrial life stage of amphibians, approx. 1-2 months old juveniles of the Common Frog (*Rana temporaria*) were caught in the field and exposed to six different plant protection products in the laboratory on bare soil for one week. For each plant protection product three application rates, including the practically used field application rate, were selected to determine the LR50. Our results show that the majority of the plant protection products caused a mortality between 20-100% at field application rates. Therefore, our data demonstrate that the current risk assessment is not protective for amphibians.

TH 055

Assessing atrazine toxicity to the Australian striped marsh frog, *Limnodynastes peronii*

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Abstract

Atrazine is one of the most extensively applied herbicides in Australia and frequently detected in surface and ground water samples. In overseas studies, it has been reported that atrazine can cause reproductive and developmental abnormalities in exposed amphibians. Limited studies have been carried out in Australia, resulting in subsequent gaps in knowledge particularly among Australian species. To explore the effects of atrazine to native frogs, the striped marsh frog, *Limnodynastes peronii*, was exposed to a range of low environmentally relevant concentrations of atrazine, including, control, solvent control, 0.1, 1, and 10 µg/L for 21 days. Results showed that there were no significant differences in total length, snout vent length, body weight and time to metamorphosis across the treatments ($p > 0.05$). No mortality was observed in any treatments. Only 4.4% of tadpoles exhibited abnormal kinky tails in 1 µg/L and 2.2% in 10 µg/L atrazine. No such abnormalities were found either in controls or solvent controls. Histological analysis of gonads is underway and the implications for management of atrazine impacts to native amphibians will be further discussed.

TH 056

Toxicity of eucalypt and pine leaf leachates in zebrafish (*Danio rerio*) and iberian frog (*Pelophylax perezi*) early-life stages

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Whilst deforestation of natural forests represents one of the major causes of biodiversity loss, the extent of plantation forests has increased, especially in Mediterranean countries. Represented by vast areas of monoculture this trend in afforestation has raised concern for biodiversity conservation. Afforestation of Pine and Eucalypt is associated with increased stream acidity and a reduction in base cations whilst the short rotation harvesting of trees deprives stream systems of inputs of large woody debris. In the present study, the toxicity of eucalypt and pine leaf leachates that may be particularly damaging in intermittent streams, was evaluated using embryo toxicity testing with *Danio rerio*, a tropical cyprinid used in toxicological research, based on the OECD draft guideline on Fish Embryo Toxicity (FET) and with the iberian frog *Pelophylax perezi* based on the ASTM guideline on Frog Embryo Teratogenesis Assay-Xenopus (FETAX). The endpoints monitored included mortality and sub-lethal parameters in embryo development such as pericardial edema, tail deformation and hatching rate. Five concentrations of each leachate were tested, ranging from 0.64 to 20,48 and 1,6 to 32 g L⁻¹ of Eucalypt in zebrafish and *P. perezi*, respectively; and from 1,6 to 32 g L⁻¹ of Pine in both species. Simultaneously, the water physico-chemical parameters and phenolic content were evaluated in eucalypt and pine leachates. In general, leachates were found to reduce water dissolved oxygen and pH, and increase dissolved phenolics and conductivity. The results obtained suggest higher toxicity of eucalypt leachates for zebrafish (LC50-96 h = 3,32 and 12,25 g L⁻¹ of eucalypt and pine leaves, respectively), while for iberian frogs the different leachates presented similar toxicity (LC50-96 h = 14,97 and 13,26 g L⁻¹ of eucalypt and pine leaves, respectively). The embryo developmental alterations registered in both species will be presented and discussed considering the type of leaf leachate tested and their respective water physico-chemical parameter characteristics and phenolic content. These data demonstrate that leachate toxicity may contribute to the impact of pine and eucalypt plantations on aquatic biodiversity.

TH 057

Alterations of embryonic development and mRNA expression in *Xenopus laevis* caused by perfluorooctane sulfonate (PFOS)

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Perfluorooctane sulfonate (PFOS) is a synthetic chemical that has been used in industrial activities for decades. This compound has been detected throughout the world in numerous environmental samples and is considered a pollutant of concern because of its potential toxic effects, persistence and bioaccumulation properties. Nevertheless, few data exist about the effects of PFOS on amphibians. In this study, *Xenopus laevis* embryos were exposed for 96 hours to various concentrations of PFOS (0.5, 6, 12, 24, 48 y 96 mg/L), from early gastrula stage to larvae. Survival and deformities rates were monitored daily during the test and head-tail length was measured at the end of the assay as an indicator of growth. In addition, at 24 and 96 hours of exposure, mRNA expression levels of genetic markers involved in general stress responses (hsp70, hsp47, crf and urocortin), apoptosis (p53 and bax), oxidative stress (cat and sod), and differentiation and lipid accumulation (ppar β) were analysed using RT-qPCR. Deformities rate was significantly higher in embryos exposed to the highest concentration of PFOS than in controls, at 48 (+43%), 72 (+49%) and 96 (+60%) hours of exposure. The most common deformity found was axial shortening. Growth inhibition was observed in embryos exposed to PFOS concentrations $\geq$ 48 mg/L. After 24 hours of exposure significantly higher expression levels were found for hsp70 at concentrations $\geq$ 24 mg/L, for hsp47 at concentrations $\geq$ 12 mg/L, and for p53 at concentrations $\geq$ 48 mg/L, relative to controls. After 96 hours of exposure, significant increases in expression levels were found for hsp70 at concentrations $\geq$ 12 mg/L, for hsp47 and crf at concentrations $\geq$ 0.5 mg/L, and for p53 at concentrations of 12 and 48 mg/L. Alterations in mRNA expression indicated a stress response to PFOS at 24 and 96 hours of exposure, as well as alterations in apoptosis regulation. This work was supported by the projects CTM2010 19779-C02 and RTA2010-00004-C02-01. LSS acknowledges her FPI-INIA fellowship.

TH 058

Assessment of potential toxicity of wastewater treatment plant effluents in a *Xenopus laevis* embryo test using gene expressions as early response biomarkers

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This study assessed the potential toxicity of wastewater treatment plants (WWTPs) effluents with classical endpoints, such as mortality, malformations rate and growth, as well as evaluating the expression levels of several genes as response biomarkers of early effects of complex samples in *Xenopus laevis* embryos.

Sixteen samples from WWTPs effluents around the city of Madrid (Spain) were evaluated using a *Xenopus laevis* embryo test. The embryos were exposed to three dilutions of each effluent sample (1, 10 and 25%) for 96 hours. At the end of the assay, mortality and malformations rate were recorded and larval length was measured. Embryos for genetic analysis were collected after 24 and 96 hours of exposure and the mRNA expression levels of genes involved in embryonic development and chemical stress were investigated using semiquantitative RT-PCR. To value the sensitivity of this gene expression approach, the transcriptomic results were analysed and compared with morphological abnormalities rate and length embryo data.

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TH 059

Effects of larval estrogen exposure on adult reproductive potential in the amphibian model, *Silurana tropicalis*

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Amphibians are receiving increasing attention in test method development for chemical safety assessment, in particular with respect to detection of endocrine disrupting activity. While there is evidence that exposure to exogenous estrogens in the larval phase can cause aberrant sexual differentiation in anuran amphibians, consequences for reproductive fitness in the adult phase remain unclear. Assessing impacts of gonadal abnormalities, such as testicular oocytes, requires models in which incidence of these phenomena at individual level can be correlated with apical endpoints indicative of reproductive fitness that might have population level consequences. We used an emerging amphibian model, the West African clawed frog (*Silurana tropicalis*) in which the adult phase is relatively short, to assess the impacts of larval exposure to ethinyl estradiol (EE2; 10, 50, 100ng, 500, 3000 ng l⁻¹), on metamorphic and adult reproductive endpoints. Exposure of larvae to EE2 from stage 51/52 until stage 66 (completion of metamorphosis) at two temperatures (25°C and 28°C) skewed the sex ratio toward females in the exposed groups, though at completion of metamorphosis there were a significant number of undifferentiated individuals in all treatment groups. At sexual maturity, sex ratio, based on gonad histopathology, was female-biased in EE2 exposed treatment groups, and absence of one or both oviducts was also observed in female frogs that had been exposed to EE2 at concentrations $>$ 50 ng l⁻¹. Regressed testicular oocytes were observed in males from all treatment groups (including control) and incidence appeared to be influenced by parentage not EE2 exposure. Mature testicular oocytes and presence of oviduct-like ducts in frogs with testes were only seen in treatment groups exposed to EE2 $>$ 10 ng l⁻¹. Cluster analysis of computerised sperm motility measurements indicated presence of two sub-populations of sperm in frogs from all groups: one group typified by lower VSL and STR/higher VAP (slow and circular); the other by higher VSL and STR/lower VAP (fast and straight). Frogs exposed to 10 ng l⁻¹ EE2 had significantly more sperm in the former group (slow and circular) than other treatment groups. These findings suggest that further development of partial life cycle methods employing *Silurana tropicalis*, including sperm analysis, may be warranted in order to support identification and characterization of endocrine disrupters in chemical safety testing.

TH 060

Exposure to cadmium: evaluation of the effects on the larval epidermis of *Lithobates catesbeianus*

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Since amphibian integument directly interacts with the environmental constituents (water, air and soil), we investigated the acute toxicity of the cadmium (Cd, 1ppb-48h) on the skin of bullfrog tadpoles (25 Gosner stage, Gosner, 1960), *Lithobates catesbeianus*. Enzyme activities (CAT, catalase and GST, glutathione S-transferase), and also LPO (lipid peroxidation), MT (metallothionein) and GSH (glutathione reduced) levels were evaluated. Tadpoles were randomly divided into 2 groups: controls, exposed to water free of cadmium (n=8), and cadmium-exposed animals: exposed to CdCl₂, 1ppb-48h (n=8). After exposure, tadpoles were sacrificed and the skin was removed to assays. LPO (7.33 $\pm$ 1.2 nmol/mg), MT (100,13 μ molSH/mg protein) and GSH (0,08 mM) concentrations and CAT (26.49 $\pm$ 2.37 UB/mg protein) and GST (244.59 $\pm$ 15.22 mU/mg of protein) activities were significantly higher were than those from the control group. The results obtained were of ecological relevance, suggesting an adaptation to stress resulting from acute exposure to metal that directly or indirectly, generates reactive oxygen species (ROS). In contrast, an antioxidant system deficiency will induce a precarious state, making biological species more susceptible to toxic agents. GSH and MT, which represents the bulk of non-protein thiols, are synthesized in the liver and exported to other tissues functioning in the detoxification of H₂O₂, other peroxides and free radicals, being considered the predominant defense against ROS. MT has an important role in the detoxification of toxic metals such as Cd, and in the maintenance of the homeostasis of essential metals. The increased antioxidant enzyme's activity suggests that bullfrog tadpoles can develop adaptations to the pro-oxidant challenges provoked by environmental contaminants signaling increased H₂O₂ and LPO and/or other organic xenobiotics levels. These results indicate frog's epidermis can function as an adaptive system equipped with chemical and structural supports that can counteract, within certain limits, the effects of exposure to some environmental pollutants such as, cadmium. Considering that the skin represents the organ which is more directly exposed to environmental toxicants, the effects of cadmium on tadpoles are more likely to be due to injuries induced by the toxicant on the liver and the kidney, considering that these organs, usually involved in metabolizing and excreting harmful substances, are not completely developed at that stage.

TH 061

Integrated assessment of the effects posed by historical exposure to chemical contamination in natural populations of the Perez's frog, *Pelophylax perezii* (Seoane, 1885)

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Most works assessing the effects that chemical contamination may pose to amphibians have been centered on laboratory assays with organisms originated from non-impacted populations. However, amphibians may respond differently when exposed to contaminants in field or in laboratory. In addition, more holistic and integrated studies are essential to promote ecological relevance of generated knowledge. This work aimed at an interdisciplinary assessment of the effects caused by a long-term exposure to chemical contamination on adults of *Pelophylax perezii*. For this, four populations were sampled: two at chemically contaminated sites and two at reference sites. Sixteen adult organisms (8 females; 8 males) were captured at each site and transported chilled to the laboratory, where were euthanized with MS222 and its morphometric parameters measured. Afterwards organisms were dissected to collect gonads, kidney, liver, muscles, spleen, lungs and bones, which were deep frozen for posterior analysis. For each organism, biomarkers of oxidative stress (catalase, glutathione S transferase, lactate dehydrogenase) and endocrine disruption (vitellogenin-VTG), metallothioneins (MT), molecular markers, histological effects and metal levels in tissues were analyzed. Chemical analysis of surface waters revealed higher values of some elements (B, Be, Ca, K, Li, Mg, Mn, Na, Sr) at the contaminated sites comparatively with the reference ones. Metal analyses in sediment, soil and tissue samples are still being processed. Significant differences in enzymatic activities and in VTG levels were detected among the four populations, but could not be associated with different levels of the metal contamination in water. Histological analysis on male gonads revealed testicular oocytes (TO) in the impacted populations (25% and 12.5%) while no TO were detected in reference populations. Differences in the mean seminiferous lobule (MSL) diameter were observed between reference and contaminated populations, and as well between the two contaminated populations. Analysis of MT and histology of spleen, liver, lungs and kidney are underway. Also, the genetic diversity within and between populations is being addressed using a combination of neutral, non-neutral, genomic and mitochondrial DNA markers. All these data will be integrated and presented in the poster.

TH 062

Development of new non-destructive biomarkers in the loggerhead sea turtle: first evidence of induction of CYP1A in skin biopsy slices treated with PAHs, OCs and PBDEs

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Induction of CYP1A has been proven by a large number of studies to be a sensitive and specific biomarker of exposure to some classes of lipophilic contaminants. However,

the use of this biomarker has been mostly destructive, since the liver is the main target organ for analysis. This study is part of a larger project aimed at developing non-invasive biomarkers in the endangered species of *C. caretta* in the Mediterranean. In particular it focuses on developing and validating the induction of CYP1A as a non-invasive biomarker of exposure in skin biopsy slices. During the first part of the study, the Western blot method to evaluate protein expression of CYP1A in this species was set up and applied in liver and skin sampled from two specimens that died in a turtle rescue center. We detected a strong band at about 59 kDa of molecular weight in both tissues, in accordance with the findings in liver of the freshwater *C. picta picta* by Yawetz et al in 1998. In order to semi-quantify CYP1A, a series of CYP450 calibration curves from the liver (ranging from 0.5 to 5.0 pmol of CYP450) of *Caretta caretta* were analysed. In addition a skin standard was analyzed within the curves and maintained as an internal standard in subsequent WBs. Semi quantitative analysis was performed for each WB with Quantity-One software (BioRad) using Adjusted Volume (Intensity*mm2) as a quantitative parameter. In the second part of the study, skin biopsy samples were taken from 7 free-ranging specimens, and subaliquots (slices) were incubated in vitro for 24 hours with different doses of PAHs (0.5 µM BaP + 10 µM BnF and 12.5 µM BaP + 250 µM BnF, plus a acetone (0.1%) control), OCs (0.01 µg/ml, 1 µg/ml and 25 µg/ml, plus a DMSO (0.05%) control) and PBDEs (0.1 µg/ml and 0.05 µg/ml, plus a nonane (0.01 µg/ml) control) in order to investigate the potential for CYP1A induction. Western blotting of CYP1A showed a dose-related induction in skin biopsy slices treated with the different compounds. A higher induction was found after treatment with PBDEs, indicating a strong potential of these emerging chemicals to interfere with bioactivation enzymatic systems in *C. caretta*. In conclusion this study, which we believe is the first of its kind in the literature, demonstrated the presence and inducibility of CYP1A in skin biopsies of *C. caretta*, and validated this non-destructive biomarker for application in field studies.

TH 063

Effects of lead shot pellets contamination on oxidative stress biomarkers and plasma biochemistry in turtles

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Lead (Pb) shot pellets spent in hunting activities have been accumulated in wetlands worldwide, with densities in the upper 20 cm of wetland sediments above 100 shot/m² at many locations. Currently, a large number of countries are making significant efforts to reduce or eliminate the input of Pb shot in most wetlands. However, the problem often persists because spent Pb shot remains in soil for 100-300 years. During this time the pellets are slowly buried by sedimentary accumulation, or dissolve over time to disappear, affecting local sediment Pb concentrations. Although studies carried out on the effect of Pb exposure in wildlife through ingestion of pellets accumulated in sediments are numerous, those that address the effect of Pb exposure due to contamination associated with its dissolution in the environment are scarce. In order to fill this gap, we used non-destructive biomarkers to study the exposure and effect of Pb on turtles (*Mauremys leprosa* and *Emys orbicularis*) from two Spanish wetlands. The Medina lagoon (ML), where the highest densities of Pb shot pellets of Spain has been described, and Dulce lagoon (DL), a non-contaminated lagoon located in Doñana National Park. The mean whole blood Pb concentration (PbB) from ML turtles was higher than in DL (F_{1,97}=42.541, p<0.01; 29 and 5 ng/ml, respectively), both in males and females (F_{1,45}=20.130, p<0.01; F_{1,50}=26.704, p<0.01, respectively). The Gaminolevulinic acid dehydratase (ALAD) ratio was negatively related with PbB (F_{1,93}=4.661, p<0.05), regardless of the lagoon or sex. Plasma Total Antioxidant Status (TAS) was positively related with PbB (F_{1,81}=6.723, p<0.05), and also differed with lagoon (F_{1,81}=4.333, p<0.05; higher in DL), and sex (F_{1,81}=5.683, p<0.05, higher in females). Finally, three biomarkers of oxidative stress in red blood cells statistically differed with PbB and lagoon: lipid peroxidation, estimated as thiobarbituric acid-reactive substances (F_{1,67}=7.450, p<0.01; F_{1,67}=14.523, p<0.01, respectively); total sulphydryl (F_{1,77}=6.387, p<0.05; F_{1,77}=19.982, p<0.01, respectively); and glutathione in oxidized form (F_{1,63}=12.004, p<0.01; F_{1,63}=6.469, p<0.05, respectively). Additionally, the ratio reduced/oxidized glutathione was negatively associated with PbB (F_{1,63}=5.138, p<0.05). Our results evidenced that turtle living in Pb shot contaminated wetlands showed sublethal effects. Nonetheless, further research is needed to elucidate Pb effects on individual and population health and dynamics.

TH 064

Transition metals contamination on marine turtle eggshell from Thailand

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Elemental analysis of eggshells in 3 marine turtle eggshell from Thailand; green turtle (*Chelonia mydas*), hawksbill turtle (*Eretmochelys imbricata*), and leatherback turtle (*Dermochelys coriacea*), were studied by scanning electron microscope (SEM) with secondary electron imaging (SEI), backscattered electron imaging (BEI), and energy dispersive X-ray analysis (EDX) detectors. Results of elemental analysis in all 3 marine turtle types showed mainly contained carbon (C), oxygen (O), calcium (Ca), sulfur (S), sodium (Na), chlorine (Cl), magnesium (Mg), silicon (Si), potassium (K), aluminum (Al) fluorine (F) and phosphorus (P). Among elements detected, carbon, oxygen, and calcium were found at high percentages. It was found that calcium was accumulated in the outer cuticle layers (P<0.05). Interestingly, it was found contamination of hazardous metals and non metals in turtle eggshell samples. Transition metals and other metals; including lead (Pb), palladium (Pd), and molybdenum (Mo), were detected in all 3 eggshell types at the percentages of 0.1-0.5%. Also, copper (Cu) contaminated in leatherback eggshells and cadmium (Cd) was found in green turtle samples. Contaminations of these heavy metals were always seen in the outer layers except for Mo. Finally, there were other elements found in the eggshells which were bromine (Br) and Iodine (I). This work showed the merit of SEM. It is a powerful and comfortable to analyze percentage of trace elements accumulation and transition metals contamination. Moreover, this finding would be the fundamental data for further works to management of marine turtle conservation.

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TH 065

Analytical microstructure on eggshells of green turtle (*Chelonia mydas*), hawksbill turtle (*Eretmochelys imbricata*), and leatherback turtle

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Analytical microstructure on eggshells of green turtle (*Chelonia mydas*), hawksbill turtle (*Eretmochelys imbricata*), and leatherback turtle, were studied by scanning electron microscope (SEM) with secondary electron imaging (SEI) detectors. All marine turtle eggshells composed of 3 layers, which were outer cuticle layer or calcareous layer, middle layer or middle multistrata layer, and inner fibrous layer. Generally, morphology of the outer layer (cuticle layers) that composed of the aragonite form of calcium carbonate (CaCO₃). It is thick and porosity. The middle layer is compact thick that composed of CaCO₃. While, the inner layer is compact thin. However, there also were different characteristics among 3 eggshell types. The cuticle layers of green turtle eggshell contained loose nodular units forming as clusters of branching needle-like crystals without interlocking attachments. The leatherback turtle eggshells had a cuticle layer which was similar to the green turtle but their crystals were more tapering and smaller. For hawksbill turtles, the cuticle layers contained ball-shape and needle-like crystals. The middle layers and the inner layers consisted of fibers that were firmly forming as network thorough the layers. For the middle layers, sodium chloride was also observed as complexes in the compositions. The outer layer thickness of green turtles, hawksbill turtles, and leatherback turtles were 129.88±14.22, 117.51±16.19, and 83.67±17.15 m, respectively. While the middle layer thickness were 108.06±13.00, 118.32±20.06, and 144.20±2.20 m, respectively, the inner layer thickness of green turtles, hawksbill turtles, and leatherback turtles were 11.38±3.07, 3.72±0.86, and 9.69±2.53 m, respectively. This work reviewed the utilization of SEM that classified marine turtle eggshell for management of marine turtle conservation.

Acknowledgement: This research was funded by grants from Silpakorn University Research & Development Institute (SURDI) and Faculty of Science, Silpakorn University, Thailand. We are also grateful to the Sea Turtle Conservation Centre, the Air and Coastal Defense Command, the Sattahip Naval Base for their kind support in providing marine eggshell turtle samples.

TH 066

Will global warming have an influence on the adverse effects caused by pesticides? A first approach using amphibians as a model

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Over the past thirty years, climate change has produced an enormous biodiversity loss as well as a shift in species distribution ranges. Amphibians epitomize the modern biodiversity crisis, and global climate change may be directly and indirectly responsible for amphibian's decline. Although amphibians are considered less sensitive to direct temperature shifts than other groups, small changes can trigger cascade effects when thermal disturbance is combined with pollutants. Among these indirect consequences, temperature changes have been demonstrated to modify the lethal and sublethal effects of contaminants in biota. Most studies addressing the adverse effects posed by contaminants to several amphibians species have been performed at moderate, constant ambient temperature of approximately 20 °C, which is an over simplification of what happens in the environment (for example, temperatures as high as 40°C may occur, with diurnal variations of 5°C). In fact, there is fragmental but strong evidence suggesting that temperature induces changes in acute toxicity of chemicals, hence, the effect under laboratory conditions may substantially differ from that observed in real ecosystems.

The aim of this project, addresses the question: Could be that changes in environmental temperature regimes, due to climate changes, influence the adverse effects caused by pesticides on amphibians?

Future results from small scale experiments (laboratory experiments) may be validated by conducting studies at large spatial and temporal scales with a higher ecological realism (mesocosms). The underlying mechanisms responsible for the changes observed in experimental systems at a large scale, not replicable, may be examined at lower levels, such as subcellular levels (biochemical biomarkers), individual level (survival, growth/development, behaviour) or population level (recruitment).

EC02P -Contaminant pathways, trends and biological effects in a warmer Arctic

TH 073

Occurrence and seasonality of cyclic volatile methyl siloxanes in Arctic air

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Cyclic volatile methyl siloxanes (cVMS) are high-volume production chemicals occurring in a wide range of technical applications and personal care products. Due to the high vapor pressures of cVMS, volatilization to the atmosphere is their main mode of emission to the environment. In the atmosphere, cVMS have half-lives ranging from 6.9 to 10.6 days due to reaction with hydroxyl radicals, indicating that they are persistent in the atmosphere according to the definition by US EPA. In addition, model simulations have predicted cVMS to have potential for long-range atmospheric transport. However, measurements of cVMS in the atmosphere of remote areas, such as the Arctic, remain scarce. Thus, there is a critical need for more empirical data to assess their long-range atmospheric transport potential and atmospheric behavior. At high latitudes large seasonal variations in the amount of sunlight are followed by corresponding large variations in atmospheric hydroxyl radical concentrations. In addition, cold temperatures in the winter season slows down chemical reaction rates. As a consequence, air concentrations of cVMS are expected to vary substantially in the Arctic atmosphere, with relatively high concentrations in winter (polar night) and low concentrations in summer (midnight sun period). This has been predicted by model simulations, but still remains to be validated by observations. In this study an active air sampling method using solid phase extraction cartridges, previously validated and applied for decamethylcyclotrisiloxane (D3) in Sweden, was adapted for Arctic conditions and further evaluated for hexamethylcyclotrisiloxane (D3), octamethylcyclotetrasiloxane (D4) and dodecamethylcyclohexasiloxane (D6) in addition to D5. The method was applied to collect air samples at the Zeppelin observatory in Ny Ålesund (79°N), Spitsbergen, Norway, to assess the atmospheric burdens of cVMS and to investigate if the predicted seasonality is occurring. Results from various sampling campaigns will be presented and discussed, including results from method validation.

TH 074

Emerging persistent organic pollutants in arctic air, surface water and snow in Ny-Alesund, Svalbard

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Emerging persistent organic pollutants (ePOP) may reach ecologically sensitive Arctic environment via atmospheric and/or oceanic long range transport. They are subject to a variety of processes in the Arctic environment such as degradation, bioaccumulation and interaction between the atmosphere, snow, water and soil. Additionally, climate change may significantly influence the transport and environment fate of ePOPs in the Arctic. As a part of collaborative German-French program at joint French-German Arctic Research Base (AWIPEV) in Ny-Alesund, Svalbard, this project is proposed to investigate the occurrence and long term trends of ePOPs in arctic air, water and snow.

Integrated high-volume air samples were taken on the roof of IPEV station using a high-volume pump operated for 7 days to obtain a volume of ~2000 m³. A glass fiber filter is used to trap the airborne particles and the gaseous contaminants are collected with a PUF/XAD-2 resin column. Surface snow samples were collected on the glaciers in Ny-Alesund and seawater samples were obtained in Kongs Fjord in May of 2011. All samples were analyzed for neutral and ionic perfluorinated compounds, brominated flame retardants, dechlorane plus and current-use pesticides. Data achieved from this study may improve models to predict the environmental progression and assess the effect of climate change on the long-range transport and the fate of the ePOPs in the Arctic ecosystem.

TH 075

Brominated flame retardants in ice cores from Svalbard: an east-west comparison

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Ice cores from Svalbard have proven to be useful indicators of contaminant conditions in the European Arctic. Our previous work has shown that ice cores from eastern Svalbard (the Austfonna site, 79.48°N, 24°E, 740 m.a.s.l.) shows a larger number of current-use pesticides in its profile than observed in western Svalbard (the Høltedahlfonna site, 79.13°N, 13.27°E, 1150 m.a.s.l.). Since our earlier work, comparisons between these sites for other contaminants have been of interest. In 2007 we drilled a short core (12 m) at Austfonna in order to identify the burden and inputs of brominated flame retardants at that site, providing comparable data for Høltedahlfonna. Two of the most abundant BFRs to appear in marine and glacial samples from Svalbard include decabromodiphenyl ether (BDE-209) and hexachlorocyclododecane (HBCD). The results for these BFRs at Høltedahlfonna show growing inputs over time since the 1970s, reaching 322 pg cm⁻² yr⁻¹ and 910 pg cm⁻² yr⁻¹ between 1995 and 2005. Results from Austfonna cover 1995 - 2007. Because of larger sample size, we were able to obtain 3 samples over this period from 1995 - 2000, 2000 - 2004, and 2004 - 2007. Our results for BDE-209 show fluxes of 1.95, 47.9 and 28.3 pg cm⁻² yr⁻¹ from 1995 and ending in 2007, the surface about 10% the input found at Høltedahlfonna. For HBCD, the Austfonna inputs over the same time intervals are 272, 315 and 804 pg cm⁻² yr⁻¹ over the intervals from 1995 - 2007. The HBCD results at Austfonna are lower, but between 2004 and 2007 are about 88% of the Høltedahlfonna flux from 1995 - 2005 (910 pg cm⁻² yr⁻¹). The burden of BDE-209 at Austfonna is 466 pg cm⁻² while at Høltedahlfonna over similar time period it is 3232 pg cm⁻² yr⁻¹, about 7 times more. The burden of HBCD is 8348 pg/cm² at Austfonna (1995 - 2007), while at Høltedahlfonna it is 9078 pg cm⁻² yr⁻¹, about 9% more. These results clearly show that our earlier observations of a greater pesticide input at Austfonna than Høltedahlfonna are not true for BFRs, at least after 1995. Different classes of compounds likely have different sources in the region; pesticides may be delivered in the gas phase while BFRs are certain to be on particles. These and other characteristics of long-range atmospheric transport in this region of the Arctic likely play a role in the contrasting observations we have made between pesticides and BFRs.

TH 076

Atmospheric deposition of legacy and emerging persistent organic pollutants in Northern Sweden

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The Arctic is an ideal indicator region for Persistent Organic Pollutants (POPs) because it is far from sources of these contaminants. To reach remote areas such as the arctic, POPs undergo long range transport via multiple cycles of atmospheric deposition and re-volatilization, known as the grasshopper effect. Thus, atmospheric deposition is believed to be an important pathway for arctic contamination. The aim of this study was to quantify the flux of legacy POPs from atmosphere to land in the Arctic as well as screen for emerging POPs new to the arctic.

Bulk atmospheric deposition samples using Amberlite IRA-743 as an adsorbent for hydrophobic pollutants were taken every two months for one year at two different locations in northern Sweden. The first sampling site was a subarctic site approximately 60 km from the city of Umeå. The second site was within the Arctic Circle, in a remote area near Abisko National Park. A method was developed for the analysis of various legacy and emerging POPs in these samples based on GC-HRMS. Two current use pesticides and four flame retardants, which have scarcely been detected in the arctic, were identified in the samples: trifluralin, chlorothalonil, TBECH, hexabromobenzene, BTBPE, and Dechlorane Plus. Hexabromobenzene, BTBPE, and Dechlorane Plus were detected in all samples and TBECH and Trifluralin were detected in more than half of the samples. The ratio of the two isomers of Dechlorane Plus was similar to the technical product at the first site; however, the more remote site exhibited a reversal in the dominant isomer indicating isomer selective degradation during long range transport. Legacy compounds including isomers of HCH, PCBs, DDT and metabolites, and chlordane related compounds were quantified in levels comparable to similar studies. PCB congener profiles differed between the two sites as well, with a lower proportion of PCB 180 and a higher proportion of PCB 52 depositing at the remote site. Nearly all compounds at both sites showed an increase in deposition during the months of June and July correlating with increased precipitation.

TH 077

Assessing the potential influence of global climate change on the long-range transport and accumulation of organic contaminants in the Arctic environment in relation to physical-chemical properties

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The potential influence of global climate change (GCC) on the fate, transport, bioavailability and bioaccumulation of organic contaminants is rapidly becoming an area of interest for scientists engaged in environmental chemistry and toxicology research. The Arctic region is of particular concern due to changes to the cryosphere that are already occurring (e.g. reduction in sea-ice cover and volume, melting of permafrost and glaciers), the fact that climate change models project the greatest warming to occur in this region in the future and finally that human inhabitants of this region harvesting local food resources (e.g. seals) have an elevated exposure to many organic contaminants of concern (i.e. POPs) (1-3). Global climate change could potentially influence exposure to organic contaminants via direct (e.g. altered distribution of chemicals in the environment) and indirect (e.g. increased primary productivity = altered organic carbon cycling = altered fate in aqueous environments) pathways (1). For human beings, lifestyle changes (e.g. dietary transition) facilitated by changes in the environment (reduced sea-ice = increased delivery of imported food items) could also be highly influential in the long-term (4). The purpose of this investigation is to explore the potential influence of changes to the physical environment (e.g. temperature, precipitation rate, sea-ice cover, primary productivity) on the long-range transport and accumulation of neutral organic compounds in the Arctic using a global-scale fate and transport model. Simulations were conducted for hypothetical chemicals covering a wide range of partitioning properties for a 20-year constant emission period followed by a 20-year depuration (i.e. zero primary emission) period. While sensitivities to the GCC scenario parameterizations considered here are relatively low (typically within a factor of two), responses can differ substantially depending on i) partitioning properties, ii) compartment (e.g. air, water, soil) and iii) stage of emission (uptake vs. depuration).

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TH 078

Increasing environmental concentrations of POPs in the Arctic in a future warmer climate

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The Danish Eulerian Hemispheric Model (DEHM) was applied to study the impact of climate change on contaminant levels in the Arctic. DEHM is a 3-D atmospheric chemistry-transport model developed over the last 20 years for studying the long-range transport of SO₂, SO₄, and Pb to the Arctic. All important source regions for the Arctic are included in the model domain, which covers the Northern Hemisphere. This model has been developed further to include mercury species/chemistry, and Persistent Organic Pollutants (POPs). The model has a spatially detailed 3-D atmosphere up to 15 km over the surface. In addition, it has four surface compartments: a 75 m thick ocean layer, a 15 cm thick soil layer, and dynamically evolving vegetation and seasonal snowpack compartments.

The model system has been run with meteorology obtained from ECHAM5/MPI-OM (SRES A1B scenario) for two different decades: 1990-1999 and 2090-2099. In this climate scenario the global temperature is predicted to increase continually with 3 °C by the end of 2100 relative to the period 1971-2000.

The model system was run initially for a period in order to spin-up the concentrations of POPs in the ocean and soil compartments. The concentrations in air, ocean water and soil of all the species from this simulation were used as initial concentrations for three different model runs with constant emissions (from year 2000) for the decades: 1990-1999, 2090-2099 and 2190-2199 using input from the ECHAM5/MPI-OM climate model. Simulations were also performed without spin-up concentrations. The differences between the results for the three decades are hence only due to changes in the meteorological as predicted by the ECHAM5/MPI-OM model system.

The results produced with the DEHM model system shows that the atmospheric concentrations of the studied POPs will increase. As the surface concentrations (soil and ocean water) also increases, this indicates that there will be an increased transport to the Arctic in a future warmer climate. This study has shown that it is useful to use meteorological output from climate models to investigate the exposure levels of contaminants under future changed climate conditions.

TH 079

Concentrations and potential sources of some semi-volatile organic contaminants in the air at Little Fox Lake, Yukon Territory, Canada

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In an investigation of the levels and potential sources of Semi-Volatile Organic Contaminants (SVOCs) at Little Fox Lake (LFL) in Canada's Yukon Territory, air samples were collected from 2007 to 2009. Air was drawn through a PM10 sampler inlet to exclude snow and large particles, then a Glass Fibre Filter (GFF) and dual Poly-Urethane Foam (PUF) plugs to trap the analytes. The sampling media were changed every 24 hours over two two-week periods in the late summer of 2007 and 2008, while week-long samples were collected the remainder of the deployment. After solvent extraction, column clean-up and fractionation, samples were analyzed by GC-ECD and GC-NCI-MS for a suite of SVOCs including some chlorinated pesticides, benzenes and industrial by-products. The median detection frequency for all compounds was 11%, ranging from 1% to 99%. Concentrations of most substances appeared to be independent of one another, but a weak but significant correlation appeared between levels of α -Endosulfan and trans-Nonachlor (concentrations were log-transformed, $r^2 = 0.50$, $p < 0.001$). After correcting for sample volume, concentrations of analytes ranged up to 22 pg m⁻³ for α -Hexachlorocyclohexane (α -HCH), considerably lower than the levels of α -HCH reported for the same station in 2000-2003, which was detected in 97 percent of samples (%OS). This was followed by pentachloroanisole (19 pg m⁻³, 19% OS), pentachlorobenzene (17 pg m⁻³, 99% OS), α -Endosulfan (10 pg m⁻³, 58% OS), trichloroveratrole (9 pg m⁻³, 97% OS) and a handful of others with low concentrations or low detection frequency. Hexachlorobenzene was present in nearly all samples but could not be quantified due to PUF breakthrough. The measured concentrations were combined with back-trajectories to elucidate potential source areas of these contaminants. The method is conceptually a Concentration Weighted Trajectory analysis, but trajectory endpoints above 700m elevation were filtered out and a 'geodesic' grid was employed to avoid skewing near Earth's poles. Most analytes were not revealed to have specific source regions. The method does suggest that the primary source of γ -HCH to the air at LFL is the Bering Strait, but the Chukotka region in Easternmost Siberia was highlighted as well. Tri- and tetra-chlorinated benzenes correlated with air-masses that originate in Inuvik, Northwest Territories.

TH 080

Heavy metals and nutrients in soils of the Yamal Peninsula (West Siberia) as indicator of ecosystem stability

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The development of gas fields on the Yamal peninsula (West Siberian Arctic) leads to disturbances of landscapes. Vegetation of the Arctic tundra is the most changeable component prone to surficial and subsurface impacts. Vegetation and related peat stabilize the thermal regime of Arctic soils and thus prevent permafrost from degradation. The patterns of vegetation and recovery depend directly on soil chemistry. Vegetation on soils depleted in major and trace elements is poor, and such landscapes are weakly sustainable. Thus, the sustainability of plant-soil systems largely depends on their chemical composition.

The biogeochemistry of permafrost plant-soil systems has been studied in soil and plant samples collected through environmental profiling in the Arctic tundra of the Yamal Peninsula. Sampling was along profiles that traverse different geomorphic levels and different types of disturbances (drilling sites, staffsettlements, quarries of mineral ground). Soils in northern West Siberia are deficient in many plant nutrients necessary for rapid and efficient revegetation in disturbed areas. Disturbance to peat topsoil reduces the store of organic matter, potassium, and nitrogen. Mechanic damage and pollution cause degradation or extermination of plant communities, while the remediation efficiency depends on the availability of mineral nutrients. Technogenic loads at development sites change dramatically the geochemical framework of landscapes. Specifically, soils at drilling sites are contaminated with Ba, Sr, Pb and petroleum hydrocarbons.

The chemical composition of different types of soils varies greatly. Zonal tundra peat-gley soils are depleted in P and have low cation exchange capacity (CEC) and total absorbed bases (TAB), alluvial soils have highest P and K enrichment, while peat in bog-tundra and fen soils is the richest in nitrogen.

The sustainability as an ability of rapid recovery after disturbances ("elasticity") is the maximum in alluvial ecosystems and depends on the contents of plant nutrients in soils.

TH 081

The 2012 assessment report on persistent organic pollutants in the Canadian Arctic

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The Northern Contaminants Program (NCP) has been documenting levels and trends of persistent organic pollutants (POPs) in the Canadian Arctic since 1991. The program includes measurements of legacy and new POPs in high volume air samples at Alert on Northern Ellesmere Island as well as more limited measurements using passive air sampling. The same chemicals are determined in samples of arctic biota (seabirds, beluga, seals, lake trout, burbot, arctic char) from multiple locations in Nunavut, the Northwest Territories and the Yukon. As a result of annual sampling of selected species initiated in 2004, temporal trend data sets for legacy and new POPs in biota are improving in statistical power. Scientific information about legacy and new persistent organic contaminants in the Arctic that is generated under the NCP has been contributed to circumpolar assessments of the Arctic Monitoring and Assessment Program (AMAP). These arctic data have played an important role on addition of new substances to the Stockholm Convention and the Convention on Long-Range Transboundary Air Pollution, and the implementation of these international agreements, particularly for the purpose of assessing their effectiveness. Because contaminants are accumulated in the Arctic primarily from long-range transport, data on new chemicals from arctic monitoring is considered as critical evidence when assessing the need to add new substances to these conventions. The issues have been addressed in the NCP have also provided the scientific base for Canada to evaluate the significance of the issue and it equipped the Government of Canada to advocate on the international stage for legal agreements on ban or phase out of POPs that end up in the Arctic.

In this study, we will present highlights from the recent assessment of spatial and temporal trends of POPs in Canadian Arctic that assessed results over the period 2003-2010. This assessment noted declining concentrations of legacy POPs such as chlordane, DDT, HCH and toxaphene and more limited declines of PCBs and HCB in the atmosphere and in biota. Trends of new and emerging POPs during 2003-2010 varied with increases (HBCD, BTBPE), plateauing levels (PBDEs) and peaks followed by declines (perfluorinated chemicals; PFCs) in biota and slow increases of PBDEs and PFCs in air.

TH 082

POPs in the Arctic & climate change, Part C: hemispheric transport of air pollution, UNECE

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The Arctic region is ideal to observe intercontinental transport of Persistent Organic Pollutants (POPs), since it has few regional POPs sources. Conversely, it is crucial to understand the emissions of POPs from Northern Hemisphere continents and transport to the Arctic before projecting exposures to toxics within the Arctic. We present here key findings of an assessment of the transport of air pollutants across the Northern Hemisphere conducted by the Task Force on Hemispheric Transport of Air Pollution (TF HTAP) for the consideration of the Convention on the Long Range Transport of Air Pollutants.

This presentation will emphasize the TF HTAP integrated approach to measurements, modeling, emissions and impacts, but focus on measurements and modeling, with special attention to climate change, which may alter POPs cycling between environmental compartments. Most scientific projects are not coordinated in a way to facilitate

synergy and integration. The TF HTAP collaboration enabled communication between specialists and identification of the most important cross-dependencies and research gaps. Furthermore, the review permitted the collating and sharing of an immense amount of data, among which recent IPY observations played a crucial role. Modeling enabled comparison between diverse and, sometimes, competing approaches with the benefit that model parameterizations could be exchanged. This intercomparison led to better appreciations of the robustness of models and the importance of uncertainties.

Mindful of overlap with other international organizations, the TF HTAP is initiating a new four-year work plan with a multi-pollutant co-benefit approach that harmonizes experiments, model parameterization and exploitation of results. Collaborations are voluntary, and key areas, like global POPs emission estimates, remain bottlenecks for further progress. Global estimates have often been provided in forms that cannot be used by models. For instance some emission estimates under UNEP have been reported by country, not location, substances are sometimes grouped together, and sometimes emissions are given without uncertainties. Continued monitoring will be essential to discriminate between the effects of climate change and progress resulting from emission regulations. The five TF HTAP volumes provide an extensive synthesis of the state-of-science and a guide to future research and policy needs. http://www.htap.org/activities/2010_Final_Report.htm

TH 083

Brominated flame retardants and polychlorinated biphenyls in the Antarctic food web

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Antarctica is a pristine desert continent on which industrial and agriculture activities never existed. Therefore, contaminants primarily reach the continent by long-range atmospheric transport (LRAT). Since semi-volatile persistent organic pollutants have a documented LRAT potential these substances are of special importance for Antarctic research. Their lipophilic and persistent nature allows accumulation in organisms and magnification throughout the food web. In this study various organisms have been analysed for the dioxin-like PCB congeners, polybrominated diphenyl ethers, alternative brominated flame retardants as well as Dechlorane Plus in order to investigate the contamination in the food web in Antarctica and estimate the risk for the species analysed imposed by PCBs (calculation of the toxic equivalents -WHO).

The samples were collected during in November 2010 to January 2011 around the Chinese "Greatwall-Station" on King George Island. The freeze-dried biota samples were extracted in a Soxhlet apparatus using dichloromethane. Extracts were purified by gel permeation chromatography to remove large molecules such as fatty acids, and further by silica-gel clean-up to remove polar compounds. Gas chromatography coupled with mass spectrometry was used for instrumental analysis.

The results indicate biomagnification throughout the food web from the herbivores to the carnivores. The detected concentrations were in the high pg/g to the low ng/g wet weight range.

TH 084

POPs affect the plasma steroid levels in polar bears, *Ursus maritimus*

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The Polar bears, *Ursus maritimus*, is the ultimate top predator of the arctic food chain. Due to biomagnification, polar bears are therefore exposed to high levels of persistent organic pollutants (POPs). Such POPs may potentially exert endocrine effects on sensitive pathways such as the steroidogenesis. Genital deformities in polar bears from the Svalbard region indicate that vital processes such as growth and reproduction may be impaired. Such impairments may potentially be caused by POPs. We have implemented a recently developed GC-MS/MS method for the simultaneous detection of 9 steroids (pregnenolone, progesterone, dehydroepiandrosterone, androstenedione, testosterone, dihydrotestosterone, estrone, 17 α -estradiol, and 17 β -estradiol) in plasma from 35 adult and subadult polar bears (3-23 years) collected in April 2008, Svalbard, Norway. POPs and their metabolites levels (HCB, α -HCH, β -HCH, oxychlordane, trans-nonachlor, mirex, p,p'-DDE, p,p'-DDT, PCBs, OH-PCBs, BDE-47) were also analysed in plasma. This allows for the first time an assessment of the impact of POPs on the overall polar bear steroidogenesis. Relationships were investigated using Principal Component Analysis. Significant impact of POPs on steroid plasma levels was observed. The coupling between POPs and steroid hormone levels may have serious implication for reproduction in polar bears. Thus, combined with climate change, POP exposure may exert detrimental impact on polar bear growth and reproduction.

TH 085

Temporal trends of legacy and new persistent organic pollutants in landlocked char in High Arctic Lakes in Canada

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Landlocked char are important sentinel species in Arctic lakes. As the only top predators in most high latitude Arctic lakes, char are good indicators of changes in inputs of bioaccumulative contaminants such as persistent organic pollutants (POPs). In this long term study landlocked Arctic char are collected annually from three lakes near the community of Resolute Bay (74° 42' N; 94° 51' W) on Cornwallis Island (Amituk, Char and Resolute) and in Lake Hazen (81° 49' N; 70° 42' W) in Quttinirpaaq National Park on Ellesmere Island. Results from 10 (Char) to 15 (Resolute) years of sampling are available with collection numbers ranging from 7 to 25 adult fish (>200 g) per lake except in Char Lake where the range has been 3 to 10 fish annually. Homogenized muscle (+skin) samples have been analysed for PCBs, chlorobenzenes, DDTs, chlordanes, dieldrin, and toxaphene, as well as new POPs (endosulfan, HBCD, HCHs, hexachlorobutadiene, PBDEs, PFOS) to assess trends over time. Σ PCB concentrations declined significantly in Char Lake (1993-2010), Amituk (1992-2010) and Hazen (1990-2010), but not in Resolute (1997-2010). Σ HCH also declined significantly and at a more rapid rate than other POPs. Toxaphene concentrations have also declined although the trend is significant only in Lake Hazen. Endosulfan (including endosulfan sulfate) concentrations increased during the 1990s until about 2004, particularly in Amituk and Resolute Lakes and have since declined. Concentrations of tetra- and penta-BDE congeners increased significantly and rapidly in most of the lakes during the 1990s-early 00s. The year for maximum concentrations varied from 2003 in Amituk, 2005 in Resolute to 2008 in Lake Hazen. While local contamination, i.e. from waste waters or dumpsites, does not appear to have influenced levels of most POPs, this is not the case for PFOS and related chemicals used in fire fighting foams in Resolute Lake. This lake is downstream of the Resolute airport where these chemicals appear to have been used in the past. On the other hand the perfluorocarboxylates (PFCAs), were present at similar low concentrations in all lakes. Preliminary temporal trend results suggest that PFC concentrations have plateaued in Amituk, Char and Hazen lakes and are now declining.

TH 086

Monitoring and modelling reveals time trends of POPs in Arctic biota

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Protecting Arctic ecosystems against potential adverse effects from anthropogenic activities is recognized as a top priority. In particular, understanding the accumulation and effects of persistent organic pollutants (POPs) in these otherwise pristine ecosystems remains a scientific challenge. A typical feature of data bases that contain monitoring data on tissue concentrations of POPs in Arctic biota is that these are fragmentary, i.e. not all POPs have been measured in all species. Here, we present a new approach to predict tissue concentrations in species for which no data exist by using the available tissue concentrations, a bioaccumulation model and time trend modelling. We demonstrate this approach for the Barents/Norwegian Sea ecosystem and show that the legacy-POPs decreased by a factor of 10 between 1985 and 2010, which reflects regulatory efforts to restrict these substances. In contrast, concentrations of fossil fuel derived PAHs in lower trophic levels (invertebrates and fish) increased 10 to 30 fold over the past 25 years and now dominate the summed POP burden in these biota. Our findings indicate that the debate on the environmental impacts of fossil fuel burning should move beyond the expected sea water temperature increase and examine the possible environmental impact of fossil fuel derived PAHs.

TH 087

Persistent organic pollutants in Antarctic Krill (*Euphasia superba*)

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Krill is a key node in the Antarctic ecosystem and the most important source of food to higher trophic levels in the Antarctic Region. Few Studies have reported legacy and emerging POPs concentrations in krill samples and when done it has for a reduced number of samples and covering a limited spatial extension. Hexachlorocyclohexanes (HCHs), Hexachlorobenzene (HCB), Polychlorinated biphenyls (PCBs), dibenzo dioxins and furans (PCDDFs) and Polybrominated diphenyl ethers (PBDEs) concentrations were reported previously in the bibliography. The objective of this work is to report concentrations of PCBs, HCHs, HCB, PBDEs and PCDDFs in Antarctic Krill (*Euphasia superba*). Samples were taken in February-March 2009 in the Weddell Sea, Bransfield Strait and Bellinghausen Sea around the Antarctic Peninsula. The common pattern between the different families of POPs and the influence of biogeochemical factors and proximate ice regions will be discussed.

TH 088

Effects of a warmer climate on temporal trends of persistent organic pollutants in Greenland biota

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Persistent organic pollutants (POPs) have been used extensively in industry, agriculture and consumer products and have reached the Arctic via atmospheric and ocean transport. They include organochlorine compounds such as polychlorinated biphenyls (PCBs) and DDT, which are regulated by the Stockholm Convention and were banned in most industrialised countries about 30 years ago, as well as fluorinated and brominated compounds, such as perfluorooctane sulfonate (PFOS) or hexabromocyclododecane

(HBCD), recently phased out or still unregulated. These compounds have in common to bioaccumulate and biomagnify through the Arctic food chain, with biomagnification factors depending on the properties of the compound and species-specific metabolic capacities. In Greenland, systematic monitoring of POPs has covered the last 16 and 28 years for organochlorines and polybrominated diphenyl ethers (PBDEs), respectively. In general, organochlorines have decreased in biota of the marine and the freshwater environment, however, with indications of stabilisation since approximately 2000. Brominated compounds have been shown to increase, following their increasing use in industry and a wide range of consumer products, but decreasing PBDE concentrations have been observed in adult seals from East Greenland over the last 5-10 years, a trend which might also be expected for other species, due to recent regulations. Climate change and its consequences for the Arctic might also have an impact on the fate of POPs, in terms of e.g. changes in transport pathways and feeding behaviour of Arctic wildlife. POP time series in ringed seals from Disko Island (West Greenland) are currently being studied, in combination with climate information and data on trophic level. Local climate indices include winter ice coverage in Disko Bay and summer water temperatures and salinity at Fylla Banke near Nuuk. In addition, the Atlantic Oscillation index is used as a more global climate index. Furthermore, data are included on stable nitrogen isotopes in ringed seal muscle, to give information on trophic variability.

Acknowledgements

The authors wish to thank Arctic Station and the University of Copenhagen for providing the data on daily ice coverage in Disko Bay. We also thank Mads Hvid Ribergaard, Danish Meteorological Institute (DMI) for providing the data on sea water temperature and salinity at the top of Fyllas Banke.

TH 089

Impact of dense shelf water cascading in the transfer of organohalogenated contaminants offshore Svalbard, Arctic Ocean

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The formation of dense shelf water cascading depends on the evaporation or cooling of the sea surface, which depends on air temperatures. Only if the air-sea surface gradients become large enough, sea-ice freezing may lead to the formation of dense waters at Storfjorden (Svalbard Archipelago). The Arctic Ocean warming may have an influence on the production of cascading water masses flowing from the Storfjorden shelf area to the continental slope, which may contribute to the rapid transfer of persistent organic pollutants into the open marine sediments.

To date no studies have considered the influence of these cascading processes in the transport of contaminants from the continental shelves to the deep marine environments, and there are no records of organohalogenated contaminants in open sea sediments from the Arctic Ocean. For this purpose, sediment cores were collected at 500, 1000, 1250, 1500 and 2000 m water depths along the Storfjorden area and the continental slope during two oceanic cruises (July 2010 and July 2011). These organohalogenated compounds encompass organochlorine pesticides and industrial products, as polychlorobiphenyls and polybrominated diphenyl ethers (PBDEs). The results show detectable values of all analysed compounds, with higher concentrations of HCB and 4,4'-DDE considering organochlorine compounds, and higher levels of BDE-47 and BDE-100 congeners, relating PBDEs.

TH 090

Biogeochemical and physical controls on legacy persistent organic pollutants in the Antarctic atmosphere, seawater and plankton

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Legacy Organochlorine compounds including Polychlorinated Biphenyls (PCBs), Hexachlorobenzene (HCB) and Hexachlorocyclohexane (HCHs) are ubiquitous pollutants that reach remote ecosystems through atmospheric transport and deposition. Persistent organic pollutants were detected in Antarctica for the first time in the 60s by Risebrough and coworkers, but despite these pioneering work few studies have been centered in the marine environment. Antarctic studies have reported occurrence of some of these chemicals in seawater, the atmosphere, and in zooplankton but no data are published for phytoplankton. In addition, previous studies did not assess their simultaneous occurrence in air, water and plankton and the fluxes between these compartments. Current paradigms of the controls on the cycling and sinks of POPs in remote areas take into account the effect of temperature and organic matter cycling. Lately, the biological pump and physical pump (subduction of water masses) have also been postulated as important processes affecting the inputs of POPs to polar environments even though large uncertainties remain on the relative importance of all these processes affecting the occurrence of POPs in Antarctic waters and biota. The objectives of this work are: i) to measure the simultaneous occurrence of Legacy POPs in gas and aerosol phase, dissolved seawater and phytoplankton concentrations around the Antarctic Peninsula (Weddel Sea, Bransfield Strait and Bellinghausen Sea) and ii) to assess the physical and biogeochemical controls driving their occurrence in Antarctic seawater and plankton. This was carried out through two sampling campaigns in the austral summers of 2007/8 and 2008/9. The results show low levels in all phases, but the lower levels in plankton are observed in the high productivity regions of the Weddel and Bellinghausen sea. The potential role of ice melting plays on levels in air, seawater and plankton was assessed at the ice margin role of the Weddel Sea and Wilkins platform.

TH 091

Relationships between mercury, organic matter, and climate change

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Recent studies in the remote pristine lakes in the Canadian High Arctic has shown that temporal trends of mercury (Hg) may significantly correlate with the labile, algal-derived organic matter (OM) over past decades and centuries. This correlation is attributed to increase in phytoplankton productivity during the latter half of the 20th Century; suggesting that the dynamics and fate of Hg in High Arctic lakes may have been seriously confounded by increased algal productivity as the result of recent climate change. The important questions facing this hypothesis are (1) the degree that climate change and resulting algal productivity influence the Hg flux into the aquatic systems (2) whether or not this phenomena is limited to Arctic region and what is the role of depositional environment in the observed pattern (3) how atmospheric flux of Hg as related to the local/regional anthropogenic source(s) may influence the Hg-OM correlation observed in the sedimentary records.

This study examines the historical records of Hg and OM from 40 high-resolution lacustrine/marine sediment cores representing a wide range of depositional environment, climatic, and geographical conditions across Canada. The results show that the nature of the relationship between sedimentary Hg and OM may significantly change depending on the degree of Hg influx, and quantity and composition of the OM content of the sediments. It appears that OM, in particular, labile, mainly algal-derived OM primarily controls the Hg distribution in the sediments when the Hg influx (atmospheric deposition, runoff, etc) is within or close to background levels (e.g., as in Arctic lakes). When the Hg influx exhausts the capacity of OM scavenging (in the case of high anthropogenic input), excess Hg can be found in sediments, showing a clearly different temporal pattern to its organic counterpart. Only in these cases will sedimentary Hg profiles follow the history of anthropogenic Hg deposition. Given the relative homogeneity and paucity of OM in many Arctic and Sub-arctic lakes, small changes in climate may induce dramatic shifts in the rate of suspended OM scavenging of Hg, which is reflected in the sedimentary record of Hg.

TH 092

Temporal trends of mercury in landlocked char in High Arctic Lakes in Canada: Possible influence of climate warming?

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As the only top predators in most high latitude Arctic lakes, char are good sentinels for changes in inputs of bioaccumulative contaminants such as methyl mercury. In this long term study, landlocked Arctic char have been collected annually from three lakes near the community of Resolute Bay (74° 42' N; 94° 51' W) on Cornwallis Island (Amituk, Char and Resolute) and in Lake Hazen (81° 49' N; 70° 42' W) in Quttinirpaaq National Park on Ellesmere Island. Results from 10 (Char) to 15 (Resolute) years of sampling are available with collection numbers ranging from 7 to 25 adult fish (>200 g) per lake except in Char Lake where the range has been 3 to 10 fish annually. Char muscle samples have been analysed for total mercury (THg) by cold vapour atomic absorption spectrometry after acid digestion in a high pressure microwave oven or by Direct Mercury Analyser. In addition the tissue digest was analysed for selenium and 30 other elements using ICP-MS. Geometric mean (length adjusted) concentrations of THg ranged from 0.12 to 0.30 ug/g (wet wt) in Resolute and Hazen lakes and from 0.5 to 1.6 ug/g ww in Char and Amituk Lakes. Although THg levels were generally higher in samples from the mid-00s compared to the 1990s, no statistically significant trends were found in all 4 lakes using length adjusted geometric means for each sampling year. Results for Resolute Lake, with 15 sampling years, were closest to achieving the desired goal of detecting a 5% change (with a power of 80% at P=0.05) at 16 yrs and power of 65%. These trends are in contrast to those observed in lake trout and burbot in Canadian sub-arctic lakes and rivers where increasing THg has been observed, and also to some mid-latitude lakes in the Great Lakes region where THg has been shown to be declining in fish. Preliminary comparisons with climate related data for the period 1990-2011 showed that THg in char in Resolute Lake were weakly correlated with average maximum air temperature in June and July with higher temperatures and average THg in 1997-98 and in 2005-06. However additional annual sampling is needed to further evaluate this tentative relationship.

TH 093

Temporal trends of cadmium in landlocked Arctic Char (*Salvelinus alpinus*) in the Canadian High Arctic: the possible influence of climate change

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The Austrian-Canadian research cooperation "HighArctic 1997-2010" investigated the effects of short-term and long-term climate change on freshwater ecosystems in the Canadian High Arctic and in the Austrian Alps. The ongoing multi-year study has shown that global warming may endanger fish populations from Arctic and alpine lakes by leading to an increase of both metal accumulation and stress. Comparison of metal levels (e.g., cadmium, zinc) and biochemical stress indicators in Arctic char collected from Canadian Arctic lakes revealed marked seasonal and inter-annual trends in the turnover of metals, as well as stress responses in the liver. Results indicate metal accumulation and level of stress to be higher the warmer the summers are in the Canadian Arctic. Predictive relationships between lake temperature and metal uptake were similar for high Arctic lakes and previously studied Austrian high mountain lakes, thus confirming water temperature to be a driving force of metal accumulation in Arctic char from these sensitive ecosystems. The observed effects provide clues as to what would happen to the extremely vulnerable land-locked char populations in the event of a longer-term, synoptic warming trend associated with global climate change. We speculate that the projected warming conditions could be a serious threat to the stability of Arctic char populations in high latitude and high altitude lakes. The results illustrate that fish from high latitude and high altitude lakes appear to be sensitive bioindicators of the interactive effects of pollution and global climate change

TH 094

Modeling pathways, processes and trends of mercury contamination in the Arctic

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A three dimensional process based global mercury model - GRAHM (Global/Regional Atmospheric Heavy Metals Model) is applied to study the pathways, processes and trends of mercury in the Arctic. Environment Canada's mercury model GRAHM simulates meteorological processes and Hg related physico-chemical processes in the atmosphere. The model simulates transport and transformations of mercury in the atmosphere and its exchange with Earth's surfaces on global scale. Hg emissions in the model include global anthropogenic, natural and reemission of previously deposited Hg from land and oceans. The model includes mercury transport, chemical reactions and bi-directional surface exchanges of mercury. Proper treatment of the transformations of mercury deposited onto snowpacks is important to model the impact of changing climate in the Arctic. In the springtime, Atmospheric Mercury Depletion Events in the Arctic are accompanied by high levels of deposition of mercury to the cryosphere. A significant portion of the deposited mercury to the snowpacks is observed to be revolatilized rapidly depending on the surface characteristics and meteorological conditions. A combination of physical and chemical environmental factors may result in trapping or losing a substantial portion of the deposited mercury within the cryosphere. This mercury may enter the meltwater and make it to the oceans or other aquatic bodies. A comprehensive dynamic scheme of air-ice-snowpack-melt water exchange of mercury based on extensive review of measured data was developed and implemented in the model to specifically address the impact of warming Arctic on mercury accumulation in the Arctic. The new version of the model is applied to determine the net accumulation of atmospheric mercury to the Arctic soils and Ocean. Relevance of mercury deposition to the snowpacks in the Arctic was analyzed. Atmospheric pathways, source attribution and trends of mercury concentrations and deposition were analyzed. The model description and results will be presented at the conference.

TH 095

Identification of degradation products of quinalphos in rats: an in vivo study

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Pesticides are among the few toxic materials deliberately disbursed into the environment to preserve the agricultural produce. Besides their use as agrochemicals the pesticides are known homicidal and suicidal agents because of their easy availability. According to a WHO estimate as many as three million people per year are poisoned by pesticides resulting in around two hundred thousand deaths. Therefore, the various monitoring programmes need to fulfil the obligation to know as to what is the behaviour of pesticide in the human system. In order to evaluate the deleterious effects of exposure to quinalphos a comprehensive study was planned to follow the pharmacokinetic in vivo degradation in rat. A rapid HPLC procedure has been developed employing an octadecyl endcapped RP-C18 column and an ultraviolet detector. Several procedures for the extraction of quinalphos from biological samples were scanned and judged on the basis of recovery. Methanol was found to be the most suitable extractant with a recovery of 95±5%. All the samples viz. blood serum and urine collected at various time points were lyophilized, extracted and analyzed. Also the metabolic intermediates of quinalphos in serum and in urine of albino rats at different time intervals were identified using GC-MS after dosing the animals with 5 mg kg⁻¹ body weight. The decay was measured for a maximum of twenty four hours in rats. Control studies show that the degradation of the pesticide followed a first order kinetics. The profiles of concentration observed in biological fluids suggest that quinalphos is rapidly absorbed and reaches a peak concentration in 2 hours after dosing and thereafter it is metabolized in blood and urine with a half life of 3.8 and 4 hours, respectively. Among the important metabolites identified in vivo, a majority of them appear to be formed mainly through hydrolysis and oxidation. In each case quinalphos oxon, O-ethyl-O-quinoxalin-2-yl phosphoric acid, 2-hydroxy quinoxaline and ethyl phosphoric acid are formed. The results on metabolites may be helpful in assigning possible contributors towards the toxicity of quinalphos in addition to parent pesticide. The results further indicate that the metabolites, 2-hydroxy quinoxaline and oxon which are more toxic than the parent compound seem to persist for a longer time. The presence of these metabolites in the concerned biological fluids can help in the identification of quinalphos as the source of poisoning.

EC03P - In situ remediation of metal(loid) contaminated soils: exploration of emerging technologies and long-term effects of previously remediated sites

TH 097

Helianthus tuberosus L. grown with multiple pollution in greenhouse conditions

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Helianthus tuberosus L. (Compositae) offers wide technological interest as a bio-ethanol due to its high potential for biomass production. Recently studies showed the tolerance of this crop to heavy metals. These characteristics make it a potential candidate plant for phytoremediation of soils and to obtain renewable energy in polluted areas with moderate levels of heavy metals that can no longer be used for agriculture applications. The present work reports data concerning to the tolerance of two cultivar-clones of *H. tuberosus* (VR and D19) grown in hydroponic culture with a multipolluted solution of heavy metals, in order to evaluate their use as energetic crop in polluted sites. The experiment was carried out in a greenhouse under controlled conditions. Tubers were recollected in field and transferred to plastic trays filled with turf during two weeks. Then, the plants were carefully rinsed in distilled water and transferred to hydroponic culture with coarse perlite. Plants were fertilized with a modified Hoagland's solution and treated with heavy metals: (i) Treatment 1: 30 mg-L⁻¹ of As (V), Cd, Cr (VI) and Ni; (ii) Treatment 2: 30 mg-L⁻¹ of Cu, Zn, Pb and Cd; (iii) Treatment 3: 30 mg-L⁻¹ of As (V), Cd and Ni. Control plants were fertilized only with nutrient solution. The plants were harvested after two weeks, separated into leaves, stems, roots and tubers, dried in an oven at 70 °C for 72 h, and dry weight was recorded. Results showed the leaf, stem and root dry weight were significantly reduced in both cultivar-clones as comparison of the control when plants grown in presence of a mixture of As (VI), Cd, Cr (VI) and Ni. Biomass of both cultivar-clones showed no significant differences when treatment with Cu, Zn, Pb and Cd was applied. VR was not affected by As (V), Cd and Ni, while D19 biomass was significantly reduced with this treatment, in the case of leaves and roots by 50% in comparison to control. From this preliminary experiment we concluded that both cultivar-clones of *H. tuberosus* seem to tolerate mixtures of Cu, Zn, Pb and Cd. Related to cultivar-clones, VR tolerated concentrations of As (V), Cd and Ni in solution better than D19, and both cultivar-clones were sensible to the presence of Cr (VI). Acknowledgements: EIADES PROGRAM S2009/AMB-1478, RTA000150-00-00-INIA; Contratación de Doctores INIA-CCAA and FPI-INIA to support the predoctoral grant Montalbán B.

TH 098

Numerical modeling of a Phyto-extraction process

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The phyto-extraction is an in situ remediation technique involving plants that extract contaminants and accumulate them in plant tissues. Despite its low cost and low impact presents difficulties in estimating the efficiency of the process and the time required for remediation. The results depend on a complex network of interactions between plants, soil and atmosphere in the presence of contaminants. This research aims to estimate the time required for remediation of soil. Pedologic and climatic data from Rio de Janeiro were used. The initial contamination levels were determined based on real cases of industrial activities and waste disposal. The root contaminant uptake model was previously calibrated using data from greenhouse experiments. The simulation was organized in three phases: pre-contamination (determination of hydrological conditions and water table), contamination (evolution of the plume of contamination before planting), remediation (remediation process simulation). Although the results were specific to the chosen scenario, it was observed that the efficiency of the phyto-extraction decreased with depth reached by the plume of contamination. The proposed methodology, despite the need for calibration enhancements and models for vegetation, provided indications that may be useful in the design of phyto-extraction project.

TH 099

Immobilization of metals in contaminated sites with use of abundant waste materials exemplified in Cu and Cd binding onto sewage sludge

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Viable remediation of sites contaminated with metal(loids), should fulfil several basic requirements, of these cost-efficiency, effectiveness, abundance of a material used for remediation, easiness of management and temporal sustainability are the major ones. In these terms, stabilized sewage sludge (SS) seems to be particularly promising. In this study, binding properties of SS from a tertiary sewage treatment plant for metals, exemplified in Cu and Cd sorption, in view of SS application for remediation of sulfidic extraction waste dumps generating metal-rich Acid Rock Drainage (ARD) has been presented. Freshly generated SS was moderately acidic (pH 5.55), of hydraulic conductivity 10⁹-10¹⁰ m/s, specific surface with respect to polar adsorbate (H₂O) 216 m²/g, CEC₂₅=258cmol/kg, with predominance of Ca and Mg as exchangeable ions, OM 51% and initial content of trace metals from 4.82 mg/kg d.m. (Cd) to 3790 mg/kg d.m. (Zn), with Cu content 222 mg /kg d.m. Sorption capacity for studied metals

assessed in batch mono-metallic Me-SO₄ system at pH 4.0 accounted for 40600 mg Cu/kg and 39000 mg Cd/kg and was comparable with natural sorbents (e.g. peat). In the environmentally relevant systems, such as ARD generating sites, a number of factors may influence sorption capacity of materials, of these pH, competitive effect of other ions, adsorbent-adsorbate contact mode, dominating anions or cations in solution and the temporal transformation of adsorbent are the most important ones. Sorption experiments taking into consideration all these factors were thus conducted. The following systems were studied: (a) sorption of metals from Me-Cl, Me-SO₄ and Me-NO₃ solution, in monometallic and binary batch systems, at pH 4.0; (b) comparative Me binding onto freshly generated and weathered SS; (c) sorption in flow-through systems, in mono-, binary and polymetallic systems, also at strong domination of Fe ion, at pH 3.0 and 1.5; (d) application of a simulated "worst case" polymetallic ARD onto freshly generated and weathered SS in a flow-through system. The aforementioned factors appeared to exert a strong reducing effect on the SS sorption capacity, of these pH, Fe predominance in adsorbate and weathering transformations of adsorbent (SS) were the most important ones, able to reduce its sorption capacity up to 2 orders of magnitude. Despite this, even in the worst case, sorption capacity of SS was relatively high and allowed to substantially attenuate metal mobility.

TH 100

Bioremediation of contaminated soils by biosurfactants produced in co-culture fermentation processes

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Coculture fermentations, instead of single cultivation can be utilized advantageously, in industrial biotechnology in the production of chemicals, particularly during the use of lignocellulosic material as feedstock, since both cellulosic and hemicellulosic fractions can be employed.

The use of *Lactobacillus pentosus* and *Lactobacillus rhamnosus* allow producing biosurfactants from both fractions. Biosurfactants are amphiphilic compounds of microbial origin with a pronounced surface activity and with several advantages over chemical surfactants including lower toxicity, higher biodegradability, and effectiveness at extreme temperatures or pH. Biosurfactants are potential candidates for many commercial applications in the biomedical, petroleum, and food processing industries.

This work deals with the application of biosurfactants obtained from different agroindustrial wastes, during cocultures processes, in the bioremediation of soils contaminated with heavy metals.

TH 101

Biosorption of heavy metals from gold mine wastewaters by *Penicillium simplicissimum* immobilized on zeolite: kinetic, equilibrium and thermodynamic studies

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A biosorbent based on zeolite and *Penicillium simplicissimum* (both living and heat-killed fungal biomass) was developed for the clean up of heavy metals from gold mine wastewaters. With an initial concentration of 500 mg l⁻¹ at pH 3 - 4 for a single component system, 99% adsorption was observed for: Cu²⁺, Co²⁺, Cr³⁺, Fe²⁺, Ni²⁺, Zn²⁺, Hg²⁺. Immobilisation of fungi on zeolite yielded higher biomass, showing the potential of this study towards remediation of polluted mine sites. Desorption results showed that the adsorbent could be reusable.

TH 102

Mercury immobilization using modified mackinawite as reactive layer in in-situ capping

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Mercury and methyl mercury adversely affect human, birds, and mammals health. The effects of mercury contamination are cause of global concern; thus remediation of contaminated sites is a high priority issue. This study investigated the mercury immobilization using mackinawite modified with L-cysteine as an active layer in in-situ capping. Mackinawite (FeS) is a promise material to be used in in-situ capping due its excellent capacity for mercury uptake from anoxic contaminated sediments. The surface modification with L-cysteine increases the mackinawite oxidation resistance, becoming suitable its use in field conditions. Modified mackinawite as a reactive capping layer was studied in lab scale simulator systems using lake sediment contaminated with mercury. The mercury profile towards the cells showed that modified mackinawite is an excellent material to be used as reactive layer in in-situ capping. It was observed that the mackinawite modification have little or no influence on the mercury uptake from the contaminated sediment, in comparison to the unmodified mackinawite. The double layer system (sand layer over the modified mackinawite layer) was the best design observed for in-situ capping. Incubation experiments showed that modified mackinawite has higher mercury methylation inhibition capacity, than the observed from unmodified mackinawite.

TH 103

In situ arsenic and lead stabilization and remediation in acidic soils using green chemistry and nano iron welding fumes

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Arsenic (As) and lead (Pb) are highly toxic naturally occurring elements found in soils worldwide and is recognized as a carcinogen responsible for neurological, gastrointestinal, cardiovascular, and reproductive illnesses. Toxic concentrations of As can be released into drinking water supplies as a result of mineral weathering and human activities. Soils with toxic accumulations of As and Pb pose a public health threat particularly to children because of their tendency to consume soil via hand-to-mouth contact (USEPA, 2000) and, to a lesser extent, adults with home gardens and occupational exposures. When pseudo total As contamination levels are ≤ 2000 mg kg⁻¹ in situ remediation with Fe amendments has been successful in preventing As leaching (Akhter et al., 2000) and may under appropriate conditions be an effective long-term strategy. Likewise, the transformation of Pb to pyromorphite at low levels of contamination by the addition of phosphorus (P) compounds has become an approved strategy to protect human health in the US. However, many soils, such as orchard soils, are contaminated with As and Pb due to historical applications of the insecticide lead arsenate. In these soils, the addition of P solutions is not feasible due to the risk of As leaching into ground water or streams.

The goal of this project is to demonstrate physiochemical technology responsible for the immediate in situ long-term stabilization and remediation of contaminated soils by iron (Fe) occlusion. Arsenic and Pb remediation is achieved by occlusion of As within newly precipitated Fe (hydr)oxides minerals. The risk for As leaching will be reduced or eliminated by the application of Fe nanoparticles applied to the soil with a "green" nonionic surfactant and wetting agent derived from citrus fruits. We will present results of ligand-exchangeable and ligand-dissolvable As and Pb pools, citrate bicarbonate dithionite extractable Al, As, Fe, and Pb, as well as As and Pb X-ray Absorption Near Edge Spectroscopy. Remediation efforts that sequester As and Pb within recalcitrant occluded Fe minerals while controlling soil physiochemical factors could give risk assessors and stakeholders confidence that the danger to human health has been significantly reduced or eliminated. Concurrently, this remediation technology may achieve substantial cost savings in comparison to the standard practice of soil excavation and off site landfill deposition (Scheckel et al., 2009).

TH 104

Influence of humic-based sorbents on bioavailability of lead in soil

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Heavy metals are major pollutants in industrial soils. This study focuses on in-situ remediation of lead's soil pollution in model experiment. Commercially available potassium humate BC-HumNa produced by Russian company from brown coal as well as magnetoactive humic-based nanocomposite Fe₃O₄/HA formulated by coprecipitation method in situ were tested. Fe₃O₄ nanoparticles are spherical and their diameters vary from 7 to 16 nm. Modeling of lead pollution was conducted in pots on samples of the model soil (100 g soil; Pb 320mg/kg). Humic sorbents was introduced to achieve a concentration of 0.0025 and 0.01% (by weight). Lead water-soluble fraction was measured using Atomic Absorption Spectrophotometry, 10 days after. Toxicity was valued in relation to standardized test-cultures of living organisms (representatives of higher plants, invertebrates crustacean, and bacteria). Results are presented and discussed, indicating that both humic-based sorbents modified lead bioavailability in soil. Apparently changing in the content of water-soluble lead, and the responses of test organisms on the impact of aqueous extracts of contaminated soil humate nanostructured substances showed a higher detoxifying potential and were more effective than sodium humate.

TH 105

Preliminary results of the zinc, copper and iron content in the feral oyster *Striostrea margaritacea* and the effects of them on the lysosome membranes

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The causes, the monitoring and the control of marine pollution, particularly heavy-metal pollution, have become a very important subject to a great deal of research in recent years. The aims of the present study is to determine the degree of metal contamination in the wild oysters, *Striostrea margaritacea*, as well as in the water and sediment from selected polluted (Wilderness, Witsand) and unpolluted (Goukamma MPA, Knysna) sites on the South African Coast and also to determine the toxicity of selected metals to the oysters, by using a biomarker (lysosome destabilization) as tool. The biomarker will be tested using the neutral red retention time assay. A primary screening has revealed high amounts of Zinc, Copper, Iron and Aluminum in oysters from the polluted areas. Results of this study will possibly be used to introduce a more advanced monitoring program using bivalves as bioindicators.

TH 106

Uranium fate in crayfish after different conditions of exposure. Focus on U accumulation and speciation in the cytosolic fraction of 2 target organs: gills and digestive gland.

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Whereas uranium speciation in water column is quite well studied by modelling in correlation to its bioavailability, its speciation within living aquatic organism is poorly described despite a wide coordination chemistry and affinity for proteins. Uranium analysis at the organ and sub-cellular levels is necessary to understand biodistribution,

precipitation and specific metal interaction with biomolecules during the fixation, transport and incorporation into a biological cell or tissue. So, such information is fundamental to better explain uranium bioavailability, accumulation after direct and trophic exposures and toxicity.

In the case of uranium the elucidation of its toxicity does not only go through the characterization of target-biomolecules involved in metabolic pathways but also goes through the determination of the sequestered fractions likely to emit alpha rays in the cell.

This study deals with the uranium fate in two target organs of crayfish, i.e. gills and digestive gland, after different levels of direct exposure. As mainly considered as chemotoxic, focus was done of the soluble fraction of U in these organs. Thus, subcellular distribution of uranium was performed using ultracentrifugation followed by uranium ICP-MS measurement. Following this step, uranium distribution within cytosolic proteins was assessed using hyphenated HPLC SEC-ICPMS.

Results show that in crayfish, U accumulation in gills is higher than in digestive gland after direct exposure. Only 25 to 5% of U are in the soluble fraction of gill cells and can pretend to be studied for U speciation and the assessment of the chemotoxic fraction of this element. In digestive gland, the soluble fraction represents 60 to 30% of the uranium content.

The ICP MS chromatogram of DG cytosols shows similar patterns for control samples and contaminated samples while intensity of 3 fractions at 4, 7 and 40 kDa seems to be enhanced with the contamination. Same patterns and trends were observed with the UV detection at 280 nm.

To conclude, Hyphenated techniques were used to assess U recovery and U distribution within cytosolic proteins of cells from gills and digestive gland of crayfish after exposure. Three main protein peaks of interest have been identified and will further be analyzed to identify molecules complexed with U. These speciation studies correlated to biological effects are a key step to elucidate the chemotoxicity of this element.

TH 107

Determination of Se species in fish tissues by HPLC-ICPMS

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Se plays a major nutritional role in humans and animals as it is an essential component of a number of enzymes including glutathione peroxidase. The narrow margin between the beneficial and toxic levels of Se has important implications on human health. Due to its dual role, Se has been widely studied in the environment.

The release of Se from geological sources has been accelerated due to industrial and agricultural activities making it bioavailable to fish and other organisms in aquatic ecosystems, hence, Se has surfaced as an element of primary concern.

Se is present in many chemical forms in the environment: from inorganic Se (IV) and Se (VI) to the organic amino acids such as selenocysteine and selenomethionine. The toxicity of Se is related to its chemical form with the inorganic forms being more toxic than the organic form in general. Many enzymes and other proteins require Se for their activity and the forms required by animals are the Selenoaminoacids or Se containing proteins. Since, selenoaminoacids are mainly associated with proteins in the living organism, to release the Se species incorporated into proteins, the peptide bond needs to be broken in a way that releases the intact Se containing amino acids or their derivatives.

Although, the measurement of total Se is essential to provide elemental concentrations, it is insufficient information as the biological actions of Se are dependent on the physical properties of its various chemical forms. Speciation information is therefore required to gain an understanding of its biogeochemical cycling and metabolism.

This project investigates the extraction and determination of Se species by HPLC-ICPMS after proteolytic digestion and the derivatisation of selenoaminoacids. Low efficiency of proteolytic extractions reported in the literature could be a consequence of incomplete digestion of tissue due to insufficient unfolding of proteins, hence the inaccessibility of the enzymes to the target peptide bonds. Fish tissues are extracted using urea to denature proteins followed by reaction with dithiothreitol to break the Se-Se and S-Se bonds and alkylation with iodoacetamide to derivatize selenomethionine and selenocysteine to more stable carboxymethylated forms. This allows for the enzymatic digestion of the stabilised carbamidomethylated selenocysteine and selenomethionine allowing proteolytic digestion to extract Se species.

TH 108

Measurement of methyl mercury and inorganic mercury in sediments and fish tissues by HPLC-ICPMS

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Mercury is considered to be a major environmental contaminant on a global scale and therefore has inspired the development of methods for the determination of mercury species in the wide range of environmental matrices.

While natural inputs of mercury occur, anthropogenic inputs from combustion of coal and petroleum products account for more than 50% of the global mass of mercury.

The elemental mercury produced being highly volatile is transported in the atmosphere over long distances and in the earth's crust and oceans, wet deposition occur in the form of inorganic mercury. Dissolved inorganic mercury shows a high tendency to absorb onto suspended particulate matter and consequently, gets accumulated in the top of sediments.

Environmental and health risks for mercury derive from methyl mercury that is formed by biomethylation of inorganic mercury by microorganisms in sediment and soil.

Methyl mercury is a neurotoxin that readily crosses biological membranes which accumulates to harmful concentrations in exposed organisms and biomagnifies in aquatic food webs to concentrations of toxicological concern.

Developing a sensitive, reliable and cost effective method to measure mercury species is important for monitoring mercury concentrations in biota and sediments in order to avoid ecotoxicological risk and to understand the biogeochemical cycling of mercury species in the environment. Since methyl mercury is the most toxic species, routine analysis of this species is increasingly in demand.

This project describes a procedure for the extraction and determination of methyl mercury and inorganic mercury in sediments and fish muscle tissues using HPLC-ICPMS after extraction using 2-mercaptoethanol. A Perkin-Elmer 3 µm C8 (30 mm x 3 mm) HPLC column and mobile phase containing 0.5% v/v 2-mercaptoethanol and 5% v/v methanol (pH 5.5) at a flow rate of 1.5 ml min⁻¹, 250°C was used for the separation of mercury species. The developed extraction procedure of mercury species from the fish tissues was compared against an enzymatic extraction using Protease type XIV and similar results were obtained for both extraction procedures. The methyl mercury concentration of sediment reference material ERM CC 580 and biological certified reference materials, NRCC DORM-2 Dogfish muscle, NRCC Dolt-3 Dogfish liver, NIST RM Albacore tuna and IRMM IMEP-20 tuna fish were in agreement with the certified values.

TH 109

EDTA: a marker of anthropogenic pollution in the Suquia River basin

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Aminopolycarboxylates, such as ethylenediaminetetraacetic acid (EDTA) have been extensively used as chelating agents in many industrial and domestic applications for years.

EDTA is released to the aquatic environment mainly via runoffs of sewage treatment plants. Due to high consumption and slow biodegradability, EDTA has been found in surface waters of different industrialized countries. Our main goal was determining the presence of EDTA as a marker of anthropogenic influence along the Suquia river basin (Córdoba-Argentina). To verify the pollution we also measured concentration of metals (Li, Be, B, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Mo, Ag, Cd, Ba, Tl, Pb and Bi), pH, conductivity and dissolved oxygen (DO) at eight monitoring stations with different pollution degree from the upper to the lower river basin. Sampling was carried out during 2011, considering both dry and wet seasons. EDTA was analyzed by HPLC coupled to ESI-HRMS (QTOF), while metals were assayed by ICP-MS.

EDTA was found in at least 3 locations. Amounts of EDTA varied along the basin, showing absence of EDTA in the high basin, trace amounts in the middle basin, while the highest values were observed at the lower basin. So far, it is possible to differentiate 3 areas in accordance with anthropogenic inputs: a quasi pristine area in the upper basin, a medium polluted zone surrounded by small cities in the medium basin, and a high impacted area, downstream the sewage discharge of the main city (Cordoba). Some metals (Be, Cr, Co, Ni, Se, Mo, Ag, Cd, Tl and Bi) were below LOD throughout the basin. Conductivity, DO and other metals (Li, K, Ca, Mg, Ga, Sr, Ba) showed a pattern coincident with EDTA concentration, with increased values as anthropogenic influence rose. Additionally, concentrations of Al, Mn, Fe, Cu and Zn increased progressively after Córdoba sewage discharge. Concentrations were generally higher during the dry season. Our current results demonstrate that EDTA can be used as an efficient marker of anthropogenic pollution, in addition to metals and other water qualities parameters.

TH 110

Speciation and bioavailability of arsenic and heavy metals in contaminated stream sediments

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The application of the Selective Chemical Extraction technique on the sediments of the stream that drains the "Vale das Gatas" Mines area, allowed us to notice that: 1) the most part of the contents of Cr, W, Sn, Ni and Mn in the residual fraction, is mainly constituted by silicate minerals, non bioavailable; 2) part of the metals (Fe, Mn, Cu, Zn, Cd and Pb) are easily bioavailable in all sampling sites in large percentages; 3) all the researched metals are also potentially bioavailable, that is, they occur in organic, reducible and carbonate phases, susceptible of being set free depending on some changes of water stream physical-chemical conditions. Cu, Zn, As, Cd, and Pb are the most remarkable examples.

The characteristics of the main contamination focus, also appear as an important aspect, conditioning the support phases of the researched chemical elements. Thus, in station where the water stream contamination is essentially owed to the Acid Mine Drainage (AMD), a great part of the elements appear preferentially associated to exchangeable and moderately reducible fractions. That is a result of the affinity of these elements in being adsorbate and its easiness of coprecipitating with Fe oxides; while in stations where the contamination is owed not only to the AMD of tailing but also to solid load transportation, exchangeable and moderately reducible fractions lose some importance, increasing the tendency of association with fraction 5 (sulphides) and with fraction 6 (residual).

TH 111

The occurrence of Arsenic-Glutathione complexes in Chlorella vulgaris: off-line elemental (ICP-MS) and molecular (ES-MS) mass spectrometric detection for liquid chromatographic separation

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One response of plants to exposure to arsenic is the production of Glutathione (GSH), although its part in the detoxification mechanism is still unclear. Due to the strong affinity of arsenic for sulphur, glutathione (GSH) and phytochelatin (PC) tend to form complexes with As(III) and to a lesser extent, As(V). However, the identification and quantification of these complexes has proven to be elusive due to the instability of the metalloid complexes and it represents a challenge for modern speciation techniques. Arsenic speciation instrumentation has received much attention recently and reliable speciation has been achieved. In contrast, relatively little progress has been made to improve sample storage and extraction protocols. In particular, extraction of As in the microalgae *Chlorella vulgaris* remains poor impeding As-GSH complexes identification in these cells.

The aim of this investigation was to determine the presence of As-GSH complexes in *Chlorella vulgaris* after exposure to arsenic and contribute to elucidate the mechanisms of arsenic detoxification used by microalgae. For this reason synthetic As-GSH complexes were prepared. *Chlorella vulgaris* cells were exposed to As(III) and As(V), concentration ranging from 0-200mg/L.

The quantification and determination were performed by extraction with HPLC-ICPMS and HPLC-ESI-MS. The same chromatographic conditions were used for both instruments. Quantification by ICPMS does not ensure unequivocal identification of molecules but allows the determination at low detection limits and is not significantly affected by matrix interference. The use of ESI-MS alongside ICPMS allows both low concentration quantification and unequivocal identifications of molecules of interest. In order to understand the mechanism of detoxification employed by living cells exposed to inorganic arsenic, reliable methods for extraction, quantification and detection must be developed. In this study, sample preparation and extraction procedure appropriate for the nature of the analyte has been applied to minimise oxidation and preparation time. Trace analyte quantification with low limits of detection and low matrix effects by ICP-MS and correct identification of the As-GSH with ESI-MS was achieved in *Chlorella vulgaris* cells exposed to inorganic arsenic.

TH 112

LC-ICP-MS speciation of selenium compounds in Se-enriched green algae

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Selenium is an essential element and has been known to be a necessary component of the human diet. It is important e.g. for protection of cells against effects of free radicals and for the normal function of immune system. Selenium is also toxic and its toxicity and bioavailability is related to concrete selenium compound. Usually organic selenium species (Se-amino acids) exhibit higher bioavailability and lower toxicity than inorganic selenium compounds. Se-enriched biomass of green algae *Scenedesmus* and *Chlorella* might become a source of organic bound selenium. The aim of this work is to develop a method for identification and quantification of selenium species in algal biomass.

For this purpose various types of extracts were studied by coupling of anion exchange liquid chromatography with inductively coupled plasma mass spectrometry. Acid and enzymatic hydrolysis of proteins in algal biomass was applied for determination of total content of selenomethionine and selenocysteine. Extraction with buffers and alkaline media was used for identification of non-protein bound selenium amino acids and inorganic ions.

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TH 113

Speciation of arsenic and selenium in surface water

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Within the framework of various international River basin programs arsenic pollution is monitored in Dutch surface waters, among other matrices. Selenium is monitored on a national basis. To determine the impact on the aqueous environment of the amounts present (national) environmental quality standards are used. Current research into the speciation of both elements should lead to a better understanding of the extent of pollution, because toxicity of the various species differs extensively. This approach is expected to be incorporated into future legislation frameworks.

TH 114

Ion chromatography with XSERIES 2 for metal speciation applications

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1. Introduction

Here, the coupling of the Dionex ICS-5000 ion chromatography system to XSERIES 2 is described. This combination provides a single powerful, flexible solution for both ion chromatograph and ion exchange chromatography applications as well traditional HPLC methods. The combined system is ideally suited for trace metal speciation as it provides a completely metal free pathway reducing contamination risks and improving robustness when used with potentially aggressive mobile phases. Together with the wide selection of chromatographic columns applicable, appropriate solutions for all types of trace metal speciation studies can be found.

2. Materials and methods

In this study, speciation analysis of different trace metals was carried out using hyphenation of the Dionex ICS-5000 chromatography system coupled to XSERIES 2. Different analytical columns were used including anion exchange chromatography and reversed phase chromatography.

3. Results and discussion

Different application examples of the system will be shown, including from ion chromatography (e.g. Cr speciation), ion exchange chromatography (speciation of different As compounds), and also reversed phase chromatography for the separation and quantification of organotin species. Particular emphasis will be made on the use of smaller internal diameter columns that significantly lower the consumption of mobile phase (and therefore sample) without compromising separation efficiency.

4. Conclusions

The results show the possibilities offered by the hyphenation of the Dionex ICS-5000 LC system to XSERIES 2 for trace metal speciation analysis. Due to the high flexibility offered by the system, different applications could be successfully run on the same instrument and showed high separation efficiency together with reduced sample and mobile phase consumption.

TH 115

Speciation of Bromine in water samples using HPLC-ICP-MS

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Foods and agricultural products contain various concentrations of nutrients, which can range from trace amounts to percentage levels. However, whilst most of their nutritional content is intended for maintaining good health, the benefits of the desired mineral content of such products can be compromised by concentrations of elements deemed to be toxic to humans and/or animals.

ICP-MS is a flexible tool for matrix, minor and trace elemental analysis. In combination with separation techniques, ICP-MS allows the determination of the chemical form of elements present in the sample and thereby a differentiation between essential nutrient and toxic to human or animals. For elements such as As, Cr, Se and Br it is well known that it depends on the oxidation state or the present compound if an element is harmless or toxic to humans. Bromate e.g. is formed by the ozonation of water in purification processes. Bromate is potential carcinogen whereas bromide is essentially non-toxic. Therefore monitoring of the total bromine concentration in water will not indicate the risk for human health. The coupling of a chromatography technique to an ICP-MS provides a powerful and very sensitive tool to separate bromine and bromate.

The presentation will imply the required sample preparation steps, flexibility and sensitivity for the determination of different bromine species in water samples using the Bruker auroraM90 ICP-MS. The capability of ICP-MS for this kind of analysis will be shown with the help of different sample types.

TH 116

New approaches for the traceable determination of PBDEs (in coastal water samples) according to the Water Framework Directive (WFD)

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Polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants in polymeric materials. Owing to the adverse effects linked to the exposure to these compounds, the EU has included PBDEs (congeners 28, 47, 99, 100 153 and 154) in the list of priority substances that need to be measured in all surface waters covered by the WFD. Environmental Quality Standards (EQS), equivalent to the maximum allowable concentrations, have been defined at very low levels for PBDEs (Σ PBDEs ≤ 0.5 ng/L). Analytical methods intended for their monitoring must meet certain minimum performance criteria in terms of uncertainty ($\leq 50\%$ at EQS, 95% confidence) and limits of quantification (LOQ $\leq 30\%$ of EQS) for reliable measurements of PBDEs in complex environmental water matrix at EQS level. The overall low concentrations of the target analytes in water together with the challenging requirements defined by the WFD necessitates the development of suitable methodologies combining sensitive detection approaches with accurate and precise analytical techniques.

In this regard, inductively coupled plasma mass spectrometry (ICP-MS) or electron capture negative ionization (ECNI) mass spectrometry provide interesting possibilities as it offers high sensitivity for the detection of heteroatoms such as bromine, especially when using gas chromatography (GC) as sample introduction technique. Furthermore, ICP-MS has been widely used in combination with Isotope Dilution Mass Spectrometry (IDMS) for the accurate and precise determination of other priority pollutants.

In this work, some preliminary results on the determination of PBDEs in water by elemental IDMS are shown. Ultrapure water samples fortified at the EQS level were spiked with ⁸¹Br-labelled PBDEs, liquid-liquid extracted with isooctane and injected in a GC-(ECNI)MS. Obtained results met the uncertainty requirements of the WFD in river water samples but LOQs still need to be further improved. Instrumental settings of the GC-ICP-MS coupling were optimised for the highest bromine sensitivity for its future use in IDMS in an attempt to meet the WFD requirements.

This work has been performed within the scope of an EMRP Researcher Grant for the development of a traceable measurement approach for monitoring PBDEs in coastal water, awarded in accordance with the EURAMET process to complement the JRP "Traceable measurements for monitoring critical pollutants under the European Water

TH 117

Improved extraction, preservation and analysis of hexavalent Chromium from soil extracts and water

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Chromates are oxyanions (e.g. CrO₄²⁻, Cr₂O₇²⁻) of chromium in oxidation state +6. They are strong oxidizing agents that are considered toxic and potentially carcinogenic. Hence, they are regulated in the environment and as a primary drinking water contaminant in the US.

Drinking water standards are regularly re-evaluated by the US EPA. In 2008, they conducted a comprehensive review of the health effects of chromate based on toxicity studies done by the National Toxicology Program. In September 2010, the EPA released the Toxicological Review of Hexavalent Chromium. Based on that report the state of California's Office of Environmental Health Hazard Assessment in the recently issued a new public health goal (PHG) for chromate in drinking water of 0.02 µg/L.

Currently, dissolved hexavalent chromium is measured according to EPA Method 218.6. This method is based on anion exchange chromatography on an IonPac AS7 column (4 mm format) and detection after postcolumn reaction with diphenylcarbazide to yield a compound with visible absorbance at 530 nm. This permits a method detection limit (MDL) for chromate in drinking water at 0.3 µg/L. Modifications including lower eluent and postcolumn reagent flow rates, larger reaction coil, and larger injection volume significantly increased the sensitivity over Method 218.6, resulting in an MDL of 0.02 µg/L.

However, the modified method does not allow sufficient sensitivity for routine analysis at the proposed PHG (California) level of 0.02 µg/L. This presentation describes modifications including the use of the column in the 2 mm format and a smaller reaction coil to increase method sensitivity. The modified method permits a MDL of 0.001 µg/L, and a quantitation limit of 0.003 µg/L, which is more than sufficient for analysis at the proposed California PHG level. It also discusses alternatives for delivering of the post column reagent and other eluents that have been proposed. We are working with the US EPA Office of Ground Water and Drinking Water to create a new EPA method based on these final modifications, EPA 218.7

TH 121

Class 1 and 2 integrons, sul resistance genes and antibiotic resistance in Escherichia coli isolated from the East River, South China

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Extensive use of antibiotics by humans and animals have resulted in the development of antibiotic resistance in various bacteria and dissemination of antibiotic resistance genes (ARGs) into the environment. The objective of this study was to investigate antibiotic resistance profiles in Escherichia coli isolated from surface water of the East River catchment in both dry and wet seasons, and to characterize selected ARGs including sul genes and integrons to better understand the dissemination of resistance genes and integrons in surface water.

89.1% of the isolates were resistant and 87.5% showed resistance to at least three antibiotics. Tetracycline and streptomycin had the highest resistance frequencies of 60.1% and 56.3%, respectively. Multiple antibiotic resistances (MAR) index ranged from 0.16 to 0.54 in different parts of the East River catchment. sul2 was detected most frequently in 89.2% of 1403 SXT (sulfamethoxazole/trimethoprim)-resistant isolates. The presence of integrons (class 1 and 2) was frequently observed (82.3%) while no class 3 integron was found. In these integrons, 21 resistance genes of 14 gene cassette arrays and 10 different families of resistance genes were identified. Three gene cassette arrays, aac(6)-Ib-cr-aar-3-dfrA27-aadA16, aacA4-catB3-dfrA1 and aadA2-lnuF, were detected for the first time in surface water. Integrons could play a key role in developing multiple resistance amongst microorganisms by transferring various resistance genes simultaneously, which is clearly demonstrated by a strong correlation between the MAR index and number of integrons (p<0.0001). Principle component analysis and cluster analysis demonstrated that human activities, especially discharge of wastewater are the main factor leading to the wide dissemination of resistance bacteria and genes in the aquatic environment. The findings reflect the importance of surface water as a reservoir and as a medium for the dissemination of antibiotic resistance genes and resistant bacteria in the environment.

EP01P - Antimicrobial resistance in the environment

TH 122

Antibiotic contamination and promotion of antibiotic resistance in microbial communities of three reservoirs

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Pharmaceuticals are continuously released in the environment from discharges of wastewater treatment plants or through run-off from fields after application of sewage sludge and animal manure. They attract increasing attention as contaminants because of their potential threat to the ecosystems and public health, even at their very low concentrations and persistence rates. Among these compounds, antibiotics may pose a risk to the environment since they are designed to have a biochemical effect in the microorganisms, and thus can have a significant impact in the processes controlled by them in aquatic ecosystems. One of the greatest concerns about the presence of antibiotics in the environment is the escalation of antibiotic resistance as a consequence of chronic exposure of naturally occurring bacteria to sub-lethal doses of these compounds and the potential impact in public health.

The objectives of this study were first to determine the levels of antibiotics in different environmental compartments (water, sediments and fish) of three reservoirs in Catalonia: La Llosa reservoir was chosen for its pristine condition, whereas Sau and Foix are respectively a moderate and a highly polluted reservoir. Antibiotic concentrations were correlated with the composition and functioning of the natural bacterial communities in the reservoirs as well as with the presence of antibiotic resistance genes, not only in water and sediment but also in fish intestine.

A general chemical characterization showed a clear difference between the reservoirs in terms of eutrophication and chemical pollution, with exceptional high values in Foix reservoir. Foix samples showed the highest antibiotic content in water and sediment, as it was expected according to the pollution gradient of the target reservoirs. Load was particularly high for macrolides family, which is consistent with data related to antibiotic consumption last years.

Antibiotic concentration found was consistent with the quantity of qnrS and sul gene copies (index of antibiotic resistance for the quinolone and sulfamide family, respectively) found in the samples. Resistance genes were detected in Foix Reservoir at much higher levels than in the other two, less affected by anthropogenic contamination. The combination of chemical and biological data provided a broader perspective to assess the impact of antibiotics released into the environment and their potential effect as promoters of antibiotic resistance.

TH 123

Persistence of heavy metal and antibiotic resistance downstream a point source pollution of a river ecosystem

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The spread and evolution of antibiotic resistance in the natural environment is still not well understood. There is evidence that heavy metal contaminations contribute to the spread and persistence of antibiotic resistance genes in the natural environment. Lab experiments identified heavy metals as selective agents for the co-selection of antibiotic and heavy metal resistance due to co- and cross-resistance. However, field studies are rare and the results of some field studies did not support the hypothesis that heavy metals co-select for antibiotic resistance. Thus, the impact of heavy metal pollution on the persistence of antibiotic resistance in the environment needs further investigation. The Western Bug River (Ukraine), a river ecosystem that was supposed to be highly contaminated by a heavy metal point source due to industrial and urban waste water was chosen to investigate the persistence of antibiotic and heavy metal resistance along a heavy metal gradient. Therefore, the heavy metal concentrations of water and sediment samples from ten sampling sites along the Western Bug River were analysed. Furthermore, sediment and freshwater bacteria were isolated and identified. Microbes belonging to the genera Aeromonas and Pseudomonas were tested to their susceptibility towards a wide range of antibiotics and heavy metals. First results showed that elevated concentrations of the heavy metals Cu, Cd, Cr, Ni and Pb were detected over a long distance downstream the source. Furthermore, the bacterial resistance to Cu, Cd and Ni correlate to the analysed concentrations of the corresponding metals in the river. The concentration of Cu and Cd also correlate to the bacterial tolerance to the antibiotics cefotaxime and cefepime. Those first results lead to the conclusion that the wide ranging contaminations of copper and cadmium might support the persistence of the tolerance to the respective metals as well as to the antibiotics cefotaxime and cefepime.

Investigations on the genes responsible for the resistance as well as the location of those genes within the genome will contribute to a deeper understanding of the co-occurrence of antibiotic and heavy metal resistance genes in the Western Bug River.

TH 124

Organic pollution and spread of multi-drug resistant Escherichia coli in the Bug River, Ukraine

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The emergence and the dissemination of antibiotic resistances constitute a global threat for public health and an emergent environmental problem. Rivers and streams constitute a reactor for evolution of new resistances because bacteria from different sources such as urban, industrial waste water, or farms, are mixed with indigenous species. Genetic exchange between allochthonous and indigenous bacteria appear to be promoted by inorganic and organic pollution; however current knowledge is largely incomplete. As a case study, we investigated the antibiotic resistance features of the Gram-negative populations of the Western Bug River (Ukraine) and its main tributary Poltva, a waste water treatment plant effluent. Hydro-chemical criteria, benthos abundance and Escherichia coli load, determined in five sampling sites, highlighted a high organic load from the urban area. A total of 247 isolates were studied for their susceptibility to the clinically relevant β-lactams, aminoglycosides and ciprofloxacin. Generally higher resistances were found in the most polluted sites, thus in the Poltva and, downstream, in the urban area of the Bug. Nineteen drug resistant and three multi-drug resistant E. coli were isolated. The genetic analysis of the acquired resistances showed the presence of blaTEM-1 in 18 out of the 19 resistant E. coli. In the Poltva and downstream in the Bug, four β-lactams resistant E. coli harbouring blaCTX-M-14 and one blaCTX-M-1 were found. Additionally, the acquired fluoroquinolones resistance gene qnrS was detected in three isolates. Class 1 integrons were present in 16 of the resistant E. coli. For two isolates, the insertion sequence ISEcp1, involved in

the mobilisation of blaCTX-M genes, was found ahead of blaCTX-M-14 gene. RAPD analysis indicated a relatedness between four E. coli harbouring blaCTX-M-14 of which two were isolated from different sampling sites. The MLST profile indicated that the E. coli harbouring blaCTX-M-14 belonged to the ST131 and the one harbouring blaCTX-M-1 belonged to ST156. This study, integrating environmental and clinical approaches, is the first report of antibiotic resistance species isolated from water bodies in the Ukraine. Furthermore, this is an important case study demonstrating how organic pollution of rivers may represent a clinical risk: the water acts like an infectious sources and reservoir of antibiotic resistances.

TH 125

Distribution of novel beta-lactam resistance genes of ampC family among environmental aquatic bacteria

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In addition to medical setting, antibiotic resistance is wide spread in environmental bacteria. One factor contributing to rapid spread of antibiotic resistance is horizontal gene transfer. For detecting the horizontal transfer, phylogeny of the resistance genes has to be compared to the phylogeny of the hosts. In the current work we focused on the ampC genes in environmental aquatic bacteria and detected several events of potential horizontal gene transfer. Presence of beta-lactamases was tested among bacteria isolated from a lake and its run off river. The bacteria were isolated on antibiotic (including ampicillin) containing selective media. The isolate collection was obtained from samples collected in years 2005 to 2008. The lake is potentially exposed to several antibiotics from use in farming and diffuse countryside settlements while there is a larger town (100 000 inhabitants) at the runoff river. The isolates were phylogenetically characterized by 16S rRNA gene sequencing and presence of various ampC subclasses (LAT, DH4, ACC, ACT, FOX) as tested using multiplex PCR. Only the FOX subclass gave PCR signal. This subclass was found among Gammaproteobacteria and one Betaproteobacterium. The total number of FOX containing isolates was 44. The most prominent group of FOX gene carriers was genus *Aeromonas* (n = 26), most of them the fish pathogen *Aeromonas salmonicida*. On the other hand, not all isolated *Aeromonas* bacteria (n ~ 60) had FOX genes. In addition, the FOX gene was abundant among *Pseudomonas*. According to the sequences the FOX genes found clustered to form a novel subclass, related to the plasmid borne FOX (FOX-3, -4, -8 and -9) sequences found in the Enterobacteria *Escherichia coli* and *Klebsiella* spp. We also found that the phylogeny of FOX genes does not coincide with the phylogeny of the host bacteria. In conclusion, we have identified a new subclass of ampC genes where transfer between different bacterial groups has been relatively frequent.

TH 126

Emergence of antibiotic resistant bacteria in Baltic Sea aquaculture

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Aquaculture environment has been suggested to be a reservoir of antibiotic resistance genes due to prophylactic and therapeutic use of antibiotics. It has been observed that antibiotic resistance genes are persistent in the sediment below aquaculture farms even in the absence of selection pressure. Here, HPLC analyses were conducted to monitor the selection pressure in the aquaculture farm sediments.

Sediment samples were collected from two medium sizes of marine aquaculture and pristine areas in the northern Baltic Sea during summer time for six successive years.

HPLC measurements revealed a high concentration of oxytetracycline in the sediment samples of 2011. There is a therefore a high selection pressure in the aquaculture farm sediment. These results suggest a mechanism for the emergence of antibiotic resistant bacteria in the Baltic Sea aquaculture environment.

In future studies, resistant bacteria from sediment, fish feeds, and fish guts will be isolated by using dependent-bacterial culture methods to further clarify the relation between resistant bacteria and fish pathogens. In addition, molecular methods such as PCR and qPCR will be performed to qualitatively and quantitatively analyze antibiotic resistance genes, respectively.

TH 127

Diversity and antibiotic resistance of *Acinetobacter* spp. in water from the source to the tap

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Acinetobacter spp. are ubiquitous bacteria in the environment and hypothetic reservoirs of antibiotic resistance in drinking water. *Acinetobacter* spp. isolated from a drinking water treatment plant and from tap water, served by that plant, were identified and typed based on the analysis of the *rpoB*, *gyrB* and *recA* partial gene sequences. Antibiotic resistance was characterized, using the disc diffusion method, and each isolate was classified as wild or non-wild type, according to the observed phenotype.

The strains of *Acinetobacter* spp. could be affiliated to 11 different species, four of which represent presumably new species. Most of the isolates were related to the species *A. johnsonii* and *A. lwoffii*. These two groups, as well as others related to the species *A. parvus* and *A. tjernbergiae*, were detected both in the water treatment plant and the tap water. Other strains, related to the species *A. pittii* and *A. beijerinckii*, were isolated only from tap water. Most of the isolates (80 %) were susceptible to all of the 12 antibiotics tested. In contrast, non-wild phenotypes for tetracycline, meropenem, ceftazidime, streptomycin, among others, were observed in water-treatment plant samples and/or in the tap water samples. Although, in general, this study reports a low antibiotic resistance risk of the *Acinetobacter* spp. in water, the potential of these bacteria to acquire and disseminate resistance via drinking water is demonstrated.

TH 128

Resistance to broad-spectrum antibiotics in aquatic systems: do anthropogenic activities affect the dissemination?

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Significant amounts of antibiotics and antibiotic-resistant bacteria are released daily into the environment. The prevalence and molecular diversity of resistance genes may be an indicator of water quality, useful to assess the risk of environmental resistance to public health. Production of extended-spectrum beta-lactamases (ESBLs) is one of the major resistance mechanisms that Gram-negative bacteria have developed against broad-spectrum antibiotics such as cefotaxime (CTX). The association of ESBL genes with genetic elements that potentiate their capture and expression have contributed to their highly efficient mobilization and expansion, being reported frequently worldwide and thus becoming a serious clinical threat. The aim of this study was to compare the prevalence and diversity of CTX-resistant (CTX^R) bacteria within polluted (P) and unpolluted (UP) ecosystems. Twelve rivers from the Vouga River basin (Portugal) were sampled and physicochemical and microbiological parameters of water quality were determined. Results classified 9 rivers as UP and 3 as P. Bacterial counts were 1.9 X 10⁶ CFU/L in P and 0.68 X 10⁶ CFU/L in UP streams, of which 8.8% and 0.6%, respectively, grew on CTX-supplemented agar. Antimicrobial susceptibility testing against 16 antibiotics of 6 different classes revealed that 60% (P) and 40% (UP) strains were multiresistant. ESBL production was detected on 39 strains (27P and 12UP). ESBL genes were screened by PCR and blaCTX-M was the most frequently detected. Analysis of the blaCTX-M genetic context revealed the presence of different mobile genetic elements previously described in clinical strains. Clone libraries of blaCTX-M amplified from environmental DNA were constructed to compare the diversity of these genes in P and UP sites. Clear differences were detected with the P library presenting a much higher diversity of CTX-M-like variants (14 in P and 4 UP). Also, UP library was mainly composed by sequences that affiliated with chromosomal ESBLs. In conclusion, results showed clear differences between P and UP lotic ecosystems, concerning prevalence, phylogenetic diversity and antimicrobial susceptibility profiles of CTX^R bacteria and production of clinically relevant ESBL genes. This validates the hypothesis that anthropogenic activities modulate the environmental resistance gene pool and are promoting antibiotic resistance dissemination. Also, it suggests that blaCTX-M-like genes may constitute pollution indicators.

TH 129

Abundance of class 1 integrons and diversity of their associated gene cassettes in hospital and urban effluents

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The spread of antibiotic-resistant bacteria is a growing problem and a public health issue. Wastewaters are considered to be sources for antibiotic resistance dissemination by horizontal gene transfer. Mobile integrons (MI) are genetic elements that acquire, exchange and express antibiotic-resistance genes embedded within gene cassettes (GC). Class 1 MI are the most frequent with more than 130 GC conferring resistance to almost all known antibiotics. The aim of this study was to assess the class 1 MI relative abundance and their GC composition in an hospital and an urban effluents, as well as in the influent of the WWTP receiving sewer from the urban area, comprising the hospital & the urban effluent.

Effluents from a 2000-bed French hospital, effluent from an urban area and the wastewater treatment plant (WWTP) influents receiving wastewater from 280,000 inhabitants equivalent were sampled. After a 24h flow proportionate sampling, effluents were concentrated and total genomic DNA was extracted using the PowerWater™ DNA isolation kit (MoBio). Class 1 MI were quantified using a qPCR, and normalized by the 16S DNA gene. The GC variable region of class 1 MI was PCR amplified from total DNA and cloned in pGEM-T easy vector (Promega) to construct a library from which 60 clones, chosen at random, were sequenced for characterization.

The relative abundance of class 1 MI in hospital effluents was 9 and 7 fold higher than in urban effluents, suggesting that hospital effluents are an important source of bacteria harbouring class 1 MI. GC harbored by the class 1 MI belonged to GC involved in resistances to aminoglycoside, beta-lactams, trimethoprim, chloramphenicol, erythromycin, and quaternary ammonium compounds. The Chao abundance based Jaccard similarity index was used to compare the shared GC pool between samples. Indeed, the gene cassette composition of the urban effluent and the WWTP influent was the most closer. These results suggest that the influent from the WWTP is more influenced by the urban effluent than the hospital effluent.

To our knowledge, this is the first study which has assessed the impact of hospitals effluents on the WWTP. Even if the prevalence of MI was higher in hospital effluents, it appeared that this impact on the WWTP influent was attenuated by the dilution effect in the sewage network

TH 130

Occurrence of antimicrobial resistance among aquatic environmental bacteria of fish farms and pond sediments

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Antibiotics are the most important therapeutic agents used in control of bacterial infectious diseases. The use of a wide variety of antibiotics in human medicine and animal husbandry, including aquaculture, has led to the emergence of antibiotic resistant pathogens. In the present study bacteria from water and sediments related to aquaculture were collected from Chile (200 isolates), Norway (100 isolates), Pakistan (127 isolates) and Tanzania (135 isolates). The bacterial isolates were screened for the presence of prevalent antibiotic resistance genes by a disc diffusion assay, followed by Southern hybridization and further confirmation by PCR and gene sequencing. The marine aquaculture industry in Chile uses antibiotics extensively while the marine aquaculture industry in Norway uses low levels of antibiotic treatment. Freshwater aquaculture in Pakistan and Tanzania has no history of antibiotic use. The frequency of resistance genes was, however, higher in fresh water isolates than in marine isolates. The prominent resistance genes found in the bacterial isolates studied were Tet A, Tet G and Tet E operons (7.6 % of isolates), *sul1*, and *sul2* (23.5 % of isolates), *dfrA1*, *dfrA5*, *dfrA7*, *dfrA12* and *dfrA15* (56.4 %), *blaTEM* (10.2 %), *strA-strB*, and *aadA1* (10.7 %), *CAT-1* (2.1 %) and *mefA* (1.5 %), causing resistance against tetracycline, sulphonamides, trimethoprim, amoxicillin, streptomycin, chloramphenicol and erythromycin, respectively. The *Int1* gene was also found at all four sites in association with gene cassettes. Carbapenem resistant fresh water environmental isolates were screened for the presence of the *blaNDM-1* (New Delhi metallo- β -lactamase) gene, but *blaNDM-1* was not found. All isolates were also negative for plasmid mediated *qnrA*, *qnrB* and *qnrS* genes. Extensive use of some antibiotics in Chilean marine aquaculture and in Norwegian fish farming during the 1980s and early 1990s appear to have altered the aquatic environment by selecting antibiotic resistance genes. Use of manure and poultry waste is common in freshwater aquaculture in South East Asia. We assume that in Pakistan integrated freshwater fish farming practices utilizing domestic farm and poultry waste along with antibiotic residues may have contributed to creation of a pool of resistance genes in the aquaculture facilities. These antimicrobial resistance genes persist in aquatic environmental bacteria and help in further dissemination of resistance genes.

TH 131

Increased antibiotic resistance levels in Swiss lakes are related to human activities

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Antibiotic resistance in clinical environments is a major concern in human and veterinary settings as it interferes with infectious disease treatment and raises health costs. Moreover, antibiotics and antibiotic resistant bacteria are currently discussed as a new class of environmental pollutants as they are released in high amounts from treated animals and waste water facilities into natural environments, including natural waters, where their fate and impact are yet unknown. Their elevated discharge has likely lead to an increase in the natural resistance background level of the exposed environments, such as surface waters and soils.

The aim of the presented study is to evaluate the prevalence of antibiotic resistance genes in different lakes in Switzerland, a country with comparatively low antibiotic use. Furthermore, we want to investigate the correlation between increased level of resistance and measures of human impact on the lakes, e.g. degree of eutrophication, presence of intensive agriculture or waste water treatment plants in the watershed, etc. .

We have sampled 20 Swiss lakes varying in size, trophic status (P content) and land use pattern which are currently evaluated for the prevalence of several antibiotic resistance genes, including sulfonamides (*sul1*, *sul2*), tetracyclines (*tetB*, *tetM*, *tetW*) and fluoroquinolones (*qnrA*) using solely non-culture-based approaches. The respective target genes are amplified directly by PCR and qPCR in environmental DNA extracts. As a reference gene we amplified 16s ribosomal gene fragments.

It is hypothesized that an increased occurrence of antibiotic resistance genes in lakes is due to increasing anthropogenic activities surrounding the lake and that the more eutrophic the lake (highest phosphorus concentration) the higher the prevalence density of resistance genes. The dataset will provide a basis to determine background levels of the natural antibiotic resistance versus antibiotics resistance pollution and to propose suitable indicators.

TH 132

Differential gene expression and biofilm formation of multiple antibiotic-resistant *Pseudomonas aeruginosa* in municipal wastewater

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As antibiotic resistance is commonly found in wastewater systems, its persistence seems to be obvious. The factors determining the persistence of antibiotic resistance are largely unknown. Antibiotics or substances with a likewise mode of action may provide selection advantages for resistant bacteria. *Pseudomonas aeruginosa* is a clinically important, opportunistic pathogen, which exhibits high intrinsic resistance to numerous antimicrobial agents. The persistence of multi-resistant *Pseudomonas aeruginosa* carrying up to seven acquired therapeutically relevant antibiotic resistance mechanisms was studied. Such multi-resistant *P. aeruginosa* were enriched by clinical as well as municipal wastewaters. Agar diffusion testing and molecular biology confirmed their multi-resistances. Pulsed Field Gel Electrophoresis (PFGE) genotyping resulted in different groups of *P. aeruginosa* isolates depending on sampling points and numbers of resistances.

Differential gene expression analyses via quantitative SyBrGreen PCR were performed to demonstrate wastewater matrix dependent impacts on molecular responses in *P. aeruginosa*. A number of different genes were selected being involved in biofilm formation, signal molecule syntheses, antibiotic resistance, and virulence. The expression of these gene targets were analysed in presence of wastewater or tap water as a control. Additionally, epifluorescence microscopy analyses in combination with different staining methods were applied to quantify live/dead percentages in *P. aeruginosa* biofilms and visualized differences in biofilm formation (biomass, structure) under wastewater influence.

The results are important to understand selective pressures of wastewater xenobiotics on *P. aeruginosa* regarding resistance as well as persistence in aquatic environments.

TH 133

Occurrence of antibiotic resistance genes in wastewater used for irrigation in the Mézquital Valley

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Wastewater (WW) reuse for irrigation is widely practised in agriculture to alleviate water shortages. WW contains a huge amount of potentially harmful compounds, such as pharmaceuticals and microbial pathogens. We investigated the risks of WW irrigation in the Mézquital Valley (60 km north of Mexico City) for environment and human health. The Mézquital Valley is the world's largest WW irrigation field. WW from Mexico City is reused for crop irrigation, which poses risks for farmers and the consumers of the agricultural products. We followed the pathway of the WW from Mexico City to the Mézquital, starting with the WW effluent of two hospitals in Mexico City. In addition we took samples from the two major WW channels which transport the WW out of the city to the Mézquital. The influent and effluent of a dam in the Mézquital, and the reclaimed water after passing through the soil were sampled subsequently. Sampling was done in March and September 2011 to study differences between dry and rainy seasons. We selected two frequently used antibiotics, sulfamethoxazole and ciprofloxacin, and five antibiotic resistance genes (*sul1*, *sul2*, *qnrA*, *qnrB*, *qnrS*) that mediate resistance against these antibiotics. Furthermore, the *blaSHV5* gene that is frequently found in bacterial isolates from patients in Mexican hospitals was selected. This gene encodes for Extended-Spectrum Beta-Lactamases (ESBLs). Furthermore, we determined the concentration of the faecal indicator *Enterococcus* spp. and the total bacterial concentration. Total DNA was extracted from all samples and was used for quantitative PCR specific for each resistance gene, total bacteria (16S rRNA gene) and *Enterococcus* spp. All of the resistance genes were present in high copy numbers (10^3 - 10^7 gene copies/100 ml water) in the WW samples. Two antibiotic resistance genes (*qnrA* and *qnrB*) were completely removed after soil passage, but the other four genes were still found in the reclaimed water: *qnrS* decreased by 4 logs from 10^5 to 10^1 copies/100 ml, *sul1* decreased by 5 logs from 10^9 to 10^4 copies/100 ml, *sul2* and *blaSHV5* both decreased by 5 logs from 10^7 to 10^2 copies/100 ml. The concentration of total bacteria decreased by 3 logs from 10^9 to 10^6 copies/100 ml and the concentration of *Enterococcus* spp. by 4 logs from 10^7 to 10^3 copies/100 ml. The recovered water still contains a high concentration of total bacteria and faecal indicators. Therefore, without further treatment it is not suited for crop irrigation.

TH 134

Development of treatment methods for the degradation of antimicrobial compounds present in wastewaters

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The intensive use of antibiotics and disinfectants has been leading to the environmental contamination with these micropollutants or with their toxic degradation products. The widespread distribution of these contaminants may have harmful effects, such as the reduction of microbial diversity and the spreading of antimicrobial resistance (AR). The increase of AR leads to a generalized claim that micropollutants with antimicrobial activity must be removed from industrial, hospital and domestic wastewaters. Among the different mechanisms that bacteria can use to resist the antibiotic action, degradation is one of the most often used to tolerate beta-lactams. The diversification of beta-lactamases in the environment has been observed over the last years and, curiously, such a property can also be of interest to attenuate antibiotics contamination. Indeed, the implementation of degradation methods is urgently needed.

The present study aims at developing low cost efficient treatment systems to remove beta-lactams from wastewaters, by using microorganisms able to degrade these antibiotics. Biodegraders thriving in wastewater treatment plants have been enriched using amoxicillin supplemented culture media. Mixed cultures able to transform approximately 100 % of 30 mg L⁻¹ amoxicillin to non-active degradation products were obtained. The members of these cultures were identified and their antibiotic resistance phenotype was determined. The genetic determinants responsible for their degrading activity were also characterized. The diversity of bacteria and of genes involved in these processes will be also discussed.

TH 135

From the hospital effluent to the municipal wastewater treatment plant: bacterial communities and antibiotic resistance

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Given the intensive use of antibiotics, hospital effluents, which often do not have any dedicated treatment and are discharged into the municipal collectors, are suspected to contribute to the dissemination of AR bacteria into the environment. In spite of this suspect, studies on the fate of AR bacteria from the hospital effluents to the receiving sewage treatment plants and the environment are scant. The main objective of this work was to assess the impact of the bacterial load from a raw Hospital effluent in the municipal wastewater treatment plant (WWTP) into which it is discharged.

Antibiotic resistance was assessed through successive sampling campaigns of raw hospital effluent and raw and treated wastewater of the municipal wastewater treatment plant to which the hospital effluent is directed. The presence of antibiotics and heavy metals in the samples was determined in the different samples. The microbiological analyses comprised the enumeration of total heterotrophs, enterobacteria and aeromonads on different culture supplemented or not with amoxicillin or ciprofloxacin (1).

Furthermore, the influence of the hospital effluents on the bacterial communities of the WWTP was investigated using 16S rRNA PCR DGGE. Cell counts and amoxicillin resistance rates were similar between the hospital discharge and the point of inflow to the WWTP. In contrast, ciprofloxacin resistance was detected to be about three times higher in the hospital effluent than in the raw municipal wastewater. The hospital discharge seems to have a significant impact on the levels of ciprofloxacin resistance that enter the WWTP. The treated effluent displayed a reduced cell density of about 100-1000 times for both total and AR bacteria. However, the treatment did not cause significant variations on the rates of amoxicillin and ciprofloxacin resistance. This study confirms hospital discharge as a significant source of AR into the environment. The variation in bacterial communities in the hospital effluent and in the WWTP raw and treated effluent is analyzed.

1. Novo, A and Manaia, CM, (2010), Factors influencing antibiotic resistance burden in municipal wastewater treatment plants, *Applied Microbiology and Biotechnology*, 87:1157 - 1166. <br type="moz" />

TH 136

Phylogenetic diversity of quinolone resistant *Escherichia coli* isolated from wastewaters

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Antibiotic resistance dissemination has become more important in the last decades. The species *Escherichia coli* comprises different subgroups which may have different roles in antimicrobial resistance dissemination. In previous studies, wastewater treatment was observed to promote an increase on quinolone resistance, presumably due to the differential removal rates of distinct genotypic groups (Figueira et al., 2011). The current work aimed at investigating the relationship between quinolone-resistance genes and the phylogenetic group of the bacteria. Seventy *Escherichia coli* strains isolated from raw and treated municipal wastewaters were characterized based on a multi locus sequence typing (MLST) approach. The presence of quinolone resistance determinants was assessed based on the detection of mutations in the *gyrA* and *parC* genes and on the presence of the genes *qnrA*, *qnrB*, *qnrS*, *aac6*, *qepA*, *qnrC*. The distribution of the MLST groups and the type of quinolone resistance genetic determinants is discussed.

TH 137

Antibiotic resistance and bacterial communities in a wastewater treatment plant

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Although it is still difficult to establish clear cause-effect relationships, it is widely accepted that chemical pollution in the environment can influence the spread of antibiotic resistance among bacterial communities.

Domestic wastewater is a complex mixture of environmental and human commensal bacteria, which come in contact with a considerable amount of antimicrobial agents and other substances with unknown effects on microbial communities and on bacterial metabolism.

In this work we studied the bacterial communities, based on DGGE patterning and used multivariate analysis to infer about the influence of some environmental parameters (antimicrobials concentration, percentages of antibiotic resistance among culturable bacteria, and climate or plant operation conditions).

This study was designed to identify the factors observed to influence or be interdependent of either the bacterial communities and/or the antibiotic resistance rates. A total of 14 composite samples of raw and treated wastewater were collected from an activated sludge wastewater treatment plant over a period of about six months. Total DNA was extracted from raw and treated wastewater and the bacterial communities were compared using DGGE analysis. Antibiotics and heavy metals were determined in the inflow and in the treated effluent. The culturable antibiotic resistant rates were documented on Novo & Manaia, 2010 study. Some of the most relevant DGGE bands were selected for further identification.

Based on the obtained DGGE profiles, bacterial communities formed coherent groups according to the time of the year. Among the bacterial taxa varying over these periods were, for example, *Arcobacter*, *Streptobacillus*, *Firmicutes* and *Clostridiales* in raw wastewater and *Sulfurimonas*, *Bacteroidetes*, *Leadbeterella*, *Gammaproteobacteria*, *Arcobacter* and *Streptobacillus* in treated wastewater.

After Canonical Correspondence Analysis, it was possible to assess the influence of some factors and to find significant relationships between DGGE bands, antibiotic resistance rates and antimicrobials concentrations. However, the direct influence of a specific antibiotic resistant bacterial group or antimicrobial could not be identified in the community structure.

The results suggest that the wastewater communities are affected by the presence of antimicrobial agents and that these substances affect the bacterial metabolism which may be related to antibiotic resistance dissemination.

TH 138

Quantification of resistance genes and isolation of resistant bacteria for last resort antibiotics from wastewater treatment plants

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Antibiotic resistance is increasing rapidly among bacteria as antibiotics are used in increasing quantities in treating humans and animals. Due to bacterial multiresistance to different antibiotics, we are heading back to pre-antibiotic era where there is no treatment for common bacterial infections. One major concern is the microbial resistance for the last resort antibiotics, such as vancomycin, carbapenems and quinolones.

In wastewater treatment plants antibiotics, other pharmaceuticals, heavy metals and xenobiotics mix up with bacteria from human, animal and environmental origin.

Wastewater treatment plants are known reservoirs for various resistance determinants and serve as hotspots for horizontal gene transfer. Resistance genes for different antibiotics are usually associated with mobile genetic elements that are able to horizontally transfer between different bacteria. Dense bacterial populations and biofilms in the sludge promote horizontal gene transfer and different chemicals can pose a selection pressure promoting the survival of the resistant bacteria. Horizontal transfer of certain mobile genetic elements, such as Tn916, has also been shown to be inducible by antibiotics. The resistant bacteria from the wastewater treatment plants may eventually end up in the environment either in the effluents or in the sludge.

In this study we quantified different resistance genes for the last resort antibiotics, such as carbapenems, 3rd & 4th generation cephalosporines, quinolones and vancomycin, from different compartments of the wastewater treatment plant using quantitative PCR. Moreover resistant bacteria were cultured on ciprofloxacin and meropenem containing media and the resistance pheno- and genotypes were characterised.

Our results show that many resistance determinants and resistant bacteria can be found from the wastewater treatment plants. Resistance genes and resistant bacteria are even found from the effluents via which they are released to the environment.

TH 139

Vancomycin and ciprofloxacin resistance in enterococci from a Hospital effluent and in the receiving Municipal Wastewater Treatment Plant

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The increasing incidence of resistance to a wide range of antibiotic agents in human commensal and pathogenic bacteria has become a major concern over the last decades.

Enterococci, important human commensal bacteria are also frequently associated with nosocomial infections. Given the intensive use of antibiotics, untreated hospital effluents released to the municipal sewage collectors are suspected to constitute a major source of antibiotic resistances. In spite of this suspect, the influence of hospital effluents on the levels of antibiotic resistance in the municipal wastewater treatment plants is not very well understood.

This study was designed to verify if hospital effluents may be a possible source of ciprofloxacin and vancomycin resistant enterococci and how these resistances are affected by wastewater treatment in municipal wastewater treatment plants.

Raw hospital effluent and raw and treated wastewater of the municipal wastewater treatment plant to which the hospital effluent is directed were collected and analyzed during successive campaigns. The analyses included the enumeration and isolation of enterococci on appropriate culture media supplemented or not with vancomycin or ciprofloxacin. The resistance phenotypes of the isolates were determined by diffusion agar method, using the antibiotics amoxicillin, gentamicin, ciprofloxacin, tetracycline, erythromycin and vancomycin. Isolates were also examined for the presence genes possibly responsible for the observed phenotypes.

The percentage of resistance to ciprofloxacin was generally higher than that to vancomycin and both decreased from the hospital effluent to the wastewater treatment plant raw inflow, presumably due to dilution. The wastewater treatment in the municipal facility also decreased the prevalence of ciprofloxacin and vancomycin resistances. Frequently, vancomycin resistance was not detected in the final effluent. The impact of hospital effluents in the prevalence of antibiotic resistant Enterococci and antibiotic resistant determinants in the environment is discussed.

TH 140

Occurrence of beta-lactamases, namely GES-5 carbapenemase, among Gram-negative isolates from wastewater samples in Northern Portugal

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Background: Antimicrobial resistant pathogens are profoundly relevant to human health and many were the studies that focused on their spread. However, natural and human associated environmental reservoirs of antibiotic resistance (AR) are yet poorly understood. The main goal of this study was to evaluate some AR mechanisms in Gram-negative bacteria isolates from surface and raw and treated waste water environments.

Methods: Water samples were collected from different aquatic environments within an urban water cycle in the region of Northern Portugal (1, 2). Screening of antimicrobial susceptibility of 48 Gram-negative isolates (20 *Escherichia coli*, 8 *Citrobacter* spp., 7 *Klebsiella* spp., 6 *Kluyvera* spp., 2 *Enterobacter* spp., 1 *Hafnia alvei*, 1 *Pantoea agglomerans*, 1 *Pseudomonas luteola*, 1 *Rouletella ornithinolytica*, 1 *Serratia* spp.) was performed by disk diffusion method. The search for antibiotic resistant genes was based on the interpretative reading of susceptibility profiles. PCR and sequencing were used to screen and identify *bla* and plasmid-mediated quinolone resistance (PMQRs) genes. All isolates were also screened for the presence of class 1 integrons.

Results: Overall, 13 isolates were multidrug resistant, suggesting a great diversity of AR mechanisms. Two isolates showed non-susceptibility to carbapenems, one of the last resorts on the antimicrobial therapy. Their phenotypic and molecular characterization revealed the expression of a chromosomal metallo-beta-lactamase in *P. luteola* and the presence of a GES-5 encoding gene in a *Klebsiella pneumoniae* isolate. Furthermore, a variety of beta-lactamase encoding genes were observed, specifically 12 *bla*_{TEM-1} with

distinct promoters, 4 *bla*_{SHV} (2 *bla*_{SHV-1} and 2 *bla*_{SHV-11}) and also different chromosomal AmpC beta-lactamases, namely CMY-65. Class 1 integrons were detected among 6 of TEM-1-producing isolates. Together, these beta-lactamases explain the level of beta-lactam resistance. None PMQR genes were detected.

Conclusion: In conclusion, this study provides the first description of a class A carbapenemase in an environmental setting in Portugal, in addition to several other beta-lactam resistance mechanisms. The study highlights the need of surveillance of these AR mechanisms in environmental backgrounds, since it represents a liable reservoir of potential pathogenic resistant bacteria.

1-Figueira V et al. 2011. Sci Total Environ, 409:1017-23.

2-Figueira V et al. 2011. J Water Health doi:10.2166/wh.2011.002.

TH 141

Pseudomonas spp. in drinking waters - diversity and antibiotic resistance

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Pseudomonas are common inhabitants of aquatic environments, including drinking water. The problems associated with multi-resistance in clinical isolates of *P. aeruginosa* are well known, but a lack exists in the study of the presence of antibiotic resistance phenotypes in other species and in environmental isolates of this genus. This study aimed to evaluate the diversity and antibiotic resistance phenotypes of *Pseudomonas* spp. present in drinking water. Isolates from a drinking water treatment plant, household taps, cup fillers of dental chairs, a drinking water biofilm and bottled mineral waters were examined. Based on the analysis of the 16S rRNA gene sequence, it was observed that the isolates belonged to 20 species. Taxonomic diversity was further inferred based on the analysis of six housekeeping genes (16S rRNA, *rpoD*, *rpoB*, *gyrB*, *recA*, *ITS*) and PFGE profiles. Susceptibility to eight classes of antibiotics was tested using the ATB PSE(08) and disk diffusion method. *Pseudomonas* spp. were not detected immediately after the chlorination steps, in some taps and in a brand of mineral water. Twenty eight different sequence types (ST) were observed by the housekeeping genes analysis, with the genes *rpoD* and *gyrB* showing the best performance in the species differentiation. Although the same species have been isolated from different samples, the same ST was never observed in different types of water. Nevertheless, the same clone was detected in different taps. The prevalence of antibiotic resistance was, in general, low and related with the species or even with the ST. Resistance to ticarcillin, fosfomycin and cotrimoxazole were the most prevalent. Ceftazidime resistance was very rare, but was present in all the isolates identified as *P. simiae*. Resistance to nalidixic acid was observed in *P. chlororaphis*, *P. nitroreducens*, and *P. aeruginosa* recovered from tap water but not from biofilm. Intrinsic resistance was observed to cephalothin, and no resistance phenotypes were observed to ciprofloxacin, tetracycline, piperacillin, imipenem, meropenem and colistin.

This study shows that the *Pseudomonas* spp. observed in the taps may not have origin on the drinking water treatment plant. Antibiotic resistance was observed to be mainly species, rather than site-related. In general, *Pseudomonas* spp. are confirmed as potential vectors of antibiotic resistance in drinking waters, with the selection of some strains representing an important role of resistance spreading.

TH 142

Bottled mineral water as a potential source of antibiotic resistant bacteria

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The hypothesis that bottled mineral water may represent a supplier of antibiotic resistant bacteria to humans was tested. The antibiotic resistance phenotypes of the cultivable bacteria present in nine batches of two Portuguese and one French brands of commercially available mineral waters were examined. Among the 238 isolates recovered on R2A and PIA and on these culture media supplemented with amoxicillin or ciprofloxacin, most were identified (based on 16S rRNA gene sequence analysis) as Proteobacteria of the divisions Beta, Gamma and Alpha. Also present, were members of the phyla Firmicutes, Actinobacteria and Bacteroides. Bacteria resistant to more than three distinct classes of antibiotics were detected in the all batches of the three water brands at densities up to 102 CFU/mL. In the whole set of isolates, it was observed resistance against all the 22 antimicrobials tested (ATB, BioMerieux and disc diffusion), with most of the species showing resistance to three or more classes of antibiotics. Bacteria with the highest multi-resistance indices were members of the genera *Variovorax*, *Bosea*, *Ralstonia*, *Curvibacter*, *Afipia* and *Pedobacter*, yielding resistance up to seven classes of antibiotics. Presumable episodes of resistance acquisition in the aquifer or during bottling are supported by the fact that members of the same species isolated from different brands exhibited distinct antibiotic resistance profiles. The Actinobacteria presented high resistance levels to the antibiotics ceftazidime, meropenem and ciprofloxacin according to minimum inhibitory concentrations (Etest and M.I.C. Evaluator). Bottled mineral water was confirmed as a source of antibiotic resistance mechanisms, with the potential to be transmitted to humans.

TH 143

Important vectors of antibiotic resistance genes in agricultural systems

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Spreading manure on agricultural soils was recently shown to promote spreading of transmissible antibiotic resistances and residual veterinary medicines in agricultural soils. Treatment of soil with manure spiked with the antibiotic sulfadiazine (SDZ) or manure from pigs treated with SDZ resulted in significantly higher transfer frequencies compared to manure not containing SDZ. In the present study we aimed to characterize plasmids which were captured into *E. coli* recipients from soil samples of microcosm, mesocosm and field experiments by probing and antibiotic resistances conferred. The plasmids captured were mainly assigned to LowGC and IncP-1e plasmids. The diversity of antibiotic resistance genes carried on these plasmid vectors was remarkable. Our data showed that the selection of bacterial populations carrying *sul* genes often will co-select a wide range of other antibiotic resistance genes. The diversity of antibiotic resistance gene cassettes reported in IncP-1e, the ability to efficiently transfer under soil conditions and the wide host range of IncP-1e plasmids strongly suggest that these plasmids are important vectors for spreading antibiotic resistances in the agro-ecosystem. LowGC plasmids have in contrast to IncP-1e plasmids a much more narrow host range but their relative abundance is one to two orders of magnitude higher, and they are important as putative hosts are emerging pathogens of the genus *Acinetobacter*.

TH 144

Spread of antibiotic resistance determinants in wastewater irrigated soils in the Mézquital Valley, Mexico

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We investigated the spread of antibiotic resistance determinants by horizontal gene transfer (HGT) in wastewater (WW)-irrigated soils from the Mézquital Valley (60 km north of Mexico City), the world's largest WW irrigation area. There, untreated WW from Mexico City is reused for crop irrigation. WW contains a huge amount of potentially harmful components such as resistance determinants, pathogens and antibiotics. WW-irrigated soils are a potential reservoir for multiresistant organisms, which might pose risks for field workers and consumers of the agricultural products. In the Mézquital, fields have been irrigated with WW for different time periods, from 1 year up to more than 100 years. We studied the possible correlation between the irrigation time and the concentration and dissemination of resistance genes. Furthermore, we performed soil column experiments with two different types of soil (soil irrigated with WW for 100 years and rain-fed soil), to test our hypothesis that WW irrigation leads to spread of resistance genes in soils. Gene transfer scenarios were performed as follows: an *Enterococcus faecalis* donor harbouring a mobilizable broad host range resistance plasmid labelled with the Green Fluorescent Protein (GFP) [1] and a second non-mobilizable plasmid labelled with the Red Fluorescent Protein (RFP) were added to rain-fed and 100 years-irrigated soil, each in soil columns of 20 cm height and 15 cm diameter. At each irrigation 10⁹ donors were applied to the columns. The rain-fed soil was irrigated with artificial rainwater and the WW-irrigated soil was irrigated with WW once a week, in total three times. The duration of the experiment was 4 weeks. Samples were taken daily from top soil and at the end of the experiment from different heights (every 2.5 cm). During irrigation leachate water was collected and pore water was sampled at 4 heights with suction cups. Bacteria in soil and water which have acquired the mobilizable resistance plasmid via plasmid transfer are detectable by green fluorescence. Donors are identified by their green and red fluorescence. Transfer rates for both types of soil and in water will be presented. Furthermore *gfp* and *rfp* will be quantified by real-time PCR. The soil column experiment will help assess the risk posed by HGT of resistance determinants in WW-irrigated soil.

TH 145

Development of micro-scale leucine incorporation assay and its application for analysis of co-selected antibiotic resistance patterns in Cu-contaminated soil

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It is well known that Cu selects for Cu tolerance in soil microbial communities, but recently it has been documented that Cu may also co-select for antibiotic resistance. Due to the ongoing Cu accumulation in agricultural soils and the risk of human exposure to soil microorganisms, this co-selection phenomenon deserves further study. Soil is a spatially heterogeneous matrix and we thus asked whether co-selected antibiotic resistance is a general phenomenon in the entire soil matrix or whether this phenomenon can be localized to specific micro-habitats in the same long-term (+85 years) Cu-contaminated soil as used for a previous co-selection study (Berg et al., 2010; Environ Sci Technol 44:8724-8728).

To analyze spatial heterogeneity of community-level tolerance to Cu and antibiotics, we first developed a novel micro-scale [³H]leucine incorporation assay for measuring growth rates of bacteria extracted from soil. The assay allowed us to analyze [³H]leucine incorporation rates in 5-μL soil bacterial suspensions with retained sensitivity and performance as compared to the original protocol based on larger sample sizes (Bååth et al., 2001; Soil Biol Biochem 33:1571-1574). When combined with an optimized extraction and soil handling protocol, we were able to perform 96 replicate [³H]leucine incorporation incubations based on a single 5-mg soil sample.

The optimized micro-scale [³H]leucine incorporation assay was subsequently used to measure pollution-induced community tolerance to Cu and co-tolerance to selected antibiotics in 5-mg Cu-contaminated soil samples and corresponding non-contaminated control soil samples. Cu bioavailability was analyzed by a downscaled version of an existing whole-cell bacterial biosensor assay (Brandt et al., 2008; Environ Sci Technol 42:3102-3108). A low extent of spatial heterogeneity was found for both Cu tolerance and Cu bioavailability, whereas co-selected antibiotic resistance patterns exhibited a highly heterogeneous distribution (i.e. co-selection in some 5-mg soil spots, but not in others).

Our studies indicate that field exposure to Cu may constitute a persistent selection pressure for the expansion of the soil bacterial resistome, but also that co-selection

phenomena are complex and difficult to predict at the bacterial community level. The developed micro-scale [³H]leucine incorporation assay should have several possible applications within soil microbial ecology and ecotoxicology.

TH 146

Distribution of minimum inhibitory concentrations to antibiotics in E.coli and Enterococci isolated from wastewater effluents and surface waters

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Bacterial resistance to antibiotics increases the difficulty in treating both hospital and community acquired infections and are therefore of great concern to public health. Consequently, there has been extensive research in the clinical domain. There are however, few studies investigating environmental compartments, most of which have defined antibiotic resistance in terms of clinical break points. In order to understand the mechanism of resistance in environmental isolates, a more detailed study profiling the minimum inhibitory concentration (MIC) is required.

Wastewater treatment plants are a reservoir of antibiotic residues and resistant bacteria, providing probable hotspots for the dissemination of antibiotic resistance, potentially influencing the environment when wastewater effluents are discharged into receiving waters. This study presents the surveillance of MICs to amoxicillin, ciprofloxacin and clarithromycin expressed by E.coli and Enterococci isolated from effluents of a large urban treatment plant (employing activated sludge) as well as surface waters from up and down-stream of the effluent discharge. E.coli and Enterococci were selected as they are important human commensal bacteria, commonly found in the environment and frequently associated with nosocomial infections.

Standard culture methods were used to detect, enumerate and isolate bacteria from the collected samples. Bacteria isolates were identified with API identification systems. The MICs to amoxicillin, ciprofloxacin and clarithromycin the isolates expressed were measured using antibiotic gradient strips. E.coli isolated from receiving surface waters, up and down-stream of the effluent discharge, expressed MICs to amoxicillin ranging from 2-256 µg/mL. However, only 30 % are considered resistant according to clinical break points (of 8 µg/mL). Enterococci isolates did not have a resistant phenotype to amoxicillin according to clinical break points (of 8 µg/mL). However, the MICs ranged from 0.062-0.50 µg/mL. The possible significance of the MIC distribution and its relevance to environmental microbial communities is explored.

TH 147

Aspects of antibiotics resistance in Salmonella spp. isolates

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Salmonella spp., mainly some serovars of *Salmonella enterica* as Enteritidis or Typhimurium, are etiological agents of salmonellosis, the second common gastrointestinal disease in the industrially developed countries. While most cases do not require the antibiotics treatment, it can be necessary for immunosuppressed, elder or child patients. The increasing resistance of *Salmonella* spp. to antibiotics can cause complications in such cases, as well as to support the horizontal transfer of antibiotics resistance genes in the microbial community. The development and the acquisition of these genes can occur at different places under different conditions - for example by overusing/misusing antibiotics in veterinary and medical practice or in wastewater treatment plants, supported by biofilm formation. The aim of this work was to study the different aspects of antibiotics resistance in 50 strains of *Salmonella* spp. isolated in Czech Republic from different sources (wastewater treatment plant, food and clinical isolates). The isolates were typed by ERIC-PCR and REP-PCR methods and studied for their plasmids profile. Then they were tested by disc diffusion method for the resistance to 12 antibiotics (amoxicillin, ampicillin, cefotaxime, gentamicin, streptomycin, ciprofloxacin, nalidixic acid, trimethoprim, tetracycline, chloramphenicol, nitrofurantoin, kanamycin, sulfonamide). The occurrence of *tet* genes (*tetA*, *tetB*, *tetC*, *tetG*) was detected by PCR method together with the cluster SGI-1 (*Salmonella* Genomic Island-1). The strains resistant minimally to one antibiotics (tetracycline, ampicillin, streptomycin, nalidixic acid, ciprofloxacin or chloramphenicol) were examined for the ability of biofilm formation in microtiter plates in different media (LB, BHI, BHI + 2 % glucose) and at different temperatures (25 °C, 37 °C). This work was supported by the project LD11048 of Ministry of Education, Youth and Sports of Czech Republic.

TH 148

Adaption of bacteria to ameliorate the metabolic burden of carrying antibiotic resistance genes (a systems bioecology modeling analysis)

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The spread of antibiotic resistance is an important problem, and it is unclear what leads to the relatively high abundance of resistant bacteria in the ambient environment. Mathematical models are useful tools for research and management in this area. However, although many of the mechanisms underlying antibiotic action, resistance and cost of resistance are understood at the molecular level, models typically describe these processes using system-level parameters (e.g., cost of resistance). This constitutes a disconnect that limits the utility of modeling. Here we investigate if these behaviors can be predicted from basic molecular biology. A mathematical model for E. coli and tetracycline is developed based on existing models of metabolism, plasmid and antibiotic behavior. The model explicitly accounts for the action (i.e. binding to ribosome) and resistance (Tet efflux pump), and the metabolic cost of resistance, including copying the plasmid DNA, transcribing the plasmid genes, translating the plasmid proteins and the energy consumption of the efflux pump (i.e. ATP requirement to maintain membrane charge). To investigate the fitness of different strains under various conditions, individual cells are simulated using an agent-based approach. The concept of combining biological modeling (systems biology) and ecological modeling (systems ecology) in this manner is referred to as "systems bioecology". The model reproduces observations from the literature, including growth rate vs. substrate concentration, competition between resistant and susceptible strains in the absence of tetracycline, intracellular tetracycline concentrations of wildtype and resistant strains, growth rate under various tetracycline concentrations and the response of tetracycline resistance in the human gut to a 5-day tetracycline treatment. The results show that these behaviors can be predicted from molecular mechanisms. The model is then used to investigate the role of potassium uptake by the Tet efflux pump. When this feature is included, a mutation in the endogenous uptake system (Trk) reduces the cost of carrying the resistance plasmid. Carrying the plasmid is then beneficial even in the absence of the antibiotic, which is consistent with observations in laboratory evolution experiments. This finding suggests the resistance may not be reduced when antibiotic use or discharge is stopped, an important implication for the ecology of antibiotic resistance in the environment.

EP03P2 - Environmental characterisation, fate/detection, exposure, effect and risk of nanomaterials

TH 157

Toxicity of silver nanoparticles to rainbow trout - a toxicogenomic approach

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Silver (Ag) nanoparticles possess antimicrobial properties which makes them convenient adjuvant in consumer products such as clothes and medical devices. NanoAg could leach out from these products and pervade the our environment with perhaps unsuspected impacts. The purpose of this study was to examine and compare the sublethal effects of nanoAg and dissolved Ag in rainbow trout. Changes in hepatic gene expression were monitored to provide insight on the mode of action of both forms of Ag. *Oncorhynchus mykiss* were exposed to increasing concentrations of nanoAg (20 nm) and AgNO₃ for 96 h at 15°C. The liver were also analyzed with a suite of biochemical markers of toxicity. Gene expression analysis was performed using a DNA microarray comprised of 207 stress-related genes followed by quantitative polymerase chain reaction on a selection of genes for validation. The biochemical markers consisted in the determination of labile zinc, metallothioneins, DNA strand breaks, lipid peroxidation and vitellogenin-like proteins. The analysis of total Ag in the aquarium water revealed that Ag was mostly aggregated where 1 % of the total Ag was truly dissolved. Exposure to both forms of Ag led to significant changes in gene expression for 13 % of tested gene targets. About 12 % of genes responded specifically to nanoAg while 10 % of total gene targets responded specifically to dissolved Ag. The levels of vitellogenin-like proteins and DNA strand breaks were significantly reduced by both forms of Ag but DNA breaks levels were lower with nanoAg and could not be explained by the presence of ionic Ag. Labile zinc and the oxidized fraction of metallothioneins were increased by both forms of Ag but LPO was significantly induced by nanoAg only. Discriminant function analysis revealed that the responses obtained by biochemical markers and a selection of 10 target genes were able to discriminate completely (100%) the effects of both forms of Ag. It was found that genes involved in oxidative stress, protein stability and inflammation responses were able to discriminate each forms of Ag where nanoAg influenced more genes involved in inflammation and dissolved silver involved more oxidative stress and protein stability.

TH 158

Gold ingegnerized nanoparticles (Au NPs): first investigation of toxicological effects in fibroblast cell cultures of cetaceans

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Nanoparticles (NPs) are produced intentionally with the aim of developing new materials that exhibit certain specific properties. These properties are related to at least one of their dimensions, which must be less than 100 nanometers (nm). Their use has been proposed in many fields of science and industry. Several studies exist on the toxicological properties of NPs. Although the various toxicological aspects and the diversity of the NPs assessed are just beginning, many deleterious effects have been documented, particularly in animals. However, no data are available for marine top predators of conservation interest, such as toothed whales (Odontoceti). Gold (Au) NPs, also called gold colloids, are the most stable metal nanoparticles. Recently the photothermal conversion property of Au NPs has attracted a keen interest from the scientific community. In fact the Au NPs that are irradiated by a laser beam can efficiently convert the photon energy to thermal energy, which leads to the destruction of biological cells such as tumor and bacteria. Despite Au NPs are considered non-toxic by several authors, there is a serious lack of information concerning their impact on the life health and the environment. The aim of this study was to evaluate the toxicological effects of the ingegnerized Au NPs in fibroblast cell cultures of two free-ranging specimens of toothed cetaceans: striped dolphin (*Stenella coeruleoalba*) and bottlenose dolphin (*Tursiops truncatus*), sampled in Mediterranean Sea by non destructive method represented by subcutaneous biopsy. Genotoxicity of Au NPs in cetacean fibroblasts was detected by the alkaline Comet assay. Immunofluorescence technique was used for qualitative and quantitative evaluation of cytochrome P450 1A1 (CYP1A1), cytochrome P450 2B (CYP2B) and MICA expression. The first two proteins are evaluated as target of potential indicators of man-made pollutants, and the MICA as toxicological stress marker of the immune system. Finally, the Au NPs capacity to pass through the cellular barriers, was

performed by STEM. The main results of these experiments were: 1) the increase of DNA tail % compared to the control and in function of the time of exposure (4 and 24 h); 2) no significance increase of fluorescence in relation to control in the treated cells for all proteins: P450 1A1, P450 2B and MICA; 3) the presence of Au NPs into the cells with the STEM approach.

TH 159

Uptake and distribution of multiwalled carbon nanotubes in zebrafish *Danio rerio*

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Due to its unique properties, carbon nanotubes (CNT) are considered one of the most promising materials in nanotechnology. A wide spread usage is predicted. There is a lack of data regarding the ecotoxicology of this nanomaterial, which needs to be replenished soon.

We investigated the bioavailability of radiolabelled multiwalled CNT (14C-CNT) to the zebrafish *Danio rerio* by quantifying uptake over time at different concentrations after water exposure in absence and presence of dissolved organic carbon (DOC) as well as after dietary exposure. Subsequently, elimination of the material by the fish was measured. To investigate the possible interaction of CNT with fish tissue cells, imaging with transmission electron microscopy (TEM) was performed.

14C-CNT were synthesized by means of chemical vapour deposition of 14C-benzene in a small-scale batch reactor. Ultrasonication was used to disperse 14C-CNT in the media. Zebrafish were exposed via water (0.1, 0.3 and 1.0 mg 14C-CNT/L), via water with DOC (1.0 mg 14C-CNT/L & 8 mg DOC/L) and via a diet of 72 h preliminary exposed blackworms (*Lumbriculus variegatus*). Measurements were carried out after 3, 24 and 48 h. For exposure via water in absence of DOC, an additional sampling point was set to 168 h. Elimination was measured after an exposure time of 168 h, at 48, 72 and 168 h. At sampling time, the fish were dissected. The internal radioactivity of the different organs and tissues was measured via liquid scintillation counting (LSC) after tissue solubilising.

After exposure via water in absence of DOC, 14C-CNT were found in gut, gills, skin and fillet of the fish. Only little radioactivity was distributed to gonads and blood. In presence of DOC, generally little lower uptake was observed, and 14C-CNT were mainly found in gut and gills, while only low amounts were detected in the leftover fish. After dietary exposure, 14C-CNT were only present in the gut. Over the course of the elimination experiment, the 14C-CNT were largely but not completely eliminated to the water phase. By means of TEM imaging, CNT-like structures were shown to be located in the fish gut epithelium.

These results show that dispersed 14C-CNT are taken up by zebrafish via water both in absence and presence of DOC and after dietary exposure. The results of TEM imaging confirm that CNT are bioavailable for *D. rerio*, and raise concern about possible consequences of the observed interaction of CNT with gut epithelial cells of the fish.

TH 160

Co-exposure to nano-TiO₂ and ultraviolet: an ecotoxicological evaluation

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Studies about the potential ecotoxicological risks of titanium dioxide nanoparticles (nano-TiO₂) have been published but the results are still inconclusive. The properties of the diverse nano-TiO₂ must be considered in order to establish experimental models to study their toxicity. TiO₂ has important photocatalytic properties and its photoactivation occurs in the ultraviolet range. The aim of this study was to investigate the effects of co-exposure to nano-TiO₂ and ultraviolet. A South America fish (*Piaractus mesopotamicus*) was exposed to nano-TiO₂ Sigma Aldrich (25 nm, 100% anatase) under different illumination conditions: visible light (V) or visible light and ultraviolet A (UVA) at 2.7 J/cm²/h. Animals (n=8) were exposed during 96h to 0, 1, 10 or 100 mg/L nano-TiO₂. The solutions were renewed daily, and the characteristics of nanoparticles in solution were: 596.1 ± 38 nm, pdi 0.31 ± 0.09; zeta potential -19.2 ± 1 mV. Protein carbonylation, lipoperoxidation and the activities of catalase, superoxide dismutase, acid phosphatase and glutathione s-transferase (GST) were analyzed in liver. Metallothionein concentration was analysed in gills. Comet and micronucleus assays were performed with blood. The LC₅₀ was >100mg/L for both conditions tested. GST showed a significant decrease in groups exposed to 1 and 10 mg/L nano-TiO₂ under UVA compared to control under V. The results of comet assay showed the greatest damage in fish exposed to 100 mg/L nano-TiO₂ under V and 10 mg/L under UVA. For the other biomarkers there was no statistics difference between groups and control groups. Our results corroborate with literature, showing low acute toxicity of nano-TiO₂ in fish. However, co-exposure of nano-TiO₂ and UVA can cause biochemical and genetic changes. The decrease in GST activity can be related with an inhibition or reduction of expression levels, but more investigation is needed. Further studies will be carried to investigate the relevance of considering the nano-TiO₂ properties in ecotoxicological studies. Supported by: FAPESP, CNPq, CAPES, Fundunesp and Embrapa (AgroNano).

TH 161

Salinity- and pH-dependent silver nano-toxicity in post-hatch embryos of Japanese medaka

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Our group has been investigated nano-sized silver toxicology using Japanese medaka embryos and elucidated that silver nanocolloids cause severe inhibition of embryogenesis and morphological changes such as blood clots, pericardio vascular edema, tubular hearts, small eyes, shortened body, and spinal deformities at 0.5 mg/L and 1.0 mg/L.

Post-hatch embryo stage and following early-life stage of fish are well known to be more susceptible to xenobiotics than chorionated-embryos. To elucidate stage-dependent toxicity of silver nanocolloids (SNC, colloids size 43.8-66.8 nm in ultra-pure water) between embryo and post-hatch embryo stages, post-hatch embryo medaka was exposed to SNC in ERM (embryo rearing medium, pH7) for 7 days. Unlike embryos there was no observed biological effect at 0.5 mg/L and 1.0 mg/L of SNC, biological effects are significantly (P<0.05) observed at 5.0 mg/L and 10 mg/L of SNC in day 7. In terms of biological effects, body length and whole length were 6.6% and 4.5% decreased, respectively; however, tail length was 13% increased at 10 mg/L in compare with control. Eye and gallbladder sizes were also 8.7% and 35% bigger, respectively, than control. In addition, gallbladder's green color turned to yellowish, and liver formation got invisible in both SNC exposures. These results suggested that post-hatch and early-life stage of medaka are less-susceptible to SNC exposure than embryonic stage. Physicochemical properties of nanomaterials were considered to be changed by pH and salinity, and then, changed properties (e.g. zeta-potential and aggregation) would influence on environmental fate of nanomaterials. Tested SNC changed zeta-potential smaller in pH9 (-39.2 mV) from pH7 (-44.5 mV) and colloidal size larger in ERM (ca. 450 nm) from ultra-pure water (ca. 50 nm). We will demonstrate salinity- and/or pH-dependent toxicity of SNC using post-hatch embryos as well.

TH 162

Development of a test chamber to enable homogenous aqueous phase dispersions of nanoparticles for testing nanoparticle toxicity in fish

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Nanoparticles have unique properties and their behaviour in water often leads to aggregation and sedimentation of NPs out of the aqueous phase. Determination of NP toxicity in fish is important, but the heterogenous nature of static aqueous NP preparations leads to difficulties in obtaining repeatable results of NP toxicity and likely explains some of the differences in NP toxicity reported in the literature. The objective of this investigation was to develop a simple standardized procedure to enable testing toxicity of homogeneous aqueous dispersions of NPs in fish (e.g., zebrafish *Danio rerio*) that is repeatable and easily transferable across laboratories. The test system (2-L glass beaker) is designed to maintain homogeneous aqueous dispersions of NPs by stirring, and delivers water containing aqueous NPs to fish held in a semi-isolated chamber. With this we have conducted acute toxicity tests with larval zebrafish to establish 96-h LC50 values for TiO₂-NPs, Ag-NPs, and Cu-NPs, and compared to metal ion controls Ag⁺ and Cu²⁺. The ability of the NP dispersion chamber to maintain homogeneous aqueous NP-dispersions was tested by analytical chemistry (inductively coupled plasma mass spectrometry, for metals), which determined that homogenous dispersions were maintained during the exposure (total loss of NPs from water column <30% over 96 h). Without use of chamber (i.e., static exposures) the concentration of NPs (based on total metals concentration) was reduced by >90% within 24 h. The LC50 values of Ag-NPs, Cu-NPs, and TiO₂-NPs were 0.27 mg/L, 0.51 mg/L, and 897 mg/L, respectively. Of particular importance was the reproducibility of LC50 results across triplicate independent exposures which indicated a low coefficient of variation of 14.81%, 10.94%, and 12.69%, for Ag-NPs, Cu-NPs, and TiO₂-NPs. This approach has potential to become a standardized test procedure for testing the toxicity of NPs in the aqueous phase and addressing the problem of the lack of comparability of NP toxicity results across laboratories.

TH 163

Silver nano-toxicity and biological effects are dependent on cation contents and pH in tested water

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Silver nanomaterials are one of the major components of healthcare products. Their environmental fate and ecological risk are not well understood yet. Although it is known silver ion (Ag⁺) has toxic effects, it has been discussed whether nano-sized silver has its own toxicity. To elucidate this problem, silver nitrate (AgNO₃) has been used for Ag⁺ source with low pH (pH5.63 in 0.01 mg-AgNO₃/L, pH5.53 in 0.1 mg-AgNO₃/L). It is also well known that low pH aqua has some toxic effects to aquatic organisms, which not allow us to use low pH ambient for aquatic toxicology. In this study, a novel method has developed and tested to elucidate which nano-sized Ag and Ag⁺ have more toxic effects to medaka embryos. A bottom-fixed silver nanoparticles plate (nano silver plate; NSP), which is developed in this study is utilized to test toxicity of Ag⁺ released from silver. This test was demonstrated in ultra-pure water or ERM (embryo rearing medium for medaka) under different pH (4, 7 and 9). As a counterpart compound, silver nanocolloid (SNC; colloidal diameter 43.8-66.8 nm in ultra-pure water) was used. In NSP with ultra-pure water, embryos exposed to Ag⁺ at 0.57, 0.08 and 0.08 mg/L at pH4, 7, and 9, respectively, showed complete lethality in 12 hr; however, embryos in ultra-pure water without Ag⁺ exhibited 100% and 97.8% hatching in pH7 and pH9 although embryos in pH4 were completely killed in 4 days. Those suggested Ag⁺ has completed lethal toxicity at 0.08 mg/L under less-mineral ion water condition. In contrast, in NSP with ERM, embryos exposed to Ag⁺ at 0.04, 0.04 and 0.04 mg/L at pH4, 7 and 9, respectively, exhibited high hatching ratios at more than 95%. However, these embryos were observed hatch delay and decrease of heart rate, less-developed eyeball and body length. On the other hand, in exposure to SNC at 0.064 mg/L, embryos in ultra-pure water showed lower hatching ratio (0 - 62.2%) and small eyeball, shortened body length, and altered heart beat and time to hatch; and then these biological effects were moderated in ERM as well as happened in case of NSP. We estimated that embryos in ERM were relieved from biological effects of silver toxicity, which may depend on cation contents (mainly Ca⁺⁺, Mg⁺⁺, K⁺, Na⁺). NSP appeared significant biological effects to medaka embryos rather than SNC due to released Ag⁺. This study suggests that biological effects are higher by Ag⁺ not by nano-sized silver.

TH 164

Silver nanocolloids and target genes analyses in medaka embryos

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Silver nanomaterials are considered to be one of the major pollutants from nano-industries. Our group has been investigated SNC toxicology using medaka embryos and elucidated that SNC causes severe inhibition of embryogenesis and morphological changes such as blood clots, pericardiovascular edema, tubular hearts, small eyes, shortened body, and spinal deformities at 0.5 mg/L and 1.0 mg/L. This study explores the toxic mechanisms of silver nanocolloids (SNC; average particle size 3.6 diameter nm, purity 99.99%, pure water solution) using medaka embryo model. From DNA microarray analyses, 118 genes are up-regulated (>2.0 fold) and 117 genes are down-regulated (<0.5 fold). However, severe morphological defects including vascular defects, pericardiovascular edema, tubular heart, and underdevelopment of eyes and the central nervous system were all observed in SNC-exposed medaka embryos. Therefore, we chose six genes that were relevant to vertebrate embryogenesis and/or morphogenesis and well-studied in mammalian systems. Cathepsin L (ctsl), tropomyosin 1 alpha (tpm1), retinol-binding protein (rbp) and metallothionein (mt) were significantly down-regulated in SNC exposed embryos, while sarcoplasmic/endoplasmic reticulum calcium ATPase 1 (SERCA1, atp2a1) and homeobox B6b (hoxb6b) were both up-regulated. In order to understand SNC toxicology, those gene expression patterning during normal embryogenesis (from stage 12; pre-early gastrula stage, to stage 39; hatching stage) were measured with qRT-PCR. Retinol-binding protein (rbp) relative to carrier protein that bind retinol and regulate epithelial cell growth, growth of bone tissue, immune function and tumor suppression; ATPase 1 (atp2a1) relative to calcium pump on endoplasmic reticulum, heart dysfunction and abnormality; and tropomyosin 1 alpha (tpm1) relative to calcium dependent regulation of vertebrate striated muscle contraction, were 170, 2600, and 65000 folds expressed, respectively, according to embryonic developmental stages. Homeobox B6b (hoxb6b) relative to regulation of patterns of anatomical development (morphogenesis) was reached at peak (4.2 folds) in stage 35 (pectoral fin blood circulation stage), and then reduced to 1.5 folds in stage 39. Other two genes (ctsl and mt) were constantly expressed during embryogenesis (2-3 folds). Biological effects of SNC rely on biological development including expression of target genes. This information should help us to understand the toxic mechanism of SNC.

TH 165

Carbon nanospheres, multiwalled carbon nanotubes and β -cyclodextrin polymer as emerging pollutants

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The use of engineered nanomaterials is fast growing and the environmental concerns about them are also growing. The nano size (<100 nm) of nanomaterials which has resulted in their vast applications has since raised concerns about their toxicity. Carbon-based nanomaterials are one such group and have since secured vast applications in industry. Some of these nanomaterials have been specifically engineered for water purification purposes and hence their fate in the aquatic system is not yet fully understood. There is therefore a need for in vivo studies on aquatic organisms that are known to be good pollution indicators. The pollution indicators chosen for this study were *Poecilia reticulata* (fish), *Daphnia pulex* (water-flea), *Vibrio fischeri* (bacteria) and *Selenastrum capricornutum* (algae) and the carbon-based nanomaterials tested were multi-walled carbon nanotubes (MWCNTs), carbon nanospheres and β -cyclodextrin polymer. These nanomaterials were vastly characterized to determine their physico-chemical properties in their dry and wet state as per the techniques applied. All the exposures experiments were done in the presence and absence of humic acid (20, 10, 5, 2.5 and 1.25 ppm humic acid concentration) at neutral pH. Particle size and zeta potential were monitored over 96 hr (the longest time for fish exposures). LC50 (lethal concentration) and the EC50 (effective concentration) values were calculated using the Probit method. Fish mortalities were observed at 3 g/L of pristine MWCNT and 187.2 g/L β -cyclodextrin polyurethane; *D. pulex* showed an LC50 of 1.736 mg/L with oxidized MWCNTs while bacterial and algal growth was stimulated at low concentrations (2.50-1.25 ppm) of MWCNTs and carbon nanospheres. In the absence of humic acid the nanomaterial either settled or agglomerated. The nanomaterials were also well dispersed in the presence of humic acid and their hydrated particle sizes were reduced. For instance, in carbon nanospheres the presence of humic acid reduced the agglomerated size from 296 nm in "no humic acid" samples to 99.9 nm at 8 ppm humic acid and down to 31.7 nm after 96 hr. The presence of humic acid also enhanced the toxicities, especially that of carbon nanospheres to fish. At sublethal concentrations of these nanomaterials catalase, glutathione and superoxide dismutase activity was observed at in fish and daphnia, thus indicating oxidative stress to the tested organisms..

TH 166

Sublethal toxicity of titanium dioxide nanoparticles in the shore crab *Carcinus maenas*

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Ecotoxicological studies with TiO₂ nanoparticles (NP) in aquatic systems have largely focussed on freshwater biota such as crustaceans (*Daphnia magna*, *Ceriodaphnia dubia* and *Thamnocephalus platyurus*) and fish (*Danio rerio*, *Oncorhynchus mykiss*). Few studies have investigated effects of TiO₂-NPs in marine or estuarine fauna despite the expectation based on physicochemistry that accelerated aggregation and deposition of the NPs on substrates will occur at the freshwater-saltwater interface. Moreover, where reported, effects in marine invertebrates including mussel *Mytilus galloprovincialis* and polychaete *Arenicola marina* have been inconsistent. In particular, bioavailability of TiO₂ NP is unclear and has clouded interpretation of measured molecular, biochemical and physiological effects. To our knowledge, no studies have been conducted in the benthic shore crab *Carcinus maenas*, a sensitive and widely used bioindicator of coastal environmental contamination. Thus, the aim of this study is to evaluate biochemical and physiological effects of TiO₂ NP in *C. maenas* and determine NP bioavailability and accumulation in key tissues (e.g. gill and hepatopancreas). After acclimation of 15 days, adult male crabs were exposed in a semi-static test system to 0, 0.1, 1.0 and 10 mg TiO₂ NP/L (24.5 $\pm$ 10.6 nm diameter) and 10 mg/L bulk TiO₂ (134.0 $\pm$ 42.5 nm), a particle size control. For each treatment 15 crabs (5 per replicate) were evaluated. Daily, crabs were briefly removed from exposure tanks, fed with frozen cockle (*Cerastoderma edule*) in clean water to avoid dietary TiO₂ exposure and 100% exposure media exchanged. After exposure, biochemical responses associated with physiological function will be measured including gill Na⁺K⁺-ATPase activity, osmotic pressure and major ions in haemolymph as indicators of osmoregulatory competence; oxidized/reduced glutathione ratio and ferric antioxidant status in haemolymph as biomarkers of oxidative stress; pH and lactic acid in haemolymph and oxygen consumption as end points of respiratory metabolism and, O/N ratio and total soluble proteins in haemolymph as end points of energetic status. Gill and hepatopancreas will also be analysed for tissue Ti accumulation. Results are under analysis; however obtained information will likely add to our understanding of potential TiO₂ NP toxicity in an ecologically important benthic coastal species.

TH 167

Effect of silver nanoparticles on ovarian follicular cells of marine medaka (*Oryzias melastigma*)

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Silver nanoparticles (AgNPs) have gained much popularity on account of their antimicrobial properties. The increase in the production of these nanoparticles and their wide occurrence has become a growing environmental concern. While toxicity effects of AgNPs on aquatic organisms have been identified, there is a lack of data concerning their effects on marine species.

It was hypothesized that exposure to nanoparticles may potentially interfere with steroidogenesis and hence reproduction. An In vitro study was carried out to investigate the effects of two commercial AgNPs on ovarian follicular cells of marine medaka (*Oryzias melastigma*). After exposing the follicular primary cell culture to AgNPs for 48 hours, dose-dependent cytotoxicity was evaluated by an MTT assay. Modulations of the expression of the steroidogenic genes (cyp11a, cyp19a, 3bhsd and 20bhsd) were determined by measuring the mRNA concentrations by real-time polymerase chain reaction (Q-RT-PCR). Additionally, the level of mRNA expressions of the apoptotic and anti-apoptotic proteins (bax and bcl-2) were measured. At least three independent experiments were conducted.

Our results indicate that changes in gene expression pattern are elicited by AgNPs, and differences between the two commercial AgNPs were clearly evident. The data presented here provides insight that AgNPs may affect steroidogenesis and sex hormone production in ovarian follicular cells of marine medaka, which may subsequently compromise reproduction.

TH 168

Toxicity of CdSe quantum dots to marine microalgae: effects on oxidative stress, phytochelatin production and growth

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The rapid increase of nanotechnology has raised the concern about the potential adverse effects of nanoparticles on the ecosystems and living organisms. Quantum dots (QDs) are semiconductor nanocrystals, candidate to be widely used for biomedical applications and microelectronics, due to their unique optical properties. However, their environmental impact and the mechanism of toxicity still have not been fully elucidated. Phytoplankton represents the first link of the food chain in natural waters, thus the knowledge of the mechanisms of uptake, accumulation and toxicity of nanoparticles in these unicellular organisms represents a crucial point for understanding their ecotoxicity.

In this work we evaluated the effects of CdSe/ZnS QDs on the marine diatom *Phaeodactylum tricornutum*, which served as a model of cell toxicity and an indicator of potential impact to ecosystem health. Laboratory cultures of *P. tricornutum* were grown for three days in culture media at increasing concentrations of CdSe/ZnS QDs, from 0.04 to 1.00 nM. The results show a gradual decrease of the growth rate at concentrations of QDs higher than 0.5 nM. At 1 nM, it was 75% of the control. At the end of exposure, we tested the cellular Cd accumulation, the synthesis of phytochelatin (PCs) as a biomarker of the bioavailability of metal ions, the production of ROS, and the activity of antioxidant enzymes as an indication of oxidative stress. The results show that *P. tricornutum* accumulated Cd in a dose-dependent manner, but cellular Cd was not able to stimulate a comparable PC synthesis. Since the production of PCs is a specific signal of the presence of free metal ions, our findings suggest that QDs were taken up by *P. tricornutum* and were not degraded inside the cell. Measurements of the activity of the antioxidant enzymes showed that superoxide dismutase (SOD) and catalase (CAT) activities were enhanced by exposure to increasing QD concentrations, whereas ascorbate peroxidase and glutathione reductase activities were not significantly affected. SOD is considered the first line of defence against ROS, followed by other H₂O₂ scavenging enzymes, such as CAT or peroxidases. Thereby, an increase in SOD and CAT activity can be considered a symptom of oxidative stress induced by an enhanced production of ROS. This hypothesis was confirmed by the concomitant increase in the intracellular ROS concentration. Experiments are in progress to test the response of other species of microalgae.

TH 169

Effects of silver nanoparticles in the freshwater snail *Physa acuta*

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The environment is constantly being exposed to various types of contaminants due to anthropogenic activities. Recent studies showed an increase in the amount of metals, such as copper, silver or cadmium that can be found in aquatic ecosystems due to industrial effluents and domestic wastewaters disposals. Silver nanoparticles are nowadays widely being used in all kind of products, such as domestic disinfectants, pharmaceutical products, cleaning products or even contact lenses, which will lead to an increase in concentration in the environment. Therefore, this study aimed to assess the survival, reproduction and egg viability of the freshwater pulmonate snail *Physa acuta* after exposure to silver nanoparticles and also its counterpart silver nitrate in laboratory conditions. A 96h exposure test will be used to evaluate acute toxicity and a 14/28 days chronic test will be carried out where egg viability and the number of new born snails will be evaluated. Results showed a decrease in reproduction and egg hatching in higher concentrations. Silver nanoparticles and ions significantly affected all 3 endpoints measured.

This study highlights that silver nanoparticles are bringing new inputs of silver as contaminant (as ions and nanoparticles) into the aquatic ecosystems.

TH 170

Evaluation of chronic toxicity of copper oxide nanoparticles to microcrustacean marine *Mysidopsis juniae*

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The copper oxide nanoparticles are components of antifouling paints that are used for painting the hulls of boats. Their decomposition can provide both the soluble ionic form and the NPs in the marine environment, becoming an important source of contamination of aquatic ecosystems. Chronic toxicity studies in aquatic environments is a trend in environmental toxicology, since in many cases the contaminants from this group are highly diluted and do not offer sufficient concentrations to elicit acute effects but capable of causing only long-term effects. This study was aimed at assessing the chronic toxicity of copper oxide nanoparticles on the marine microcrustacean, *Mysidopsis juniae*. Tests were conducted with concentrations of 100, 75 and 50 µg·L⁻¹ and 40 test organisms with sexual dimorphism were added in each of the 2 liter tanks. The organisms were exposed for 30 days to the nanoparticles. The test was carried out in semi-static environment and the water exchange occurred once a week. The test conditions were salinity 32; photoperiod 12 hours of light and 12 hours of darkness and temperature 24±1 °C. The mysids were fed daily with *Artemia sp.* nauplii enriched with omega 3 and cod liver oil. The parameters evaluated were mortality, reproduction and total size. The laboratory maintains control charts for monitoring the sensitivity of test organisms. The data were analyzed with the softwares Statistica 7 and Minitab 16. In order to check the differences between the evaluated parameters Student's t test with a confidence interval of 95% and the Dunnett test for calculating the NOEC were used. The mysids exposed to nanoparticles suffered changes in number of mortality, fertility and size in concentrations above of 50 µg·L⁻¹.

TH 171

Effects of in vivo exposure to nTiO₂ on *Mytilus* immune parameters

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Acute toxicity of different engineered nanoparticles (ENP) underlined the potential risk of ENPs exposure to aquatic life. Apart from traditional ecotoxicity testing, more specific assays like immunotoxicity, genotoxicity, oxidative stress, may help understanding the major toxic mechanisms and modes of actions that could be relevant for different ENP types also in aquatic organisms.

We have previously demonstrated that in the marine bivalve *Mytilus galloprovincialis*, short term in vivo exposure to nTiO₂, in the mg/L range, induced significant effects on lysosomal and oxidative stress biomarkers in the digestive gland. Moreover, *Mytilus* immune cells, the hemocytes, have been shown to represent a sensitive target for the in vitro effects of different types of NPs, including nTiO₂. Biomarkers of immunotoxicity may reveal changes in the immune function induced by ENP exposure, possibly leading to changes in organisms' health.

In this work, the in vivo effects of nTiO₂ on immune function were investigated in mussels exposed to different nominal concentrations of nTiO₂ (1, 10 and 100 mg/L) for 4 days. Hemolymph immune parameters were evaluated: hemocyte lysosomal membrane stability (LMS), ROS and NO production, phagocytic activity and serum lysozyme activity. Expression of selected genes was evaluated in hemocytes by RT-Q-PCR.

Physico-chemical characterization of nTiO₂ primary particles was performed by a combination of techniques such as SEM, TEM, XRD, BET, DLS, TGA, ICP-MS, etc.. DLS analysis revealed the formation of different nm- and µm-sized agglomerates of nTiO₂ in artificial sea water (ASW). The results showed that exposure to nTiO₂ induced significant changes in different functional and molecular immune parameters in mussel hemocytes. Moreover, decreases in lysosomal biomarkers (lysosomal membrane stability and lipofuscin accumulation) were observed in the digestive gland, indicating general stress conditions.

These data indicate that exposure to nTiO₂, at concentrations in the low mg/L range, can affect the mussel immune function, and support the hypothesis that transfer of ENPs from the digestive system to the hemolymph and circulating hemocytes may occur. Biomarkers of immunotoxicity may represent sensitive indicators of how NPs may cause alterations in the organism's physiology, providing an indication of the sublethal impacts of ENP exposure, as well as an "early warning" of population level impacts.

TH 172

Method development to test toxicity of fullerenes (nC60) on sediment-dwelling invertebrate *Chironomus riparius* larvae

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The possible ecotoxicological risks of nanoparticles (NPs) have received increasing research attention in recent years as a result of the enormous economic potential of nanotechnology-related applications and the lack of information about such risks. The extent to which current standard ecotoxicological methods can be used to accurately measure the ecological risks of NPs is largely unknown due to the unique chemical properties of nanoparticles which substantially differ from those of hydrophobic organic chemicals and dissolved metals. The aim of this study was to investigate the extent to which fullerenes impact this benthic organism *Chironomus riparius* using an abbreviated version of Organization for Economic Co-operation and Development (OECD) standard method 218 to test short-term effects after exposure for 10 d. First, an environmentally realistic method to test fullerene (C₆₀) toxicity to benthic organism *C. riparius* was created by allowing suspended fullerenes to settle down creating a layer on top of the sediment (fullerene masses of 0.36 to 0.55 mg/cm²). This created an environmentally realistic exposure method which simulates a sensitive exposure route for *C. riparius* given its feeding habits. Second, two different food concentrations (0.5 % and 0.8 % *Urtica* sp.) in the sediment were tested to investigate the impact of this parameter on toxic responses. Thus, we hypothesized that higher food concentrations will decrease toxic effects. In the 0.5 % food level treatments, there were significant differences in all growth-related endpoints compared to controls. Fewer effects were observed for the higher food treatment. Fullerene agglomerates were observed by electron microscopy in the gut but no absorption into the gut epithelial cells was detected. In the organisms exposed to fullerenes, microvilli were damaged and significantly shorter. The potential toxic effects of fullerene to *C. riparius* appear to be caused by morphological changes, thus inhibiting the larvae growth.

TH 173

Multibiomarker assessment of cerium dioxide nanoparticle (nCeO₂) sublethal effects on the freshwater invertebrates *Dreissena polymorpha* and *Gammarus roeseli*

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Cerium dioxide nanoparticles (nCeO₂) are among the most widely used, principally as fuel additive for their catalytic properties. As a result, the release of nCeO₂ and the subsequent environmental exposure are predicted to be consequent, while the risks incurred are still unknown. Most of the aquatic ecotoxicological studies published from now have focused on the acute toxicity of nCeO₂, often at environmentally irrelevant concentrations, and have shown its relative innocuousness. However, with environmental concentrations in surface waters predicted to be in the low µg/L range, it is unlikely that we could extrapolate real impacts of nCeO₂ on aquatic ecosystems from the results of those studies, especially given that the physico-chemical properties of nanoparticles at these concentrations are expected to be quite different. Moreover, to our knowledge, no study has ever used a multi-biomarker approach to evaluate nCeO₂ impacts on aquatic ecosystems.

In this work, we filled those knowledge gaps by investigating sublethal effects of nCeO₂ on two aquatic invertebrates, the freshwater mussel *Dreissena polymorpha* and the freshwater amphipod *Gammarus roeseli*, at concentrations close to predicted environmental concentrations (10 and 100 µg/L) for 96 hours. A broad set of biochemical and histological biomarkers were used to evaluate nCeO₂ exposure effects on 1) antioxidant and antitoxic defences (catalase, GPx, GPx-Se and GST activities, metallothionein concentration), 2) cellular damages (lipoperoxidation), 3- iono/osmoregulation (Na⁺/K⁺-ATPase activity and Na⁺, Cl⁻ and Ca²⁺ haemolymph concentrations), 4) lysosomal system and 5) behavioural responses (locomotion and ventilation for *Gammarus* only). The first results showed an absence of significant adverse effects of nCeO₂ on all tested biomarkers. On the contrary, nCeO₂ seems to exhibit antioxidant properties, decreasing lipid peroxidation and catalase activity, and this protective activity will need to be more thoroughly investigated.

TH 174

Effects of TiO₂ nanoparticles on *Mytilus galloprovincialis* 48 h-old larvae

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Engineered nanomaterials are at the forefront of ecotoxicologist agendas due to their widespread use in a broad range of industrial and domestic sectors. Actually, they are manufactured in increasing amounts year-by-year. Particularly, nanoscopic titanium dioxide (nTiO₂) is used by a variety of industries mainly for catalysis and photocatalysis

applications and as an additive in paints, papers, inks, plastics and various consumer products with a rapid increase in its production, estimated in the United States to reach 106 tons per year by 2025. So, we expect an increased environmental exposure to nTiO₂ with concentrations in different environmental compartments that may induce toxic effects to the aquatic ecosystem.

Most of the literature on the ecotoxicity of nTiO₂ deals with aquatic organisms, mainly from freshwaters, such as bacteria, algae, invertebrates and fish. Little information is available about saltwater species such as bivalves that, amongst environmental quality status sentinels, are considered a potential target group and bio-indicators for nanoparticles toxicity. They are widespread in all fresh, brackish and salt water environments, having a key ecological role both as filter and deposit feeders according to various species and habitats. All stages of their life cycles (gametes, embryos, larvae and adults) have been used to define an endpoint for a specific monitoring purpose (acute, sub-chronic or chronic toxicity). Bivalve molluscs toxicity tests are generally easy to perform, highly sensitive, cost-effective and not time consuming ranging from 24 h to 48 h to obtain a first meaningful result, or even lower when some biomarker are taken into consideration.

The aim of the present work is focused on the assessment of the potential toxicity of nTiO₂ towards early larval stages of *Mytilus galloprovincialis* (LMK) - 48 h-old larvae, that are commonly known as D-shape ones. Dark and dark/light (8/16 h) scenarios were investigated to comply with standard protocol and mimic a more likely environmental exposure. Results evidenced unusual toxicity trends presenting their acmes around 6 mg/L of nTiO₂ and potential defensive mechanisms in the exposed larvae.

TH 175

Cellular and subcellular localization of Au nanoparticles and Au bioaccumulation in mussels exposed to Au nanoparticles of two different sizes

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The subcellular localization of metallic nanoparticles in invertebrates has been scarcely studied and the results are inconclusive. Here, mussels, *Mytilus galloprovincialis*, were exposed, for 3 d, to Na-citrate stabilised Au nanoparticles (NPs) of two different sizes (5 and 40 nm), as well as to bulk and ionic (HAuCl₄) Au forms. The nominal exposure concentrations were 0, 0.75, 75 and 750 µg Au/l in the four experimental sets. No mortality was recorded in mussels exposed to the different Au forms. Au in mussel soft tissues was measured by ICP-MS. Tissue Au accumulation was dose-dependent for all treatments. Lowest Au concentrations were found in mussels exposed to bulk Au. Autometallography was applied to localize metal ions (as black silver deposits, BSD) in paraffin-embedded tissue sections of the digestive gland. Black silver deposits were mainly found in the digestive cells. The volume density of BSD (VvBSD) was quantified by image analysis and there was agreement between VvBSD values and the tissue concentration of Au. Transmission electron microscopy (TEM) was used to study the distribution of silver enhanced (Aurion R-Gen kit) Au NPs in ultrathin sections mounted on Ni grids. In mussels exposed to Au NPs of both sizes, electron dense particles were found in the digestive gland epithelium, especially within late endosomes, lysosomes and residual bodies of digestive cells. The presence of Au in these electron dense particles was confirmed after electron probe X-ray microanalysis in mussels exposed to 40 nm NPs but not in those exposed to 5 nm NPs, due to the low signal recorded (5 nm NPs were sparse). It can be concluded that Au NPs are bioavailable for mussels, and are accumulated in the lysosomes of digestive cells. The study of the effects produced by these NPs is currently under research using a battery of selected biomarkers at molecular, cell and tissue-levels.

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TH 176

Metal-particle size affects metal bioaccumulation from sediment and depuration in a deposit-feeding snail

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With the increasing use of CuO nanoparticles (NPs), nano CuO may pose risks to aquatic organisms. The persistence of nano CuO in aquatic organisms has not yet been determined. In the present study, we compare the bioaccumulation and depuration of copper in the freshwater snail, *Potamopyrgus antipodarum*, exposed to sediment spiked with aqueous Cu, 6 nm-CuO, or 100 nm-CuO nanoparticles. Snails were exposed for 2 weeks to the control (0 µg Cu/g dry weight sediment) and three forms of Cu-spiked sediment (240 µg Cu/g dry weight sediment) and allowed to depurate for two weeks in clean natural sediment. Our results showed that the smaller size particles (6 nm-CuO) were associated with higher mortality of *P. antipodarum* during the exposure period, and the net uptake rates for sediment spiked with 6 nm-CuO and aqueous Cu were significantly higher (around 40–50%) than for sediment spiked with 100 nm-CuO. During two weeks of depuration, there were no significant differences in depuration rates (kd) among forms (aqueous Cu: kd = -0.12 wk⁻¹; 6 nm-CuO: kd = -0.22 wk⁻¹; 100 nm-CuO: kd = -0.20 wk⁻¹). Our results suggest that particle size has an important influence on the toxicity and bioaccumulation of Cu in *P. antipodarum*.

TH 177

The ecotoxicology of engineered metal oxide (ZnO) nanoparticles with respect to the aquatic sediment dweller, *Lumbriculus variegatus*

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Since the 1990's there has been a rapid increase in the use of engineered nanomaterials and one of the side effects of this "nano-boom" is the potential for these NP's to be released into the environment. The novel properties of nanoparticles (NPs) make them extremely useful in industry however these properties may also render them uniquely hazardous in nature. Zinc oxide NPs are used in a variety of applications including sunscreens, antibacterial lotions, lipsticks, UV stabilisers in plastics, as a catalyst for chemical reactions and as a food additive. Oligochaetes are important constituents of freshwater ecosystems as they feed on subsurface sediments which have an important role in the processing and recycling of deposited material. In areas where there is a high density of worms, reworking and recycling of the sediment materials can substantially restructure the sediment itself. Due to their feeding behaviour, *Lumbriculus variegatus* make excellent test organisms for studying the bioaccumulation and general effects of contaminants. To date very few published studies have used this species to investigate the environmental effects of engineered NPs. This study investigated the impact of zinc oxide nanoparticles on the mortality, reproduction and behaviour of *Lumbriculus variegatus* following O.E.C.D. protocol conditions. The NPs have been characterised using ESEM, XRD, BET and DLS techniques. Tests were conducted at 0, 1.25, 2.5, 5 and 10mg/L NP concentrations in U.S.E.P.A. media both in the presence and absence of 5mg/L Suwannee River humic acid (H.A.). There was a concentration effect, a particle size effect and an interaction effect between concentration, humic acid addition and particle size. All concentrations were significantly different from the controls and the 1.25mg/L concentration was significantly different in all cases from the 10mg/L concentration. The addition of humic acid did not have a significant effect on the behavioural response when compared to the tests done in the absence of humic acid. There was a significant difference between the bulk and nanoparticle effect.

TH 178

Uptake, subcellular distribution and genotoxicity of nano-CuO, micron-CuO and aqueous Cu in the benthic polychaete, *Nereis diversicolor*

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Engineered metal nanoparticles (NPs), such as Copper oxide (CuO) NPs are increasingly being used in consumer products, and human and environmental exposure is likely to increase. CuO NPs may cause adverse effects as a result of their small size (< 100 nm) and high surface to volume ratio. Engineered metal NPs have been shown to cause organelle damage, DNA-damage, oxidative stress, apoptosis, up/down regulation of proteins etc. CuO NPs released into the aquatic environment will likely agglomerate and/or aggregate upon contact in the brackish water of the estuary and subsequently accumulate in the sediment compartment. Therefore the benthic environment is a likely exposure scenario for CuO NPs. Before CuO NPs can cause potential toxic effects to the organism the particles must be accessible to the organism and able to reach the site of action (e.g., proteins and DNA) either directly or indirectly via the release of ions or production of reactive oxygen species. In this study we examined dietary uptake, intracellular distribution and genotoxicity of Cu added to sediment in different forms and particle sizes for the deposit feeding polychaete *Nereis diversicolor*. Cu was added to sediment as aqueous Cu (administered as CuCl₂ · 2H₂O) or as CuO particles of different sizes; NPs (polydispersed-100 nm) and micro particles (5 µm).

TH 179

Silver nanoparticle dissolution and Ag speciation as key parameters for toxicity of AgNP to algae

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Because of their multiple applications in consumer products silver nanoparticles may reach the aquatic environment and affect aquatic organisms. Toxic effects of silver nanoparticles to aquatic organisms may be either due to the effects of dissolved silver ions or to specific effects of nanoparticles.

Various processes need to be considered to evaluate the fate of silver nanoparticles (AgNP) in natural waters and their effects on aquatic organisms, namely agglomeration of nanoparticles, dissolution and speciation of dissolved Ag(I). Dissolution of AgNP is of fundamental importance, as dissolved Ag⁺ ions appear to be the species directly causing toxicity to algae. However, upon dissolution in the presence of ligands, Ag(I) may be complexed by the ligands. In the presence of cysteine as a binding ligand, increased dissolution of AgNP over a few hours is observed to be followed by a decrease of dissolved Ag(I), probably due to slow reduction processes. Dissolved Ag(I) in the presence of an excess of cysteine is mostly present in Ag(I)-cysteine complexes, and free Ag⁺ is very low. In contrast, in the presence of chloride concentrations lower than the solubility limit of AgCl(s), a slow steady dissolution process of AgNP takes place. Dissolved Ag(I) is in this case partly present as Ag(I)-chloride complexes and partly as free Ag⁺.

Complexation of Ag(I) by fulvic and humic acids appears to be only weak.

Toxic effects of AgNP to photosynthesis of algae are suppressed by complexation of Ag⁺ by cysteine, indicating that toxicity is mostly caused by dissolved Ag⁺ ions. Effects of chloride need to be carefully taken into account in growth media for algae and other organisms, due to the possible complexation and precipitation reactions. Fulvic and humic acids only slightly decrease the toxic effects of Ag⁺, in agreement with weaker binding by these ligands.

TH 180

Investigations on the effects of silver nanoparticles in the zebrafish embryo toxicity test, with consideration of sewage treatment processes

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Due to their antibacterial and desirable material properties, silver nanoparticles (AgNPs) are used in many consumables and everyday products. Critically, evidence is strengthening that AgNP possess a toxic potential, mainly due to the property to continuously release silver ions into the environment. The toxicity of the AgNPs themselves depends greatly on the environmental conditions and the susceptibility of the AgNPs to decomposition. During sewage treatment plant (STP) processes, most of the AgNPs are supposedly retained in the sewage sludge while the ionic silver may be released to the effluent. Both, sewage sludge and effluent can enter the environment, and AgNPs and their derivatives were shown to be present in surface water. The risks of AgNP release for men and environment, however, are still inadequately examined.

This project aims to examine the teratogenic and organotoxic potential of AgNPs and ionic silver (in terms of AgNO₃) to the model organism for aquatic vertebrates, the zebrafish embryo (*Danio rerio*). Thus, fish embryos were exposed to AgNPs and AgNO₃ for 48 h, examined for their lethal and sublethal effects, and subsequently whole-mount immunostained for the determination of motor neuron defects and muscle malformations. Additionally, to monitor the effects of STPs on the toxic potential of AgNPs, samples of sewage sludge treated with AgNPs and the effluents of simulated STP, were examined in the zebrafish embryo toxicity test (zFET).

AgNP- and AgNO₃-treated embryos displayed teratogenic effects such as a delayed development, tail malformations and edema. Most of the embryos were smaller than the control embryos and possessed a strikingly small yolk sac. A specific organotoxic potential was not detectable.

Fish embryo tests for the determination of the toxic potential of AgNPs in sewage sludge led to effects rather ascribed to oxygen deprivation. In contrast, zFETs performed with the effluent of the simulated STP resulted in the characteristic AgNP-mediated effects in higher influent concentrations. Dilutions of the samples indicated an accumulation of ionic silver in the effluents, as an increased AgNP toxicity through STP processes was observed.

This study therefore provides first insights into the toxic potential and behaviour of AgNPs in sewage plant processes.

TH 181

Partial bioavailability of a herbicide adsorbed to carbon nanotubes (CNT) for green algae: comparison of different CNT types with soot, kinetics, and mechanisms

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Carbon nanotubes (CNT) are increasingly manufactured and used [1]. Hence, exposure of our environment with CNT is an eventuality. The effects of CNT on green algae are currently not fully understood. CNT alone seem to be relatively non-toxic for green algae [2]. However, they are strong adsorbents for many organic compounds and may likely reduce their bioavailability. In environment, CNT will always occur together with a great number of organic micropollutants. The aim of our study was therefore to quantify the bioavailability of a herbicide in an aqueous ternary algae-nanoparticle-pollutant laboratory system.

Pristine and oxidized, purified and industrial grade multi-walled CNT (MWCNT) and diesel soot reference material were used. Classical desorption and adsorption kinetics experiments were performed. Ternary batch experiments adapted from [3] were accomplished with mixtures of CNT and diuron in a concentration range covering 2-3 orders of magnitude. The mixtures were pre-equilibrated, then inoculated with an algal stock culture of *Chlorella vulgaris*, and incubated for 24 h. The photosynthetic activity (PS II activity) of the algae and the aqueous diuron concentration were quantified before addition of algae and during the incubation time.

The findings suggest that the overall toxicity of diuron is reduced in presence of CNT due to strong sorption of the herbicide to CNT (Freundlich coefficients ranged from 5.60 to 6.22). However, locally elevated diuron concentration in proximity of algal cells, in combination with fast desorption kinetics of diuron from CNT, lead to partial bioavailability of the herbicide adsorbed to CNT for green algae.

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TH 182

Ecotoxicity of soils contaminated with zinc oxide nanoparticles to aquatic organisms

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The ecotoxicity and behaviour of nanoparticles in the environment is yet almost entirely unknown. Manufacturing processes, handling, use and disposal increase their appearance in air, soil, water and living matter. Nanoparticles can reach the soil through the application of sewage sludge. This agricultural practice has potential for contaminating not only soils but also waters (both surface and ground), since this compartment could be the ultimate container of contamination. The objective of this work is to evaluate whether zinc oxide nanoparticles (ZnONPs), leached from contaminated soil, exert ecotoxicological effects on aquatic organisms. To achieve this purpose, organisms pertaining to different trophic levels representative of the aquatic ecosystem, i.e. the unicellular green alga *Chlorella vulgaris*, the crustacean *Daphnia magna* and fish (in vitro test) have been exposed to extracts obtained from a soil (physical-chemical properties known) spiked with larger particles (zinc oxide), Cl₂Zn and ZnONPs, and the possible noxious effects have been compared. Toxicity to *Chlorella vulgaris* is determined using OECD 201 algal growth inhibition test. Toxicity to *Daphnia magna* is determined using OECD 202 immobilization test. Toxicity to fish is evaluated for in vitro tests, using the rainbow trout gonadal cell line RTG-2 as bioindicator of cell viability, with eight indicator dyes: ethoxresorufin O-deethylase (EROD) for cytochrome, 4-M-umbelliferone (β-gal) for stress cellular, Alamar Blue for energy metabolism, 5-carboxyfluorescein diacetate acetoxymethyl ester (CFDA-AM) for membrane function, lactate dehydrogenase-catalysed conversion of pyruvate to lactate for measuring the inhibition of cell growth (total LDH) and the membrane integrity (LDH released into the medium), 2',7'-dichloro fluorescein diacetate (H2DCF-DA) for oxidative damage, neutral red (NR) uptake for lysosomal activity and total protein assays.

Generated data concerning ZnONPs will be compared with data on larger particles and Cl₂Zn.

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TH 183

Are carboxyl CdSe/ZnS quantum dots taken up by bacterial cells?

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The ability of the engineered nanoparticles (ENPs) to interact with biological barriers is considered as one of the prerequisite for observation of the hazardous effects in the environment. In the present study we examine the interaction of the carboxyl - CdSe/ZnS quantum dots (QDs), as a model of composite water dispersible ENPs and metal resistant bacterium *Cupriavidus metallidurans*, largely used in metal decontamination. More specifically we explored the capabilities of the QDs to cross and/or damage bacterial cell membrane. QDs hydrodynamic diameter, zeta potential and dissolution in the bacterial exposure experiments were also investigated. Multiparametric flow cytometry measurements revealed increasing the fraction of bacterial cells expressing QD-fluorescence in QDs concentration- and exposure time - dependent pattern demonstrated the association of QDs with *C. metallidurans*. Transmission electron microscopy (TEM) images allowed observing electron dense agglomerates and aggregates with a size between 20 and 50 nm on the bacterial surface, periplasmic space and between individual bacterial cells. Energy dispersive spectroscopy (EDS), confirmed that the agglomerate at the surface and in the periplasmic space observed by TEM contains Zn, Cd and Se, major QD components. The presence of the QDs in the periplasmic space of this bacteria was associated with damaging of the cellular barrier as found by the 2.5-fold increase of the cells with damaged and leaky membranes observed by FCM following the QDs-exposure to the bacteria. Overall, this work demonstrates that low levels of QDs originating from incidental release or QDs disposal can be expected to affect bacterial membrane integrity. However given the relatively low percentage of affected cells, they are not expected to significantly influence their growth and thus their use in metal decontamination.

TH 184

Assessing genotoxic activity of nanosized gold, silver, zinc oxide and titanium dioxide using the SOS chromotest

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Gold nanoparticles, silver nanoparticles, zinc oxide nanoparticles, and titanium dioxide nanoparticles are included in various cosmetic products that many consumers use on a daily basis. In this study, we assessed the genotoxic activity of gold, silver, zinc oxide and titanium dioxide nanoparticles using the SOS chromotest. The SOS chromotest based on *E. coli* PQ37 has been used to screen for a variety of chemicals because of its practicality, however, available SOS chromotest data for nanoparticles is currently very limited. This study demonstrated that gold, silver, zinc oxide and titanium dioxide nanoparticles in a tested concentration range are classified as non-genotoxic in the SOS chromotest, evidenced by IFmax values of below 1.5. To the best of our knowledge, these are the first results to study the genotoxic activity of gold, silver, zinc oxide and titanium dioxide nanoparticles with the SOS chromotest. *This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2011- 0015985).*

TH 185

Polyhydroxyl fullerene can mitigate toxicity effects of cadmium on yeasts

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With inception of nanotechnology, nanomaterials are currently in the prime-line of research. Polyhydroxyl fullerene is one of the widely commercialized nanomaterials and applied in electronics and biomedical fields. Unlike fullerene, polyhydroxyl fullerene is known to be biocompatible, and recent studies reported its antioxidant properties. On the other hand, cadmium toxicity has been often attributed to the ability of this metal to induce oxidative stress. To assess antioxidant properties of polyhydroxyl fullerene, the model yeast *Saccharomyces cerevisiae* was exposed to cadmium (≤ 5 ppm, 3 levels) in the presence or absence of polyhydroxyl fullerene (≤ 500 ppm, 3 levels) at different pH values (5.8, 6.3 and 6.8). The size distribution and dispersion of polyhydroxyl fullerene in the stock suspension were measured with DLS (z-average 143 nm, Pdl 0.365). Yeast growth, plasma membrane integrity and accumulation of reactive oxygen species (ROS) were investigated in the presence or absence of Cd^{2+} and/or polyhydroxyl fullerene. Yeast growth was inhibited up to 34% by exposure to Cd^{2+} , but was not affected by polyhydroxyl fullerene. At pH 5.8, the exposure to the highest concentration of Cd^{2+} (5 ppm) and polyhydroxyl fullerene (500 ppm) stimulated yeast growth by 59 and 61% at the exponential and late exponential growth phases, respectively. Growth increased even more when pH increased to 6.8. Severe plasma membrane disruption and ROS accumulation were observed after exposure to the highest concentration of Cd^{2+} in the absence of polyhydroxyl fullerene. Membrane disruption and ROS accumulation decreased with increasing concentration of polyhydroxyl fullerene and with the increase in pH to 6.8. Overall, results indicated that polyhydroxyl fullerene is not toxic to yeasts and may potentially trigger antioxidant defense mechanisms to mitigate metal-induced toxicity.

TH 186

Genotoxicity of cotton cellulose nanofibers

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Cellulose whiskers have potential for applications in the future. These include optical document security and improvement of the mechanical resistance of thin films containing polyelectrolytes in lithium batteries, as well as reinforcement in the polymeric matrices of environmentally-friendly wrapping materials and possible use in medical applications. This work evaluates the genotoxicity of cotton cellulose nanofibers intended for application in the field of new nanocomposites. The emergence of proposals for the application of such materials necessitates detailed study of their toxicity, considering their safety in terms of the environment and living beings, as well as possible modifications of production techniques that could reduce any toxicity, where present. Furthermore, the use of these materials in the health sector could improve biocompatibility, which is an obligatory requirement of regulatory agencies. Nanotoxicological studies aim to ensure the safe application of these materials, hence increasing their acceptance and minimizing any future problems that might be associated with their large-scale usage. This work describes the genotoxicity evaluation of different cotton cellulosic nanofibers (white cotton, brown cotton, ruby cotton and green cotton). In this study we investigate the genotoxicity by the use of *Allium cepa* chromosome aberration test, comet assay, animal cell cytogenetic analysis, clonogenic assay and molecular assay to measure DNA breakdown by the nanomaterials. The results showed that the effects depend on the cotton nanofiber type, being that brown cotton produces more genotoxic effects than others cotton nanofibers. In this way, the results showed that more studies are needed in order to better understand their possible effects, including their environmental impacts when used in agribusiness applications. Supported by: FAPESP, CNPq, CAPES, Fundunesp, Finep and Embrapa (AgroNano).

TH 187

Modelling health effects from inhalation of nano-objects

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There have been many attempts to apply life cycle assessment (LCA) and risk assessment (RA) to engineered nanomaterials, i.e. products embedded with nano-objects (NO). However, the lack of data and modelling capacity for quantifying the fate, exposure and effects of NO currently prevents LCA and RA of nanomaterials to include the assessment of the environmental and health risks as well as the toxic impacts stemming from the releases of nano-objects to environment. To help abridge some of these gaps, we draw on existing knowledge in the field of (nano)toxicology to provide an approach to model the health effects from inhalation of NO, with specific focus on TiO_2 and nanosilver. We apply a physiologically-based pharmacokinetic (PBPK) model to obtain the concentrations of nanosilver and TiO_2 at the different target organs in the human body after inhalation. By using data from in vitro and in vivo studies, we establish dose-response relationships, using the formation of reactive oxygen species (ROS) as a response metrics, and relate the concentrations of NO at the target organs to respective cellular responses. This enables to localize where effects occur in the human body and identify zones, where hazard may arise, based on the hierarchical oxidative stress model. Available data does not enable to translate the cellular responses to a required whole-body response, e.g. incidence of disease. We thus provide recommendations to fill in this gap and we identify important data and modelling components necessary to achieve a full damage modelling; these include the need for chronic tests and methods to extrapolate acute in vitro assay results to chronic in vivo effects for which we discuss opportunities for future incorporation into the developed modelling framework. Overall, the proposed approach thus aims to serve as a first step towards a full assessment of the effects of NO on human health. The relevancy of the work applies to both risk assessment and LCA fields although the combination of the approach with a consistent (environmental) exposure model is still required to support proper risk and impact assessment from nanomaterials.

TH 188

Moving towards quantitative ecological risk assessment of engineered nanomaterials

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Despite the substantial potential of engineered nanomaterials (ENMs) to contribute to sustainable innovation, there is an extensive gap of knowledge regarding both their environmental health and safety profile and the suitable tools and frameworks for assessing their risks. The scientific community has been long discussing the appropriateness of standard risk assessment approaches to apply to ENMs and it has been broadly agreed that, despite the conventional framework is suitable, some implementations are needed to adapt it to their novelties [1]. In response to this need, in the last few years several frameworks and tools aimed to support and speed up the risk assessment of ENMs have been developed, but, with few exceptions, they all lead to qualitative results. In this context, the latest frameworks and tools for Ecological Risk Assessment (ERA) of ENMs have been collated and evaluated with respect to their strengths and weaknesses according to predefined criteria. In result, the development of specific approaches for quantitative effects assessment appears necessary to complement the available toolset and allow low-tier quantitative ERA. In this context, a nanospecific Weight-of-Evidence approach for the quantitative estimation of Predicted No Effects Concentrations (PNECs) has been proposed. This new approach implements important data quality control standards and at the same time can be easily integrated in standard RA procedure. Such tool, however, would require an advanced system for screening and evaluation of the available ecotoxicity database in order to guarantee high quality the input data. Considering that still substantial epistemic and technical uncertainties influence the design and performance of ecotoxicity tests with ENMs, a key aspect in the estimation of reliable and realistic PNECs is the deep understanding of the existing studies. Here we propose an overview of all the available ecotoxicity data found in open-source databases and in peer-reviewed literature, followed by a comprehensive analysis of those aspects related to the data generation that could play a key role in determining the overall quality of the studied endpoints.

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TH 189

Mass balance of nanoscale titanium dioxide in laboratory wastewater treatment plants according to OECD 303A

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Titanium dioxides are used in huge amounts as white pigment in consumer products and industrial applications. The use of nanoscale TiO_2 is predicted to increase in the near future. While the clearance efficiency of microscale TiO_2 ($> 0.7 \mu\text{m}$) in sewage treatment plants (STP) has been determined as being in the order of 70-85% (Kiser et al. 2009), no data on the behaviour of nanoscale TiO_2 in STP are available so far.

In the study presented the fate and behaviour of TiO_2 nanomaterials (P25 with an average particle size < 250 nm) in the laboratory sewage treatment plants simulation test according to OECD Guideline 303A has been investigated. The nanoscale suspensions were stabilised with sodium metaphosphate (SMP) using an ultrasonic homogeniser and added under continuously stirring in a ten-fold concentrated solution via a flexible-tube pump to reach a final concentration of 1, 5, and 10 mg L⁻¹ TiO_2 and 1 g L⁻¹ SMP. The organic synthetic wastewater (final concentration 50 mg L⁻¹ DOC) was added as a concentrate to the matrix of synthetic drinking water. After one week of adaptation the test was run for one week at each concentration. The TiO_2 in the outflow and in the surplus sludge was measured via ICP/OES after HCl, HNO₃, HF digestion.

The functionality of the treatment process in terms of DOC-elimination and nitrification was not affected. The overall TiO_2 balance indicated that 4% (trial 1) and 3% (trial 2) of the total TiO_2 added is discharged with the outflow of the STP and that more than 95% of the TiO_2 is adsorbed to the activated sludge. Glass fiber filtering of samples indicated that the main part of TiO_2 in the outflow has to be attributed to particles below $0.6 \mu\text{m}$. The TiO_2 balance showed a gap of 18% (trial 1) and 37% (trial 2) probably due to the limited analytical recovery rate of TiO_2 from the activated surplus sludge. The OECD Guideline 303A allows enough modifications for assessing the behaviour of nanoscale particles in STP. The calculation of an overall balance of the nanomaterial tested is recommended as a quality control.

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Reference:

Kiser, M. A. et al. 2009. Nanomaterial Removal and Release from Wastewater Treatment Plants. Environ. Sci. Technol., 43 (17), pp 6757-6763

TH 190

Bioaccumulation and effects of sediment-associated silver in different forms for the deposit-feeders, *Capitella teleta* and *Macoma balthica*

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Due to wide spread applications of nanoparticles, including Ag, in our daily life, these novel particles are receiving increasing attention by risk assessors. Many studies have been performed to test the toxicity of nanoparticles at the subcellular level. Despite these findings, there is still limited information at the whole organism level as to whether metallo-nanoparticles differ in toxicity from the same metals added to the environment in ionic or bulk form. In the present study, two organisms (i.e., a marine polychaete, *Capitella teleta* and a marine bivalve, *Macoma balthica*) were exposed to sediment amended with Ag in various forms (ionic Ag(I), nano-sized Ag and micron-sized Ag), and toxic endpoints were assessed at the whole organism level. After exposure for several weeks, no significant toxic effects were detected (i.e., mortality, growth rate and condition index) at the whole organism level, although all forms of metallic Ag were accumulated in our test organisms. Our results thus did not confirm findings from studies performed at the subcellular level that have found the toxicity of Ag nanoparticles was more toxic than the corresponding metallic Ag in ionic and in bulk forms. However, more studies should be performed at the whole organism level to confirm whether the toxicity of nanoparticles can be extrapolated from that of the corresponding metallic ions which have been well studied.

TH 191

Silver nanoparticle toxicity in *Caenorhabditis elegans*

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We tested a number of nanoparticles (NPs) for toxicity in the nematode model *Caenorhabditis elegans*, and found that certain silver NPs were the most toxic of the NPs that we had tested. We therefore extended our studies to characterize the uptake and toxicity of AgNPs in *C. elegans*, as well as the role of dissolution, aggregation, and environmental variables (presence of chloride and natural organic matter in the medium, and sulfidation of the NPs). Using six different AgNPs of different size and coating, we found a linear correlation between AgNP toxicity and oxidative dissolution, but no correlation between size and toxicity. Pharmacological rescue (using N-acetyl cysteine or trolox) and mutant analysis (using mutants sensitive to oxidative stress, *sod-2* and *mev-1* or deficient in metal sequestration, *mtl-2* and *pcs-1*) experiments supported the importance of dissolution in determining the toxicity of AgNPs in *C. elegans*. Some AgNPs (typically less soluble due to size or coating) also cause toxicity via generation of reactive oxygen species, an effect specific to nanoparticulate silver, but this effect was masked by the toxicity of silver ions except when dissolution was very limited. Interestingly, the toxicity of the tested AgNPs was almost never greater than would result from complete dissolution of the same mass of silver. Finally, we found that higher ionic strength, the presence of natural organic matter, and sulfidation of the AgNPs (expected to occur in many environments after AgNP release) all significantly reduced Ag NP toxicity.

TH 192

Impact of zero-valent iron (ZVI) nanoparticles on soil microbial structure and functionality: a molecular approach

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Anthropogenic contamination of soils by heavy metals, such as Pb, and Zn, occurs from many sources such as mining, atmospheric deposition, application of sludge, and mineral fertilizers and pesticides. Over the last decade, the use of binding agents containing nanoscale materials has led to a new generation of environmental remediation technologies that could provide cost-effective solutions to some of the most challenging environmental cleanup problems.

Research has shown that nanoscale iron particles are very effective for the transformation and detoxification of a wide variety of common environmental contaminants, both organic and inorganic. The introduction of nanoparticles (NP) in natural systems (e.g. soil, groundwater, sediments) leads to their interaction with unicellular organisms (e.g. bacteria or protozoa), plants, and animals. Prior to apply a new remediation method, studies must be done to arrive at predictive information of the impact of the process on biota, at different levels of complexity. Before manifestation of acute toxic effects on cell or organism level, the initial changes appear on molecular level. Thus, the elucidation of toxicant-specific molecular responses provides information on toxicity pathways already at subtoxic exposure levels.

In this work, nanoscale zero-valent iron (NZVI) particles have been used as an immobilisation strategy to reduce Pb and Zn availability and mobility in polluted soils. The application of NZVI to two soil microcosms at a dose of 34 mg g⁻¹ soil efficiently immobilised Pb (25%) and zinc (20%). Exposure to NZVI had little impact on the microbial cellular viability and biological activity in the soils. Three bacterial genes (*narG*, *nirS* and *gyrA*) were used as treatment-related biomarkers. These biomarkers ruled out a broad bactericidal effect on the bulk soil microbial community. A transcriptome analysis of the genes did not reveal any changes in their expression ratios after the NZVI treatment: 1.6 (*narG*), 0.8 (*nirS*) and 0.7 (*gyrA*) in the Pb microcosm and 0.6 (*narG*), 1.2 (*nirS*) and 0.5 (*gyrA*) in the Zn microcosm. However, significant changes in the structure and composition of the soil bacteria population were detected by fluorescence in situ hybridisation. Thus, our results showed that NZVI toxicity could be highly dose and species dependent, and the effective applicability of the proposed molecular approach in assessing the impact of this immobilization strategy on soil microbial population.

TH 193

Molecular ecotoxicology of nanosilver guided using in vitro prognosis

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To study the molecular and cellular basis of silver nanoparticle (AgNP) toxicity, we here used a recently established in vitro model of earthworm coelomocytes in comparison to the conventional in vivo molecular ecotoxicology approach. Compared to the latter where the test organisms are exposed to NPs of interest held in an environmental matrix, in vitro models benefit from the ease of controlling exposure conditions in a defined set of biochemical milieus that NPs may encounter.

The AgNPs tested in the present study originated from the same source, but to enhance the colloidal stability in the in vitro test media the NPs were pre-treated with serum proteins. In addition to physical characterisation of AgNPs, the active silver ion fraction was measured (in serum-supplemented cell culture medium and in soil pore-water). Using flow cytometry and atomic absorption spectrophotometry, we show that a specific population of coelomocytes can accumulate AgNPs in vitro and that silver uptake from the soil environment was evident at the organismal level (measured as total accumulated silver).

For the comparison of mode-of-action of AgNPs, we have selected several biomarker genes within stress and immune pathways and assessed their transcriptional interplay at a non-lethal concentration of AgNPs over time in vitro (1-6 hours) and in vivo (1-14 days). In vitro dataset displayed a transition from oxidative stress responses to immune signalling during the 6 hours exposure period. Similarly, a characteristic clustering pattern of oxidative stress response genes was observed in early time points of the in vivo dataset, with stronger association to immune genes at a later time point. Hence, our finding depicts a general response pattern initiating with regulation of stress genes and a subsequent shift to immune genes. This study suggests that AgNPs are likely to be recognised by the earthworm immune system gradually altering the host immune status as the exposure persists.

TH 194

Zero-valent iron nanoparticles for soil remediation

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Soil is a non-renewable resource and needs to be managed sustainably. It is both an important reservoir of chemical elements and a living matrix, and is essential for water and ecosystem health. According the European Environment Agency, in Europe the soil contamination affects almost 250,000 sites, and is expected to continue growing. Human activities such as mining, manufacturing, or land application of urban residues, have resulted in the contamination of our ecosystem, in particular, the soils. Among other pollutants, metals and metalloids can cause significant damage to the environment and human health as a result of their mobility and solubility. So, remediation strategies must be focus on reducing the metal bioavailability in the soil.

The use of zero-valent iron nanoparticles (nZVI) to remediate contaminated soil and groundwater (nanoremediation) has gained increasing amounts of attention within the last decade due to its potential for broader application, higher reactivity, and cost-effectiveness compared to conventional in situ methods. The aims of this study were, firstly, evaluate the efficiency of the remediation with nZVI to immobilize lead and zinc in two soils (acid and alkaline), and, secondly, determine the influence of this nanoremediation technology on physico-chemical and biological soil properties.

Soils were artificially contaminated with lead or zinc (200 mg/kg) and treated with commercial nZVI suspension (NANO FER 25S, NANO IRON, Rajhrad, Czech Republic). The bioavailability of these metals in soils was determined after a sequential extraction procedure, analyzing soluble, exchangeable, linked to carbonates and residual fractions.

In the studied conditions, the amendment with nZVI was more effective for lead than for zinc, regardless of soil pH. The immobilization process in the alkaline soil was favoured by the carbonate content. An increase of immobilized Pb of 81.6% and 29.2% for acid and alkaline soil, was observed, respectively; and in the case of zinc, the immobilization increased by 51.0% for the acid soil and 8.3% for the alkaline one. Soil properties were not adversely affected after treatment with nZVI. These results suggest that the use of nZVI to remediate contaminated soil with lead or zinc can be proposed as a promising alternative strategy to others amendments.

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TH 195

Phytotoxicity of nano-TiO₂ and effect of humus preparation

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The objective of this study was to determine the bioactivity of nano-TiO₂ and to assess their toxicity in presence of humic substances (HS) which are natural components of soil necessary for higher plants growth.

In this work we applied metal-containing nanomaterials - nanodioxide titanium (nano-TiO₂), which are characterized <75 nm in size of particles in 10 weights % of water suspension, manufactured in the company «Sigma-Aldrich», USA. Nano-TiO₂ represents oxide the titanium (IV), the mix of two known crystal modifications of dioxide titanium - anatase and rutile.

Among HS we have chosen «Pow-Humus» (Le-PhK) (K-humate, originated from leonardite), manufactured by German firm 'Humintech'.

Concentration of nano-TiO₂ was varied in range 5-500 mg/l; concentration of humate Le-PhK was 5 mg/l in water medium.

Bioactivity of preparations was examined by early seed root elongation assessment related to *Brassica juncea* L. as a test-organism. We have used a modification of the method «Phytotoxkit - Seed germination and early root growth», developed by Belgian scientists [http://www.microbiotests.be]. The probe is considered toxic if the deviation from the control of average roots length is 20 and more %.

Depressing effect of nano-TiO₂ on early root growth (4 days of germination) was marked. The inhibition of root growth was showed already at the low concentration (5 mg/l). The most effect was shown at the higher concentration: probe with nano-TiO₂ 500 mg/l was toxic because the deviation of average roots length was more than 25 % from control.

Inhibitory effect of nano-TiO₂ was reduced in presence of humate Le-PhK 5 mg/l. Samples with nano-TiO₂ 500 mg/l after humate application was qualified as nontoxic because the deviation of average roots length was only 14 % from control.

The obtained data has shown that: 1) higher plants are sensitive to the nano-TiO₂ presence in growing medium 2); the degree toxicity of nano-TiO₂ depends on the concentration of nanoparticles; 3) humus substances eliminate the toxic effect of nano-TiO₂ in water suspension which inhibits root growth.

Further research is required for the evaluation of humus substances influence on the nanomaterials which are more and more widely distributed in the environments including the natural soils [1; 2].

TH 196

Gene expression profile of *Enchytraeus albidus* (Oligochaeta) in response to silver nanoparticles and silver nitrate

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Due to its antimicrobial activity, silver nanoparticles (Ag-NPs) are among the most used NPS worldwide, yet little information is available regarding their effects, particularly in soil dwelling organisms. Enchytraeids (Oligochaeta) are important members of the soil fauna which actively contribute to the acceleration of organic matter decomposition and nutrient recycling processes. Hence, for hazard and risk assessment it is important to provide toxicity data for these organisms, and if possible to understand more in regard to the mode of action of AgNPs within organism. To study this we conducted toxicity experiments using the OECD standard test, testing AgNPs and AgNO₃, and relating this to Ag⁺ activity of the soil water solution. Population toxicity responses were assessed showing higher toxicity for the Ag-salt. In an attempt to understand the mode of action we performed transcription profiling using the microarray. Organisms exposed to similar total concentrations (AgNPs and Ag-salt) showed different gene-expression patterns. The observed differences in toxicity between the two exposure scenarios could not be explained by dissolution of the AgNPs, as measured Ag⁺ activity (Ion selective electrode) in the soil water solution.

TH 197

Role of p38MAPK in ROS induced DNA damage & apoptosis due to silver nanoparticle exposure in *Caenorhabditis elegans* and human cell line: comparative toxicity approach

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Toxicity of nanomaterial studies are among the fastest growing in environmental toxicology research, because of the widespread nanotechnology. In vitro systems are widely used to derive toxic potentiality of nanomaterials. As it is complex to extrapolate the derived toxicity data of NPs from in vitro systems to humans, use of alternative in vivo model system is unavoidable to get an actual picture for human toxicity. In this study, using the nematode, *Caenorhabditis elegans* as in vivo model system, we conducted comparative toxicity of silver nanoparticle (AgNPs). In our previous study, p38 MAPK mediated oxidative stress was observed as a toxicity mechanism in *C.elegans* and human lymphoma cell, Jurkat. In the present study, to find out the link between oxidative stress, DNA damage & apoptosis in *C.elegans* and human lymphoma cell, Jurkat, we hypothesized that the stress responsive protein, p38MAPK (PMK-1, a homologue in *C.elegans*) is conserved and unavoidable for toxic responses due to AgNPs exposure. Our approach is to investigate the role of p38MAPK *pmk-1* in oxidative stress, DNA damage & apoptosis by using p38 siRNA knock down in mammalian in vitro system and/or using *pmk-1* mutant *C.elegans* strain followed by AgNPs exposure and determine the distinguishable toxic end points. This study highlights the importance of comparative toxicity approach in extrapolation of in vitro nanotoxicity data for human and ecological health evaluation.

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TH 198

Toxicity of ZnO nanoparticles on soil receptors compared with bulk ZnO and dissolved free ionic zinc

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Due to increasing proliferation of nanotechnology in industrial applications and consumer products, there has been growing concern over effects of nanoparticles (NPs) on the environment. This study aims to study the effects of ZnO nanoparticles on soil receptors.

The adverse effects of NPs cannot be predicted from the bulk material as result of their small size and unique structure which give to nanoparticles very specific properties: chemical, biological and physical which affect their toxicity and environmental behavior. This work compares the ecotoxicological effects of three different forms of Zn²⁺ (nanoZnO, ZnO bulk, and ZnCl₂ salt) using different lethal and sublethal endpoints. Biological effects of nanoZnO, ZnO bulk, and ZnCl₂ salt at 1000 mg Zn²⁺ kg⁻¹ soil (d.w.) concentration were assessed on three taxonomic groups: terrestrial invertebrates, plants and microorganisms. The measured effects were: lethal, body weight alterations and reproduction endpoints in *Eisenia fetida*, germination and growth in three terrestrial plant species and two enzymatic activities (phosphatase and deshydrogenase) and two soil functions (nitrification and respiration) in soil microorganisms.

Bioaccumulation is other parameter depending on Zn forms present in the soil. In this work, accumulation of zinc in earthworms exposed to contaminated soils during 28 days was determined. In plants accumulation was studied in roots and shoots to determine effects of translocation. Differences in Zn uptake among plant species and fate of the different Zn forms studied will be reported in both earthworms and terrestrial plants.

In addition chemical soil analyses were performed before and after toxicity assays, using different extractants, to determine the distribution of the Zn forms in the different soil fractions.

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TH 199

Mixture toxicity of nano-TiO₂ and ingredients of personal care products

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In the last years many studies have focused on the ecotoxicology of nano-TiO₂. But only a few studies have investigated the mixture toxicity of nano-TiO₂ and organic contaminants. Mixture toxicity has to be considered during risk assessment of nano-TiO₂, because both substance groups often have the same entry pathway into the environment. Nano-TiO₂ and biocides are both ingredients of personal care products. Consequently, they have the potential to be released into waste water treatment plants and subsequently through biosolid amendment into soils.

Triclocarban (TCC, 3, 4, 4'-trichlorocarbanilide, CAS 101-20-2) is an antibacterial agent used in personal care and household products. It was identified as an emerging contaminant in biosolids and subsequently in biosolid treated soils.

In this study the mixture toxicity of nano-TiO₂ and TCC to terrestrial organisms is examined. The acute and chronic toxicity of nano-TiO₂ (10-1000 mg/kg dw) and TCC (42-675 mg/kg dw) as single substances as well as a mixture of both (TCC 42-675 mg/kg dw together with either 400 or 1000 mg/kg dw of nano-TiO₂) to *Eisenia fetida* is tested in accordance to OECD 207 (1984) and OECD 222 (2004). TCC is analyzed in soil samples and worm tissues using liquid chromatography tandem mass spectrometry (LC-MS/MS).

No mortality was observed for *E. fetida* exposed to TCC. However in the acute toxicity test TCC decreased the biomass of *E. fetida* in a dose dependent manner up to 20% after 14 days. In our study TCC showed a lower acute toxicity to *E. fetida* as found in a study from Snyder et al. (2011, LC₅₀ 40 mg/kg). Nano-TiO₂ did not show any acute toxicity to *E. fetida* up to 1000 mg/kg dw. Further tests will show if the simultaneous exposure of *E. fetida* to both substances will lead to mixture toxicity on basis of acute or chronic toxicity. Results will be presented in the poster presentation.

The investigation of mixture toxicity of nanomaterials and organic contaminants is important because only little is known about potential interactions and the related consequences for e.g. bioavailability or formation of toxic metabolites.

TH 200

The influence of soil pH on the toxicity of ZnO nanoparticles to the isopod *Porcellionides pruinosus*

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The stability of nanoparticles (NPs) in soils is known to be affected by soil properties, like pH, which can change NP surface charge and zeta potential. Consequently, soil pH influences the interactions between NPs and soil matrix, defining NP behaviour and bioavailability. In the present study, the effects of zinc oxide (ZnO), as NPs and non-nano sized particles, and ZnCl₂ were studied in the isopod *Porcellionides pruinosus* upon exposure in a forest soil with different pH values. The forest soil presented a pH of 4.5 and was amended with calcium carbonate (1 w/w %), in order to achieve an extra soil pH of 7.3. Soil was then spiked with ZnO NPs (30nm), non-nano ZnO (200nm) or ZnCl₂, resulting in nominal concentrations of 250, 500, 1000, 2000 and 4000 mg Zn/kg dry soil. Adult isopods were exposed individually, in 10 replicates per treatment and a control for each pH set. Animals were fed with alder leaves *ad libitum*. After 14 days, mortality, feeding inhibition and biomass gained were calculated. The pH_{CaCl2} of the soil was measured at the beginning and end of experiment. The results showed that Zn concentration was affecting soil pH, but in different ways depending on the Zn used. At pH 4.5, increasing Zn concentrations led to a pH increase in the soil spiked with ZnO 30 and 200nm, and a decrease in soil spiked with ZnCl₂. At pH 7.3, for all treatments soil pH decreased with increasing Zn concentration. The 14 days-LC50 values found at pH 4.5 were 1945 and 3718 mg/kg for ZnO 200nm and ZnCl₂, respectively, and it was not possible to calculate the LC50 for ZnO 30nm. At pH 7.3, the LC50 values were 1687, 1029 and 1285 mg/kg for ZnO 30nm, ZnO 200nm and ZnCl₂, respectively. For the feeding activity, no effects were observed at pH 4.5 for any of the Zn particles or salt. However, at pH 7.3, a decrease in consumption was observed at concentrations of 2000, 1000 and 500 mg/kg for ZnO 30nm, ZnO 200nm and ZnCl₂, respectively. Biomass gained was affected upon exposure to all Zn forms, with EC50 values of: 757mg/kg for ZnO 30nm, 584 mg/kg for ZnO 200nm and 315 mg/kg for ZnCl₂, at pH 4.5. On the other hand biomass gain did not differ from the control for any treatment at pH 7.3. The toxicity of NPs seemed to be influenced by soil pH, and in a different way from the correspondent ionic Zn exposure based on ZnCl₂ salt.

TH 201

Effects of different TiO₂ nanoparticles on growth of *Rhizobium trifolii* in liquid culture

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Nanoparticles (NP) gain importance due to possible widespread use and great potential also in agricultural applications. Little is known on how NPs interact with plants and beneficial soil microorganisms. Several studies have shown, that some NP can enter plant roots and are spread within the plant through the vascular system. NP show also effects on bacteria - like inhibited growth, disrupted cell membrane and shift in bacterial community - and can be taken up by them. Thus, before applying NP in agriculture, e.g. as pesticides or fertilizers, it is important to test whether they may negatively influence crops and beneficial microorganisms.

In a first step the influence of different NP on the nitrogen-fixing bacterium *Rhizobium trifolii* is tested under highly controlled conditions using a liquid medium as model system. We focus on the effects of NP on nitrogen-fixing bacteria because these bacteria play a key role in sustainable agriculture, providing up to 200 kg N per hectare per year. Here we compared the effect of four different TiO₂ NP, which differ in the crystal structure or in the coating, on the growth of *R. trifolii*. Two of these particles consist of rutile and have an AlO₃ dimethicone (silicon based organic polymer) or glycerol coating respectively, one is made of 100% anatase and the forth one contains 80% anatase and 20% rutile without coating.

Initial results with two TiO₂ NP - one with and one without coating, with concentrations between 200 mg/l and 1 mg/l - indicate that the different particles show similar effects on the growth of *R. trifolii*. Applied at concentrations between 10 mg/l and 1 mg/l, similar to those possibly used in practice, the two TiO₂ NP did not show any effects on *R. trifolii* growth as compared to controls without NP.

The objective of further experiments will be to assess possible effects of NP on two crops (clover and wheat) and on two widespread groups of beneficial soil microorganisms, rhizobia and arbuscular mycorrhizal fungi.

TH 202

Impact of sewage sludge spiked with silver nanoparticles to soil microorganisms

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The harmful effects of silver nanoparticles to microorganisms are commonly accepted. The application of contaminated sewage sludge to soil is the main potential pathway for the input of silver nanoparticles in soil. Several studies have shown that silver nanoparticles, which pass through a wastewater treatment plant, have been mostly modified into argentite (Ag₂S) and thus, will have a very small solubility and therefore a minor toxicity compared to silver nanoparticles. But is this sufficient to claim that there will be less danger from such contaminated sludge?

In our study we contaminated activated sludge with silver nanoparticles (AgNP) and silver nitrate (AgNO₃), both with 1mg Ag/L for 8 days under aerobic conditions by air supply in batch reactors. Subsequently, this sludge was applied to soil and afterwards mixed with grass seed and stored in pots in a greenhouse for 2, 4 and 6 months.

Additionally, we conducted two control treatments, a sludge-control and a total-control treatment, respectively. During the experimental period a series of soil parameters was analysed, such as microbial biomass-C, soil respiration, enzyme activities, phospholipid fatty acids, C-to-N ratio, and pH.

Our results show that - independent from any possible detoxification due to the conversion of silver nanoparticles in the sewage treatment plant to argentite - an unknown fraction of high toxicity particles remained. As a consequence, AgNP- and AgNO₃-treatments differed significantly from both control treatments over a number of months and inhibited the microbial community up to 30%.

TH 203

Do nanoparticles influence cell physiology and/or function?

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Nanoscience is expanding in many fields of technology worldwide, although research on toxicological effects of these nanoparticles (NP) has just begun. To date, existing OECD guidelines are evaluated for their applicability to test the toxicity of NP. A preliminary review indicates that the tests are suitable for NP, but that sample preparation and characterization have to be described in detail. In several studies previously performed according to those guidelines, we found neither acute nor chronic reproductive or growth effects of multi-walled carbon nanotubes (CNT) on whole organisms. However, CNT were found to be incorporated in e.g. fish gut epithelial cells and algae. In the case of CNT, it is questioned if there are analogous mechanisms to those of other fibrous particles such as asbestos, which penetrate into the lung and may persist in the tissue. The environmental expert panel of the German government stated (2011) that this question especially refers to carbon nanotubes that are long and rigid. The material used in this study is however known to be short and tangle. So far uptake of CNT in cells has been observed but the relevance of this finding is still unclear. Therefore, the possible penetration in cells has to be investigated. The aim of the present study is to investigate in vitro responses of different cells after exposure to CNT. Firstly, general cytotoxicity of CNT is investigated using three different endpoints: neutral red retention, enzyme quantification (lactate dehydrogenase) and microscopic analysis on cell density and sublethal effects. Secondly, potential endocrine effects were studied using the yeast estrogen screen assisted by enzymatic digestion with lyticase (LYES). Thirdly, the single cell gel electrophoresis assay (comet assay) is performed as a straightforward and sensitive technique for the detection of DNA damage. Finally, oxidative stress possibly caused by CNT was determined since many NP were shown to induce biological effects intracellularly. In addition to the tests with CNT material only, combination effects of CNT with triclocarban (TCC) and/or its metabolic products (3,4-dichloroaniline) are investigated in cell line tests. This work should provide more information about interactions of CNT- and combination effects of CNT- with organic contaminants at the cellular level.

TH 204

Uptake routes of silver nanoparticles in earthworms

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This study aims at quantifying the contribution of dermal and oral uptake of silver nanoparticles (AgNP) in earthworms. With the greater production and incorporation into consumer products, it is expected that increasing amounts of AgNPs will be released to the environment. These NPs will end up in soil through wastewater and sewage sludge or may directly be deposited on land, thus being of environmental relevance for terrestrial ecosystems. To assess their risk in soil, besides their dissolution and speciation it is important to investigate the mechanisms by which the different metal species (single nanoparticles, aggregates and metal ions) interact with biological membranes and enter the body of the organisms exposed. Earthworms are representative test organisms for ecological risk assessment. They will be exposed to contaminants in soil both through the skin (dermal) and by ingestion (oral). To assess bioavailability of metal nanoparticles in soil to earthworms, it is necessary to know the contribution of each pathway.

For that reason, experiments will be performed with the earthworm *Lumbricus rubellus*, applying oral sealing with medical histoacryl glue to block the ingestion of soil particles. Glued and unglued earthworms will be exposed to Lufa 2.2 soil spiked with 100 and 500 mg kg⁻¹ dry soil AgNPs for 7 days. An exposure of glued and unglued earthworms to soil spiked with 20 and 100 mg kg⁻¹ AgNO₃ will also be included to address the interaction of silver ions with earthworm tissues. By measuring total silver body concentrations at different sampling times in both exposure scenarios it will be possible to assess the contribution of the different routes of exposure. The distribution of silver in tissues of exposed earthworms will be mapped by transmission electron microscopy (TEM) and synchrotron X-ray microanalysis.

TH 205

Silver nanoparticles ecotoxicological assessment: Novel electrophysiology biomarker and the potential role of P-glycoprotein in modulating toxicity in earthworms and aquatic oligochaetes

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Silver (Ag) nanoparticles (NPs) are the fastest growing NP class. AgNPs are a new dimension of pollutants in the environment. A novel, non-invasive electrophysiological technique was used to compare the ecotoxicity of AgNP with that of silver nitrate (AgNO₃) by measuring conduction velocity (CV) in the medial giant fibres (MGF) of earthworms and aquatic oligochaete worms. In order to determine the sub-lethal dosages for the CV experiments, classical 48-h LC50 and LC10 values were determined for juvenile earthworms (*A. caliginosa*) and the aquatic worm (*L. variegatus*). The 48-h LC10 values for adult and juvenile earthworms exposed to AgNP in filter paper studies were 7.1 and 2.5 µg cm⁻² respectively and the corresponding values on exposure to AgNO₃ were 17.1 and 7.9 µg cm⁻². The 48-h LC10 values for AgNP and AgNO₃ in *L. variegatus* in water were 81 and 107 µg ml⁻² respectively. Based on the LC10 and LC50 values, AgNP was 2- to 3-fold more toxic than AgNO₃ and this was most marked in the juvenile earthworms. The CV in the juvenile earthworm *A. caliginosa* were measured at 0, 1, 3, 6, 8, 24, and 48 h following exposure to AgNP and AgNO₃

concentrations of LC0.001, LC0.01, LC0.1, LC1, LC2, and *L. variegatus* to concentrations of LC1, LC5, LC10, and LC20, with distilled water as the negative control. MGF CV decreased progressively in *L. variegatus* and to a lesser extent in *A. caliginosa* exposed to both AgNP and AgNO₃ up to 8 h and then the CV gradually increased. This could have been due either to degradation of Ag compounds or an adaptation mechanism by *L. variegatus*. To eliminate possible degradation of the Ag compounds, the AgNP and AgNO₃ solutions were renewed every 3 h and CV measurements taken at hourly intervals for 7 h. Based on the results of these studies and our experience with P-glycoprotein (P-gp) transporters in oligochaetes (not reported here), it is proposed that the oligochaetes adapt to excessive exposure of AgNP and to a lesser extent to AgNO₃ via the elimination of chemicals via P-gp transporters. In conclusion, MGF CV measurements in the earthworm *A. caliginosa* (used to monitor soil pollution) and the aquatic worm *L. variegatus* (to monitor aquatic pollution) can be used as a non-invasive, sensitive, and early-warning biomarker of exposure to NP but interpretation of chronic exposure requires further evaluation in view of the role of P-gp transporters in the excretion of excess chemicals from the body.

TH 206

A weight of evidence approach for occupational risk assessment of engineered nanomaterials

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It has been recognized that substantial limitations and uncertainties make the conventional RA infeasible to apply to ENMs today, which leaves regulators with little support in the near term. Knowledge gaps have been gradually filled by new research results and uncertainties have been reduced, but this process advances slowly and it will take decades [1], while quantitative risk assessment results are needed to support near-term risk management actions and regulatory decisions. In response to this need, a number of methodologies and tools to assess the risks from ENMs, in spite of the limitations, have been proposed. Most of them, however, are not intended to facilitate regulatory decision making, but instead to serve as preliminary hazard/risk screening and/or research prioritization tools. The present deficit of quantitative data and scientifically sound approaches will lead in the near and in the medium terms to uncertain and ambiguous, largely qualitative risk estimations based on expert judgments, which may fail to support proper risk management actions [1]. In this context a quantitative model for occupational RA and prioritization of ENMs, including probabilistic uncertainty evaluation, has been developed within the FP7-funded ENPRA project. Based on the conventional RA paradigm, it uses effects dose-response data and exposure measurements to rank and prioritize nanomaterials for further testing (in a lower tier) and qualitatively estimate occupational human health risks (in a higher tier). In order to ensure optimal integration of all available data our approach implements some non-conventional tools like the Weight of Evidence and the Multi Criteria Decision Analysis. The model has been applied to a number of commercially available ENMs considered as case studied in the ENPRA project, i.e. titanium dioxide, zinc oxide, silver and multi-walled carbon nanotubes. In order to test its stability and ensure the robustness of the assessment results, probabilistic sensitivity analysis has been performed using the Monte Carlo approach.

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TH 207

Toxicity assessment of nanoclay Clay2, on human hepatic cell line HepG2

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Nanoparticles (NPs) attract a great deal of attention due to their unique properties. Their high surface to volume ratios, specific chemical composition, surface structure and solubility, shape and aggregation results in materials that are qualitatively different from their bulk counterparts. These properties make them suitable for numerous applications in existing and emerging technologies. The most promising uses of NPs in the food industry are in food packaging. Dispersion of NPs in polymers increases the barrier function of plastics by creating a tortuous pathway for diffusing molecules, increasing shelf life and nutritional quality of food. Having these advantages, using NPs in food packaging seems inevitable. Since many packaging is wasted carelessly, a huge amount of these nanoparticles containing compounds might end up in the environment. As well as, possible migration from the packaging to the food product could also lead to human contact with the NPs. As earlier mentioned, the small size of NPs gives them unique properties, which includes a possible increased toxicological risk. Therefore, each nanoparticle requires its own risk assessment. In this work, the main objective was to study the in vitro effects of a newly developed nanoclay, Clay2 which is meant for use in food packaging, in order to understand the mechanism of action causing toxicity. A range of concentrations of Clay 2 (0-150 µg/mL) have been investigated in the human liver cell line (HepG2). Different assays (total protein content, neutral red uptake, and MTS reduction), were performed, and the results showed a reduction of cell viability with an EC50 of 88 µg/mL. Moreover, oxidative stress, genotoxicity and inflammation responses were explored.

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TH 208

Characterization of aqueous metal nanoparticles and assessment of exposure on immune cell viability and effector function

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Nanotechnology is an emerging multidisciplinary field that involves the synthesis of molecules in the nanoscale (<100nm). The small size of nanoparticles (NP) produces unique physico-chemical properties that are different from their larger bulk forms. Although impressive from a physico-chemical perspective, there is growing concern over the potential and unknown toxicity of these particles on biological systems. Since NPs can differ in their physico-chemical properties, their behavioural characteristics in solutions are typically assessed together with toxicity measurements. Thus, dynamic Light Scattering was used to characterize five aqueous metal oxide polyacrylic encapsulated NPs (TiO₂, CeO, ZnO, Fe₃O₄ and a polyacrylic nanocapsule void of a metal oxide core) in our test cell media solutions. Results indicated that agglomeration was occurring between particles that increased their hydrodynamic size from their reported 5-9 nm to more than 120 nm. To measure immunotoxicity, RBL-2H3 (RBL) mast cells were exposed to NP concentrations ranging from 0, 1, 10, 50, 100 and 200 mg/L over 48 hours and were analyzed for cellular viability and proliferation using flow cytometry. Propidium iodide was used as a fluorescent marker for cell death. Results indicated a dose-dependent decrease in viability with increasing doses of some NPs. Significant changes in cell viability were only observed after two hours of exposure. We next examined the influence of NP exposure on RBL FCε receptor-mediated degranulation by dosing RBLs with both sub-lethal and lethal doses and showed that degranulation was inhibited at all doses including those that did not decrease cell viability. The IgE antibodies used to sensitize RBLs for degranulation were also exposed to several concentrations of NPs to examine the effects of NP-IgE binding on degranulation. Antibody binding affinity assays and cell fluorescent microscopy was used to supplement the results of both cell and IgE NP exposure experiments in order to demonstrate the role NPs have on influencing FCε receptor and IgE activated degranulation.

TH 209

Cellular and genomic effects of nanoparticles on human cells

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The use of nanomaterials is spreading in commercial and industrial activities. However, the interactions between these products and living systems is largely unknown. Particularly, the assessment of environmental and human risks are lagging far behind the continuous development of new engineered nanomaterials, while literature data suggest that these materials represent potential environmental and health hazards. We studied the impact of nanostructured composites on human cells, by evaluations of various kinds of toxic effects and gene expression profile by DNA microarray analysis. Cells have been incubated with two different nanomaterials, which are possible candidates to develop nanocomposite products, such as Cloisite and Carbon Nanotubes (NC7000). The experiments have been carried out on HaCaT cells, representing an in vitro model of human epidermis. The analyses have been carried out also on a mixture of Cloisite and Carbon Nanotubes. Cytotoxicity analysis of this combination on HaCaT cells was carried out by using the MixTox approach and it has revealed a biphasic effect of the binary mixtures. In fact at high concentration there is a synergistic effect but at low concentration an antagonistic effect is evident. To study this phenomenon a transcriptomic approach was used to clarify the molecular changes that led to antagonistic effects of the NOEC concentration of the mixture of the two nanomaterials. Genomic effects have been explored using a 60 k microarray. HaCaT cells were exposed for 24h alternatively to 0.5 µg/ml Cloisite, 10 µg/ml Carbon Nanotubes, or to a mixture of the two components, each at half dose. The results demonstrate that in Cloisite exposed cells 453 genes are differentially expressed, 262 in the cells treated with nanotubes but in HaCaT cells exposed to their binary mixture there are only 263 genes differentially expressed. These data confirm an antagonistic interaction of the two components. Using a blast 2go approach, the main cell functions affected by the two nanoparticles and their mixture have been also evaluated. In conclusion, by taking HaCaT as a models of human epidermis, it was possible to established that Carbon Nanotubes appear to be safe nanocompounds, whereas in contrast Cloisite is the most toxic ones. However, Carbon Nanotubes in combination with Cloisite may produce a lower impact on cellular systems with respect to the sum of single agents at least when present in the environment at sublethal concentrations.

TH 210

The basic safety assessment of fullerene C60 after oral administration

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Summary: More recently, the development of nanomaterials with particle sizes below 100 nm is promoted extensively. These nanomaterials have been already used in various applications such as foods, medicines and cosmetics, and become essential to our daily life. Especially, fullerene C60 is one of the most promising nanomaterials as foods and medicines because of the unique chemical and physical properties. However, knowledge concerning the potential safety of fullerene C60 on human health and the environment is still fragmentary. Therefore, safety assessment of fullerene C60, following oral administration, is very important for safety assessment of food environment. Here, we

examined the safety of fullerene C60 by oral administration in mice. BALB/c mice were orally treated with 50 mg/mouse of polyvinylpyrrolidone (PVP)-enwrapped fullerene C60 (PVP-fullerene C60), which is water-soluble fullerene, once daily for 7 consecutive days. During exposure of PVP-fullerene C60, the mice were weighted. No statistically significant differences were noted in mean body weights in the PVP-fullerene C60-treated group when compared to the control group. To further evaluate the safety of PVP-fullerene C60, we examined the hematological parameters and plasma biochemical parameters. No significant hematological change was observed in PVP-fullerene C60-treated mice. In addition, PVP-fullerene C60-treated mouse have little change in the plasma levels of liver injury parameter including AST and ALT, and renal injury parameter including BUN compared to those of control mouse. These results suggest that PVP-fullerene C60 would have no effect in oral acute high dose administration and indicate that the PVP-fullerene C60 could be safe nanomaterials for oral administration. We believe that these data provide basic information that should help to create safe and effective forms of fullerene C60.

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TH 211

In vitro effects of amorphous nanosilica particles on the bone metabolism

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Summary: Recently, applications of amorphous nanosilica particles with a controlled particle size below 100 nm (nSPs) have been increasing in oral care products. nSPs exert various beneficial functions as a polishing agent and/or as a remineralization promoter for tooth, based on its unique physicochemical properties. Because high concentration of nSPs contents in oral care products (e.g. about 20%), for example, tooth paste, and we use them everyday, we are not able to avoid oral exposure of nSPs in living environment. Adversely, there are growing concerns about the possibility that unique physicochemical properties of nSPs induced health risks. However, there is little information about safety of nSPs in the context of oral exposure. Therefore, it is urgent need to identify the hazard of nSPs in oral cavity. In this study, we tried to evaluate the effects on osteoclast differentiation induced by nSPs in vitro, assuming the effects on alveolar bone metabolism, because parodontal tissue including teeth is one of the most important organs in oral cavity. Using in vitro osteoclast differentiation model, RAW267.4 cells treated with receptor activator of nuclear factor –B ligand (RANKL), osteoclast differentiation was assessed by tartrate-resistant acid phosphatase (TRAP) staining and activity. As the results, it was revealed that at doses that did not induce cytotoxicity by nSPs, TRAP activity and the number of osteoclast cells (TRAP-positive multinucleated cells) by nSPs treatment were equivalent to non-treatment group in the presence of RANKL. Collectively, the results suggested that nSPs exerts no effect on RANKL-induced osteoclast differentiation of RAW264.7 cell at the dose of this study. Now, we are examining the effect of nSPs on osteoblast differentiation as next step to evaluate effects on bone metabolism. We believe that applications of nSPs will extend to these new fields following further our careful safety study considering the realistic exposure amounts of nSPs. Moreover, we wish in vitro test like this is established as the alternative method of in vivo safety test in the future in consideration of animal welfare.

Acknowledgement: This study was supported in part by Health Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan.

Reference: Nabeshi H. et al. : Effect of amorphous silica nanoparticles on RANKL-induced osteoclast differentiation in murine macrophages. *Nanoscale Res. Lett.*, 6(1):464-468, 2011.

TH 212

Surface interaction of coagulation factor and amorphous nanosilica particles plays critical role for acute toxicity

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Summary: Recently, the development of nanomaterials (NMs) with particle sizes below 100 nm is promoted extensively. These NMs have been already used in various applications such as cosmetics, medicines and foods. Thus, the exposure against NMs is unavoidable for us in our living environment. Under this circumstance, there is increasing concern regarding the potential health risks by the unique mechanical properties of NMs. In most cases, however, the safety evaluation of NMs has been insufficient for ensuring their safety. In this regards, using amorphous nanosilica particles (nSPs), we are studying nSPs-induced hazard identification and qualitative evaluation of exposure level, such as in vivo/in vitro distribution analysis by transmission electron microscopy. In previous study, we examined in vivo distribution of dermal administration of nSPs with particle sizes below 100 nm, because nSPs were used in cosmetic products. As a result, nSPs with particle size 70 nm (nSP70) penetrate skin barrier and move to bloodstream after dermal application. These findings mean that it is essential to identify the biological effects after systemic exposure to nSPs. In this study, we examine the biological effects after intravenous injection of nSPs. BALB/c mice were intravenously injected with nSPs of sizes 70, 300, 1000 nm and then assessed for survival, blood biochemistry and coagulation. As a result, injection of nSP70 caused fatal toxicity, liver damage, and platelet depletion, suggesting that nSP70 caused consumptive coagulopathy. Additionally, nSP70 exerts procoagulant activity in vitro, which was diminished in Factor XII-deficient plasma. Collectively, we revealed that interaction between nSP70 and intrinsic coagulation factors such as Factor XII, were related to nSP70-induced harmful effects. In other word, it is suggested that if interaction between nSP70 and coagulation factors can be suppressed, the safety of nSP70 may be ensured. Now, we evaluate quantitatively hazard effect and exposure level of nSPs in the realistic exposure pathway, such as oral or nasal route. These results would be useful for the safety/risk assessment and evaluation of NMs.

Acknowledgement: This study was supported in part by Health Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan.

TH 213

Nanoparticle fate assessment and toxicity in the environment - Initial findings from phase 1 of the NanoFATE project

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This presentation will outline the main findings from the first 2 years of the EU FP7 project NanoFATE. The NanoFATE project was conceived to fill knowledge and methodological gaps currently impeding sound assessment of environmental risks posed by engineered nanoparticles (ENPs). Our vision is to assess environmental ENP fate and risk in example high-volume products for which recycling is not an option, namely: fuel additive, personal care and antibacterial products. Two market ENPs from each product (CeO₂, ZnO, Ag of varying size, surface and core chemistries) is being followed through their post-production life cycles i.e. from environmental entry as "spent product", through waste treatment to their final fates and potential toxic effects. This will test the applicability of current fate and risk assessment methods and identify improvements required for assessment of ENPs at an early stage.

NanoFATE focuses on developing a systematic understanding of fate and mechanisms of effects in a core set of ENPs and addressing how these may affect the application of current tools for ecological risk assessment. The ENPs we study are associated with commonly and widely used products to provide environmental and economic relevance to our work. Furthermore, the selected ENPs have different core and surface chemistry and physical properties allowing us to elaborate on current understanding of how ENP properties influence fate and behaviour in the environment, and their potential toxicity. This testing of how classical ecotox and risk assessment tools may need modifying to be "nano-applicable" will be achieved by systematically studying aspects that are related to ENP fate and toxicity and seeking to provide the knowledge needed to refine risk assessment tools and practices for use with ENPs.

The presentation given will present the main results and conclusions reached and highlight the lessons learnt.

TH 214

Establishing a comprehensive knowledge base on engineered nanomaterials - the project DaNa

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Numerous studies dealing with potential toxic effects of engineered nanomaterials have been published in the past years. Different types of particles in various modifications in several organisms have been investigated, making it difficult to draw general conclusions on the hazard posed by specific nanomaterials. Here, the project DaNa (funded by the German Federal Ministry of Education and Research (BMBF) and supported by the Swiss government) steps in, aiming at a comprehensive presentation of relevant material and toxicology data for nanomaterials.

Further, the DaNa-project acts as an umbrella project aiming at collecting scientific results of recent and current BMBF-projects funded in the field of nanotoxicology and nanotechnology for ecological benefits. The knowledge integrated into the database could also contribute to the prioritisation of further research needs.

This information is provided in the DaNa knowledge base, accessible via the internet database, www.nanoobjects.info. Data regarding application, material properties, exposure as well as human and environmental toxicology are included. From the pool of data short consumer-oriented articles are generated and published. To make the knowledge base easy to access by the user, the information is organised either according to the different types of nanomaterials or via relevant applications. Additionally, there is a glossary on nano-specific terms, as well as sections on the basics, on ongoing projects, news and FAQs.

In a first step, all data and results are evaluated regarding their scientific value applying the DaNa criteria checklist, which demands specification on particle properties and behaviour as well as on toxicological tests. Besides the scientific community, we also address the interested public e.g. journalists, students, scientists from other fields or consumers by an understandable presentation of complex scientific data.

An interdisciplinary group of scientists from biology, toxicology, ecotoxicology, physics and chemistry is engaged to achieve this evaluation and presentation for a broad audience.

TH 241

Setting most robust effluent level against severe uncertainty: application of information-gap decision theory to chemical management

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In the ecological risk assessment, we have to make decisions based on many kinds of imperfect knowledge. For example, when we set limitations for point sources, there may be an uncertainty on relationship between a chemical concentration in effluent and that in environment, and hence it may be difficult to predict an environmental concentration using the information about effluent. Another example is that the environmental quality criteria are determined to protect the ecosystems, but the ecosystems are much more complicated and the criteria may not protect the ecosystems effectively. We apply information-gap decision theory to set effluent limitations for point sources based on scientific underpinnings rather than based on groundless predictions even under severe uncertainty. Information-gap decision theory derives the decision that is most robust to uncertainty, by guaranteeing an acceptable outcome under the largest degree of uncertainty without requiring information about the extent of parameter uncertainty at the outset. We illustrate the application of information-gap decision theory to derive a general framework to set effluent limitations of pollutants for point sources incorporating cost of reduction in chemical use and cost to wildlife species that are affected by pollutants. Our framework enables us to derive decisions to deal with severe uncertainty in ecological risk management of chemicals.

TH 242

Does probabilistic approach relevant to derive Health occupational exposure limits?

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Current methods for risk assessment are mostly 'deterministic'. This means they treat factors such as the toxicity of chemicals as if they were fixed, and precisely known. But in the real world, factors such as toxicity are not fixed but variable. For example, toxicity is measured for only a very small number of species, so scientists have to estimate toxicity to Human and in some extends to all the other species that we want to protect. Current methods for risk assessment try to allow for variability and uncertainty by using 'fixed safety factors', but this fails to give a complete description of the full range of the possible risks. Also, it is difficult to decide how big the safety factors should be.

Probabilistic approaches enable variation and uncertainty to be quantified, mainly by using distributions instead of fixed values in risk assessment. A distribution describes the range of possible values (e.g. for toxicity), and shows which values within the range are most likely. The result of a probabilistic risk assessment can also be shown as a distribution, showing the range of impacts that are possible, and which impacts within that range are most likely. This should provide a better basis for making decisions about chemical risks, because the full range of possible outcomes can be taken into account.

For the first time to our knowledge this approach was applied to derive Occupational exposure limits professional (OEL).

OELs are the limits for concentrations of hazardous compounds in workplace air. OELs for hazardous substances represent an important tool for risk assessment and management and valuable information for occupational safety and health activities concerning hazardous substances. It is typically set by competent national authorities and enforced by legislation to protect occupational safety and health. It can be a tool in risk assessment and in the management of activities involving handling of dangerous substances.

TH 243

Calibration of hazard quotient trigger values for pesticide risk assessment for non-target arthropods

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The European Food safety authority (EFSA) recently developed a methodology to define Specific protection goals (SPGs) for the ecotoxicological risk assessment of pesticides (EFSA, 2010). The next step is the definition of specific protection goals for groups of non-target organisms in consultation with risk managers. It needs to be investigated whether the current risk assessment achieves an adequate level of protection to address the SPGs. According to EFSA (2010) "for each key driver (taxonomic group or other ecological entity) a reference tier should be identified, based on the most sophisticated experimental or modelling risk assessment method currently available that addresses the specific protection goal. This reference tier will then be used to calibrate lower tiers using simpler methods that are practical for routine use." To apply this concept for the group of Non-Target Arthropods (NTAs), we compare the hazard quotients, which currently decide whether a higher tier risk assessment for NTAs should be performed, with the results obtained in the higher tier risk assessment taking into account the new SPGs. To do so we use published data from Draft assessment reports that have recently been summarized by EFSA in a database. Results will be presented at the conference.

References: European Food Safety Authority (EFSA) (2010). Scientific Opinion on the development of specific protection goal options for environmental risk assessment of pesticides, in particular in relation to the revision of the Guidance Documents on Aquatic and Terrestrial Ecotoxicology (SANCO/3268/2001 and SANCO/10329/2002). EFSA Journal 2010;8(10):1821.

TH 244

How much of a difference in assessing ecological risk between effects of organism-level and population-level?

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Most protection goals stated in environmental regulations or policies are aimed at the population level or higher. A movement toward population-level ecological risk assessment (ERA) is gaining acceptance for support of chemical management in recent years. However, due to the reason that population-level ERA is a data-intensive analysis (it requires much more ecotoxicity data than organism-level), assessing the ecological risk of toxic chemical is mainly based on a limited number of measures of organism-level effects in laboratory toxicity tests. Thereby, there is an enormous concern in understanding how much of the difference in assessing the ecological risk between effects of organism-level and population-level. To get a rough answer to this concern, this study performs a comparative study on the difference between the derived reference values (PNECs) for risk calculation from approaches of organism-level and population-level. To derive the reference value defined as Clambda=1 for use in population-level ERA, extrapolation approach (Lin and Meng, 2009) using available acute (LC50 or EC50) and chronic (NOEC) toxicity test data is employed. From the comparative results of selected chemicals, a range of difference will be shown and discussed. This study is expected to be help in bridging the gap between the protection goals and practical ecological risk assessment.

TH 245

Do we need modelling for a conservative risk assessment? An investigation on Daphnia magna populations

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In this study, we use an individual-based model (IDamp) coupled to a TK/TD model (GUTS) to assess the risk of exposure of Daphnia magna individuals to a toxicant with several mechanisms of action (Dispersogen A), on the population level. Dispersogen A acts on reproduction by increasing the brood size at the expense of neonate length which decreases. It also affects survival, in addition to several other effects measured at higher concentrations. Effects on reproduction (neonate length and brood size) were calibrated using non linear regressions. GUTS was applied to calibrate effects on survival, using both chronic and acute datasets. Both assumptions, stochastic death (SD) and individual tolerance (IT) were tested. Validation was done using data from population tests describing population dynamics at six levels of contamination. The model was more successful in predicting population dynamics using SD approach rather than IT. The promoted reproductive effects (increase in brood size) caused a decrease in population abundance. Besides, not all effects observed on individuals were equally relevant on the population level. Whereas the promoting effects on reproduction failed alone to describe the effects on populations, the integration of both effects on reproduction and survival succeeded in describing adverse effects on populations. The additional effects observed at the higher concentrations on individuals were also important in determining population dynamics, but to a lesser extent than mortality or enhanced reproduction. Finally, population abundance was not always a direct predictor of population extinction probabilities.

We conclude that using an individual-based model with an integrated TK/TD module, we could extrapolate effects of Dispersogen A from the individual to the population level for Daphnia magna. Additionally, the promoted reproduction should be considered as adverse effects on the population level in risk assessment for Daphnia. Besides, whereas effects are considered separately for risk assessment, integrating combined endpoints is essential to achieve a conservative risk assessment and this can only be done using this kind of modelling approach.

TH 246

An approach to incorporate ecosystem service valuation in the assessment of protection goals: bridging the gap between risk and injury

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Remedial actions are typically based upon risk assessment and derived protection goals. Protection goals may or may not be overly protective. In developing a remedial action plan, it is necessary for stakeholders and decision makers to understand the potential benefits (i.e., gains in ecosystem service value) and costs (i.e., losses in ecosystem

service value) associated with the implementation of various remedial alternatives based upon prescribed protection goals. In this regard, a formal quantification of the effect that remedial actions have on ecosystem service values is rarely considered. Ecosystem services concepts applied to the identification of appropriate remedial options for contamination have been evolving in the United States for many years and more recently are being included in European legislation (e.g., Environmental Liability Directive). Approaches and tangible metrics that can describe the costs or benefits associated with the selection of remedial alternatives, and subsequently overall site cleanup, are still evolving in Europe. Approaches which meet the goals of managing risks while concurrently creating public benefit are needed to avoid 1) creating more harm to the ecosystem than is predicted by the risk assessment that drove the remedial action in the first place or 2) providing marginal benefit for the effort expended. The SuRF UK and the NICOLE Road Map promote sustainable remediation; however, although these frameworks acknowledge ecosystem services approaches they are not covered in detail. This paper provides an overview of the use of a practical approach termed net environmental benefit analysis (NEBA) that incorporates ecosystem service valuation to better understand and quantify the potential impacts and/or benefits associated with remediating to a specified protection goal. A NEBA considers the likelihood that identified risks are associated with an injury and the potential magnitude of that injury. As such, NEBA can help to bridge the gap between risk assessment and remedial planning by answering the question, "What do the identified risks mean in relation to the protection goal?" The NEBA approach is unique and innovative in that it considers risk, cleanup and natural resource issues concurrently. The process also promotes the selection of decisions that demonstrate a balanced win for the environment and the stakeholders. Case studies will be provided.

TH 247

Do threshold values for metal concentrations in soil protect the soil ecosystem properly?

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Regional geochemical data of heavy metals are commonly used for environmental risk assessment and management. The data used are often (near) total concentrations of the elements (often determined with Aqua Regia), whereas the exposure of the ecosystem is determined by the available or reactive fraction. The objective of our research was to develop a wider applicable method for quantitative hazard assessment of anthropogenic soil metal contamination, based on and illustrated with data for concentrations of Cd, Cu, Pb, and Zn from 360 locations in the Netherlands covering all major Dutch soil types.

Extraction of soil samples with 0.43 M HNO₃ gives a good approximation of the chemical availability of metals; the metal concentration in these extracts show a strong relation to the estimated anthropogenic enrichment, so we used them to assess the hazard of human-induced enrichment of these metals. To do this we used the toxic pressure concept, which estimates the fraction of biological species that is potentially affected due to the exposure to single metals or mixtures of metals. This is done using logistic concentration/response models parameterized with ecotoxicological effect data from toxicity tests and mixture models.

Hazards varied from very low toxic pressures (lower than 0.01) to toxic pressures just below 0.05 (just staying within the so-called 95%-protection criterion used in some soil protection legislations). In rare cases, the toxic pressure exceeded the value of 0.05, to an upper limit of 0.054 for Cd. Ranking the metals according to toxic pressure suggests that Cd enrichment induces the largest hazard increase. Ranking the soil types according to their susceptibility for toxic pressure by metals only yielded minor differences in enrichment hazards between soil types. Comparing the judgement of soils based on the current soil screening levels and based on toxic pressures that we estimated in this study showed that the soil screening values tend to be on the conservative side. Conservative soil screening values do indeed protect the soil ecosystem properly, but they do not always indicate an actual hazard or risk. When screening values are exceeded, refined hazard insights can be obtained, as illustrated in this study. These insights in the ecotoxic implications of metal concentrations in soils can provide a more refined basis for risk management decisions.

TH 248

Improving exposure scenario definitions within REACH: a comparative study of sources to aggregate exposure in Korea and in Denmark

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In recent years, the paradigm of chemical management system has changed from being toxicity oriented and media based to being risk oriented and receptor based. This trend is evident not only regarding environmental quality standards, but also for industrial chemical regulations. Political instruments to support a precautionary chemicals management system and to protect receptor's health have also been increasing. Since 2007, the European Union adopted REACH (the Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals): REACH makes industry responsible for assessing and managing the risks posed by industrial chemicals and providing appropriate safety information to their users (EC, 2007). However, to ensure a high level of protection of human health and the environment, there is a need to consider 'aggregate exposure' including background exposures from environment which varies in different regions. It is also needed to include information about the sustainable level of industrial emissions in REACH reporting system; this is in order to prevent chemicals from accumulating in the environmental media and biota. New approaches and frameworks are therefore needed to accomplish the goals of REACH, these at the same time can decrease historical background exposure for humans and ecosystem. A comparative review about the environmental management system such as quality goals, relevant organizations and environmental monitoring systems of hazardous chemicals in Denmark and Korea will be presented. Furthermore, the actual concentrations of selected substances (metals and POPs) in environmental media will be compared between the two countries with reference to the amount of chemicals emitted to environment by industries as reported in the national PRTR registers. Comparing the different background exposure between two countries allows in fact the definition of a common framework for improving exposure scenarios within REACH system, for monitoring environmental health, and for increasing degree of circularity of resource and substance flows.

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TH 249

Caterpillars and protection goals: the role of field margins as habitats and the effects of pesticide applications

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Biodiversity as new protection goal is specified in a Scientific Opinion of the EFSA. In this opinion it is proposed by the EFSA PPR panel to apply the protection goals for in-crop areas also to field margins. Field margins offer a habitat for many arthropods including butterfly and moth species (Lepidoptera) but they also receive inputs of pesticides due to overspray and spray drift as a consequence of the adjoining in-field applications. Our research project focused on the assessment of field margins in an agricultural landscape and their acceptability as caterpillar habitat with a special regard of the effects of different pesticide inputs.

Field margins were digitized and characterized in 4,000 ha of agricultural landscape around Landau, Germany. In a following step we recorded caterpillar communities in selected field margins of the study area. The impacts of different agrochemicals (insecticide, herbicide, fertilizer) on caterpillar occurrence were assessed in experimental field margins. Additionally, food quality for Cabbage Moth (*Mamestra brassicae*) caterpillars in three host plants treated with sublethal concentrations of two herbicides was analyzed in a laboratory study.

Concerning their length, nearly 80% of the field margins found in the studied landscape were less than 3m wide. These small field margins were used as caterpillar habitats by a range of Lepidoptera species. Next to orchards less caterpillar individuals could be found in comparison to cereal field margins and meadows which is probably a result of higher insecticide inputs in orchards. This finding is coherent with the results of the field margin surrogates in which caterpillar occurrence was reduced in plots with insecticide treatment. In the laboratory experiment, in one plant-herbicide combination the caterpillars showed statistically significantly lower weights in comparison to caterpillars feeding on untreated control plants indicating a reduced host plant quality.

Lepidoptera showed a vulnerability towards different pesticides. Especially in smaller field margins pesticide inputs can lead to reduced abundances of Lepidoptera. These smaller structures constitute a high percentage of the measured semi-natural habitats occurring adjoining to agricultural sites. It therefore should be carefully considered if these field margins should be treated as in-crop area receiving a lower protection status (no habitat provision) according to the protection goals.

TH 250

Bird community monitoring in chlorpyrifos-treated citrus. Results of years 1&2 of 3 year program in Valencia region

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Bird communities in citrus are influenced by a range of habitat features, such as the characteristics of adjacent areas. Reproductive success of birds in the field is affected by nest predation and food availability. Against this background of factors, the question is whether the insecticide chlorpyrifos has detrimental effects on the bird community including breeding success? Chlorpyrifos is applied to nearly the entire commercial citrus area in Valencia region in May-June for control of red scale. A comprehensive 3-year monitoring program in 10 citrus sites was initiated in 2010 (total area of around 100 ha). Data are available from 2010 & 2011. This long-term field study is a major post-registration monitoring initiative, for an insecticide which fails the standard Tier 1 risk assessment. Chlorpyrifos is the only active ingredient which controls red scale, which is a major economic pest causing downgrading of fruit to unsalable quality. This field monitoring program has Measurement Endpoints which match the Protection Goals of the regulatory assessment. Mist-netting was used throughout the season with birds being leg-ringed and their details recorded (species, age, sex, reproductive status, bodyweight). All nests within the citrus groves were located, and their status monitored. Motion-sensitive cameras were used. Nest boxes were also installed. For Sardinian warblers radio-tracking of adults was used for nest searching. Characterisation of the ecologically relevant factors was done. Arthropod food availability was measured. All citrus orchards were treated in 2010 and 2011 with chlorpyrifos, with an application rate in 2011 of 2.4 kg a.i./ha. As was also found in 2010, a diverse and abundant bird community was present in 2011. 5111 individuals of 72 species, including many small seed eaters (e.g. serin) and small insectivores (e.g. Sardinian warbler), were trapped. 60% of these (3052) were juveniles trapped during the breeding season indicating successful reproduction. In Total 306 active nests were found. The chlorpyrifos applications had no apparent effect on populations and breeding success. The diversity and abundance of birds indicated a high 'health status' of the bird communities. Monitoring will continue in 2012.

TH 251

Development of a 'hair tube' method to monitor potential effects of plant protection products on small mammal populations - application to a fungicide use

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Since 2005, an increase of requests of post-registration monitoring studies on e.g. birds, mammals, or bees is observed for plant protection products (PPP) newly (re-)evaluated in France. Actually, according to the outcome of the ecotoxicological risk assessment, a post-registration monitoring study can be requested by risk managers in France, in order to verify in practice the appropriate implementation of risk mitigation measures and get additional information on potential effects of the PPP at population level. As specified in the recently published guidance document based on the EFSA document (2009) concerning bird and mammals, all information coming from post-registration surveillance or monitorings can be useful to reduce the uncertainty about the level of protection provided by TER trigger values. However, at present, when post-registration monitoring studies are requested for such organisms, no standard guidelines or methods exist.

Regarding small mammals, a large spectrum of methods has been applied in a variety of published studies, but these are not necessarily applicable to study the dynamic of the populations of the different species living in croplands. In this context, a simple and innovative field technique using hair tubes (Suckling, 1977) was further developed and applied to assess effects of a fungicide treatment on small mammal populations in cereal fields. It aimed to monitor small mammals under large scale field conditions by assessing the proportion of hair tubes visited by small mammal species ('hair index') living in cereal fields treated or untreated with the fungicide. Part of the work was to adapt the design of hair tubes to allow the detection of rodents and shrews living and attempted to calibrate this method against a live-trapping method. Then, the ability to detect differences of population size between fields with different treatment regimes, under a range of different sampling strategies had to be assessed using power analyses. Finally, the hair index was estimated in cereals fields of Seine-et-Marne over a two year period and the scale of monitoring needed to detect significant changes in abundance and composition of rodents and insectivorous mammals on a short-term period was determined. The results demonstrate that this method is a very promising one able to assess small mammals' relative abundance at large scale (i.e. in 50-100 fields).

TH 252

Screening metal impacts from tissue residues for a calibrated biomonitor using DYMBAM and monitoring data in 6 Luxembourgish rivers

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Metals remain a pollution source for most rivers in urbanized areas although they have been pushed from the limelight by emerging compounds. The exposure of river biota to metals via food is rarely evaluated in routine risk assessments. We took the opportunity of recent development on the toxicokinetic model DYMBAM and the calibrated biomonitor for hydropsychyche to evaluate the metal pressure in Luxembourgish rivers. We used extensive data on suspended matter that had been collected in the years 2002-2005 to simulate the evolution of tissue residues for hydropsychyche assuming that it feeds on suspended matter (hydropsychyche is a net spinning filterer). As the uptake and elimination constants for this species are only partially known we used ranges of reported values for uptake from the water phase, ingestion rates, assimilation efficiency (as linked to suspended matter composition) and elimination rate to run an uncertainty analysis in STELLA with the DYMBAM equation for 6 differently polluted sites in Luxembourg. Results showed the dynamics of internal concentrations for the biomonitor over a life-cycle (July-June of next year) with a decrease over the winter period. Nevertheless, most sites yielded exposures to particulate metals which exceeded internal concentration thresholds for metal-sensitive species. The uncertainty of determining metal concentrations of low-flow suspended matter has a strong influence on the results. As for the DYMBAM parameters: uptake from the water phase had a negligible part in tissue residues while ingestion rate and in particular elimination rates where governing internal concentration. The approach seems interesting to evaluate the impact of metals in an isolated way. While in most of the sites classical organic pollution and eutrophication might dominate the depression of sensitive species the additive impact of metals cannot be ruled out following our calculations.

TH 253

Active monitoring programs: useful tools for monitoring effects of some plant protection products on birds and mammals - Feedback on a French approach

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Bird and mammal risk assessments for plant protection products are carried out under EU regulation 1107/2009 using the guidance document issued by EFSA¹. This document outlines the conservative first-tier assessment procedure for a large range of crop/uses dependant scenarios and proposes different options for refinement if needed. Field studies are one of the options. In the regulatory frame, these studies are designed to detect non-intentional effects of the plant protection product in fields of a particular crop, for a particular use and on focal species of birds and mammals. For some plant protection products, post-authorization monitoring data may be additionally useful to assist in the regulatory process. This is the case of many insecticides particularly in solid formulations like granules or seed treatments where the first tier assessment usually does not match the regulatory trigger by a great extent.

In this context, networks such as SAGIR² can provide useful information on acute effects of plant protection products on birds and mammals under practical use conditions. With experienced SAGIR people, a specific bird and mammal mortality monitoring program was pro-actively launched. It has been performed for 3 years during the drilling period of methiocarb-treated rape seeds from 2009 to 2011.

According to the protocol, any dead or moribund bird or mammal in or in the vicinity (up to 500m) of oilseed rape fields was collected and analyzed for chemicals applied to determine the cause of death. From this 3-year national monitoring program, 4 positive cases were recorded with only one case attributed to methiocarb poisoning. This work using SAGIR network shows the usefulness of such existing national scheme to record effects which can be product related. Although the absence of recorded incidents does not necessarily indicate a low risk, for our case, the high theoretical predicted risk is not confirmed by any findings. Complementary tools such as field experiments or modelling would be needed to estimate quantitatively the order of magnitude of the phenomena detected. Active animation of such national network of environmental workers is an important condition to perpetuate a survey on several years.

¹EFSA; Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA (Sanco/10997/2009). EFSA Journal 2009 7(12): 1-181; Available online: www.efsa.europa.eu

²French national network created by the National Hunting and Wildlife Agency

TH 254

Review of the causes of reported honeybee pesticide poisoning incidents in Germany

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Incident reporting is an important monitoring tool to receive information on the reliability of risk assessment of pesticides for bees and the effectiveness of risk mitigation measures. Learning from accidents has been proved to lead to quick reaction and adaptations of regulatory processes, e.g. following the poisoning incidents after drift of insecticidal dusts leading to contamination of flowering bee attractive plants.

Even if a strong and sudden damage to bee colonies is observed, the reason for the bee incident may not be clear at the first view and the extent to which it may be due specifically to pesticides is uncertain. For several decades an analysis of bee poisoning incidents has been conducted in Germany. A system for analysis and reporting of bee poisoning incidents possibly linked to pesticide applications is well established. Biological analyses, e.g. inspection of bee diseases and, if appropriate, chemical analyses of bee, plant and other samples are conducted by the authorities to investigate the cause-effect relationship between an agricultural treatment and the incident. For the interpretation of data expert judgement is needed and all information gained on the incident needs to be considered to conclude on the real cause of the incident. Data on the numbers of honeybee poisoning incidents in Germany, pesticide residues detected and conclusions on the causes for reported incidents will be presented.

TH 255

Year on year changes in Algerian mouse (*Mus spretus*) populations inhabiting citrus orchards treated with chlorpyrifos

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The Algerian mouse (*Mus spretus*) is one of the most abundant rodents across Spain, especially preferring arid areas. Its population densities can range from 3 up to >70 individuals per ha, and fluctuate over the seasons due to phases of maximum reproductive activity in April-May and August-September. Here we present population parameters obtained during field studies in 2009 and 2011 (June to September) from local populations in citrus orchards and adjacent habitats (macchia). Population densities over the study period differed substantially within and between the years. A potential explanation for stable and higher densities in 2011 could be that the carrying capacity of the orchards was reached early in 2011 and therefore the typical second reproductive peak in August could not be observed. Carrying capacity is dependent on the weather condition and available resources in a specific area. A population reaching the carrying capacity shows changes in population structure since i.e. birth rate slows down or the emigration rate increases. Therefore it is important to know about the population structure for interpretation of possible effects on small mammal populations through pesticide uses.

TH 256

Population analysis of the insectivorous Sardinian warbler (*Sylvia melanocephala*) in chlorpyrifos treated citrus orchards in Spain

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The Sardinian warbler is the smallest predominantly insectivorous bird species inhabiting conventionally treated Spanish citrus orchards, where chlorpyrifos is regularly applied. In order to investigate the importance of commercial citrus orchards during Sardinian warblers' reproduction period, their seasonal occurrence, site fidelity and age structure was analysed. For this purpose periodically repeated standardized mist-netting was conducted in ten citrus orchards near Valencia from April to August 2011. Sardinian warblers were present during the entire study period inside the study orchards (i.e. for reproduction). In total 712 individuals were trapped, 154 individuals (21.6%) were captured on at least two different days. Data gathered during a study in the same citrus orchards in 2010 was related to the 2011 results. The previous year 309 Sardinian warblers were ringed and 34 of these birds were recaptured in 2011. The results obtained support the conclusion that chlorpyrifos treated citrus orchards offer

habitat conditions which are obviously appropriate for breeding Sardinian warblers.

TH 257

Guttation and the risk for honey bee colonies (*Apis mellifera* L.): is the distance of bee colonies to a treated crop a necessary and a useful risk mitigation measure?

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Findings of high concentrations of bee-toxic compounds in guttation fluid from young crop plants that had been seed-treated with systemic insecticides gave rise to concerns about a potential risk to honeybee colonies posed by exposure to guttation of seed-treated crops or following granular applications. As bee colonies seem to prefer water sources in the near surroundings, a field trial was set up to gain clarification about the potential risk of guttation droplets containing residues to bee colonies at different distances to seed treated crops and also if, in case effects could be observed, keeping of which distance between hives and a treated crop would be necessary for potential risk mitigation measures.

The experimental field consisted of one plot planted with winter oilseed rape crop seed-treated (Elado®, a.s. Clothianidin) and one plot with untreated winter oilseed rape. 24 bee colonies in total were set up in an untreated winter oilseed rape crop before emergence, with the hive entrances pointing towards the treated crop. 6 bee colonies each were located at different distances, 0m (field border), and also in 10m, 30m and 75 m distance to treated crop. From August to November 2011 the mortality of bees was assessed with dead bee traps, bee brood and colony development assessed, until wintering of colonies. After overwintering in Spring 2012 the assessments mortality, colony size, bee brood and colony development will be continued after overwintering of colonies. During the whole observation period the occurrence of guttation was documented and, if guttation occurred, guttation droplets were sampled daily for residue analyses.

RA17P - Multiple stressors in a changing world

TH 258

Regional scale risk assessment using the relative risk model of threats to the surface aquatic ecosystems of the Umvoti River catchment, South Africa

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The Umvoti River is a highly developed, highly utilised, stressed aquatic ecosystem that is often referred to as a 'working river'. Potential sources of impacts include numerous urban areas and associated activities, informal settlements, agricultural and forestry activities, industries, sand mining activities and recreational activities. Recent assessments show that a wide range of stressors have been identified in the lower portion of the study area in particular. Existing stressors include water quality and quantity alterations, habitat impacts and associated disturbances to wildlife impacts. Stakeholders of the catchment require a management plan that considers nature and location of multiple sources in the catchment that takes ecosystem dynamics into account and provides threat assessment of established endpoints. The aim of this study is to carry out a risk assessment of the Umvoti Catchment to identify and quantify risks to ecosystem components or habitats in accordance with existing stakeholder objectives or endpoints. In this study the relative risk model (RRM) was applied to selected sources, habitats and endpoints of the Umvoti River system to generate risk and validation hypotheses to test for the validation of the risk outcomes. Various uncertainty and sensitivity tests were applied to the model to validate the risk outcomes. Findings of the RRM showed that while the upper and middle portions of the catchment have low risks of threats impacting on the endpoints, high risks exist in the lower portion of the Umvoti Catchment. Some additional moderate risks were also identified in the extreme eastern and western parts of the catchment. Within the high risk region, sources of stressors include; sand mining activities, sugarcane activities, heavy industries, urban areas and then sugar mill activities. Endpoints are potentially threatened by water quality, water quantity and habitat impacts. Threatened endpoints of concern include the maintenance of local biodiversity, particularly in the Umvoti Estuary, the sustainable provision of ecosystem services for existing users and a safe and clean environment for local communities. Three hypotheses were developed and tested for further validation of the risk assessment. These tests confirmed the spatial projections and nature of threats established in the risk assessment and reaffirmed threats to endpoints. This risk assessment is currently being used to direct environmental management in the area.

TH 259

Application of Bayesian networks for integrating multiple chemical and non-chemical stressors into a risk assessment using the South River, VA as a case study

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The structure of the conceptual models as in the relative risk approach to regional risk assessment lends itself to the construction of Bayesian networks (BN) for the calculation of risk. BN use conditional probabilities based on available data, models or expert opinion to construct the conditional probability table. This table describes the interactions between input nodes and the resultant child node. BNs intrinsically incorporate uncertainty into each of the variables and sets specific criteria and structure for the calculation of risk. The case study we are using to demonstrate the approach is the South River in the western mountains of the state of Virginia, USA. The study area is approximately 25 km in length and includes the catchments that directly feed the river. From 1929 to the early 1950s, Hg was deposited into the river from a manufacturing process. We are now estimating risks due to Hg and other stressors up and downstream of the manufacturing site. The BNs were constructed using Netica, and the process of setting ranks and distributions will be explained in the poster. Smallmouth bass, Kingfisher and water quality parameters are the endpoints examined to date using the BN approach. An interesting result is the interaction between water temperature and contaminant loading in generating an increase in risk far downstream of the manufacturing site. Another feature of the Bayesian approach is that it allows the calculation of restoration strategies and specific management and engineering options. The desired level of risk is set and the model calculates the required values of the input nodes. BNs are a powerful tool for understanding the impacts of multiple disparate stressors over regional landscapes.

TH 260

Towards a conceptual model of the impact zone ecology in rivers

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Discharge of untreated domestic wastewater into rivers can result in high concentrations of unionised ammonia (NH₃), nitrite (NO₂), and biochemical oxygen demand (BOD) as well as chemicals used in home and personal care products (HPCPs). In such scenarios, where aquatic communities are severely impacted by conventional waste water constituents (sanitary determinands), it may not be appropriate to conduct conventional risk assessments on HPCP chemicals. An alternative risk assessment model based on the 'impact zone' concept has been proposed for these direct discharge conditions. The impact zone, in this case, is the river reach downstream of a direct discharge in which concentrations of sanitary determinands are higher than their predicted no effect thresholds, resulting in potential impacts on community structure and function. Risk assessment i.e. estimation of PEC (Predicted Environmental Concentration): PNEC (Predicted No Effect Concentration) ratio, of HPCP chemicals is conducted at the end of the impact zone. Here, we present a conceptual modelling framework that describes how the ecosystem community composition may change through the impact zone and beyond it and postulate how this could be used to assess the potential impacts of chemicals used in HPCPs under direct discharge conditions. A key assumption is that different taxa or biological traits have differing sensitivities to various chemical stressors, which will alter the composition of the in-stream community along the stress gradient. At a basic level this can be predicted using species sensitivity distributions, but at higher tiers ecological considerations (e.g. indirect effects) are required.

TH 261

Effects of toxic and non-toxic stressors on fish community in the river Rhine

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Predicting river ecosystem and fish community responses to human activities is challenged by the diversity of contaminants (toxic stressors) and habitat alterations (non-toxic stressors) associated with these activities. Research on the impact of these combined stressors as well as on the ranking of impact related to individual stressors of different origin will result in improved field impact based risk management of aquatic ecosystems.

The fish community is a suitable indicator for the ecological condition of a river. The river Rhine has been intensively studied and much effort has been put in the (ecological) restoration. The diversity, richness and composition of the fish community in the river Rhine has changed remarkably since the 1970s due to both the species-dependent sensitivity of fishes and the introduction of invasive species. We therefore hypothesize that the sensitivity of the fish community related to the wide range of stressors has changed over time and that invasive fish species influence the overall responses of fish community to different stressors, due to their species-specific tolerance.

The present study describes the effects of toxic and non-toxic stressors on fish community in the river Rhine using monitoring data collected since the 1970s and species sensitivity distributions (SSDs). This statistical approach enables us to quantitatively compare concentration related effects (including contaminants, metals, dissolved oxygen, and salinity) with effects related to habitat alterations (like temperature, flow rate).

Therefore, in the present study, the effects of multiple stressors on the fish community consisting of native and invasive species are analyzed. The effects of low dissolved oxygen, temperature, stream velocity, and exposure to pesticides and heavy metals are studied with the aim to: (1) identify the difference in tolerance to toxic and non-toxic stressors between native and invasive fish species, and (2) rank these stressors according to the potential risk they have on the overall fish community as well as on the native and exotic component separately, and (3) determine possible changes over time in the sensitivity of the fish community and the relative importance of different stressors in the river Rhine.

The implications of our results for risk management of the Rhine as well as management of exotic fish invaders will be discussed.

TH 262

Multiple stressors in aquatic environments may profile biomarker baseline responses: a case study in *Dreissena polymorpha* at the population scale

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To face with the multi-pollution of their environment, aquatic organisms use various systems of defense, which allow them to live and to reproduce. This statement supports the hypothesis that a natural baseline level can be identified in wild populations, which corresponds to the defense level required for living in a given environment influenced by both anthropogenic and natural stressors. It was postulated that the basal defense levels in different populations cannot be the same, because they are an adaptation and/or acclimation response under influence of both genetic parameters and environmental conditions. In this context we conducted a large and pluri-disciplinary study involving ecotoxicology, proteomics and population genetics. Eight French populations of zebra mussels were sampled and used for biometric evaluations, biomarker measurements, energetic reserve assessments, proteomic and genetic analysis. Water physico-chemistry and sediment contamination were assessed in order to describe the studied sites. Analyses of data were performed by using integrative methods in order to establish links between site, population, and biomarker typologies.

At Seville in 2010, we presented the first detailed results (SETAC Abstract n°1244) of this study and today, we will present how we integrated all of these results. Altogether, the results show once again the difficulty of interpreting results of field survey and the difficulty to link biological responses to contamination data. However, we succeeded in discriminating (1) between sites based on their contamination profiles, and (2) between populations based on the shell morphology, the population genetics parameters and the proteome expression. These typologies were consistent with the biomarkers responses profile.

In conclusion our results support the initial hypothesis stating that wild populations do not have the same biological baseline response as they live in different environments, which are under influence of various multiple stressors. This is to take into account when seeking to understand the impact of multiple environmental stressors on ecosystem health.

This study is part of the SYDEPOP project supported by the French INSU-EC2CO-Cytrix program.

TH 263

Anthropogenic contaminants exert a stronger influence on bacterial communities than environmental factors; a study in estuarine sediments

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Estuarine environments are highly susceptible to contamination from surrounding anthropogenic activities. The most ubiquitous contaminants in the environment, metals and polycyclic aromatic hydrocarbons (PAHs), bind to fine particulates and settle to be stored in the seafloor where they interact with sediment dwelling organisms and pose a threat to organisms estuary wide during oxygenation. As contaminants continue to accumulate in estuarine systems, sensitive methods to monitor sediment contamination are required to further our understanding of contaminant effects on sediment communities and for determining levels of contamination before ecological impacts are observed. Bacterial communities are highly sensitive to the presence of contaminants, and therefore may provide early signals of sediment contamination, but until recently these changes were difficult to assess. Advances in sequencing technologies have revolutionised the way in which we can observe the microbes that represent a large portion and many functions of a system. We use 454 pyrosequencing to compare bacterial communities in estuarine sediments across a large spatial scale; from 4 contaminated (heavily modified) and 4 relatively uncontaminated (relatively unmodified) estuaries. We find that shifts in bacterial communities correlate strongly with changing contaminant concentrations and that this signature is stronger than changes associated with environmental variation. Sediment contamination was found to select for groups from the orders *Rhodobacterales*, *Oceanospirillales* and *Desulfobacterales*, while orders *Chromatiales*, *Cyanobacteria* and *Rhizobiales* experienced declines. It is likely that changes in bacterial community composition as a result of sediment contamination have implications for the functioning of estuarine environments.

TH 264

Temperature-dependent toxicity: is an assessment factor of 10 appropriate for ecological risk assessment?

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Laboratory derived toxicity data provide important information for ecological risk assessment (ERA) of pollutants and form the basis for deriving water quality criteria. However, toxicity tests are often conducted following standard methods and/or guidelines (e.g., 'U.S. EPA Ecological Effects Test Guidelines' and 'OECD Guidelines for Testing of Chemicals'), and thus carried out under controlled laboratory conditions. For instance, most of toxicity tests are run at a constant temperature which usually represents the optimum temperature for the test species. Yet, toxicity of a contaminant may vary in a temperature-dependent manner, depending on the physiology of the test organism and the chemical properties of the contaminant. Even though an assessment factor of 10 is often used to account for the variability of toxicity data in ERA, no one has investigated the appropriateness of this value to cover the temperature-dependent variability adequately. Here, we compared median lethal concentration (LC50) data of several trace metals and organic pollutants on a range of aquatic species at different temperatures to assess whether an assessment factor of 10 is under- or over-protective for ERA. Our preliminary results indicated a considerable variability of copper toxicity with temperature across different freshwater species. For each species, we used the temperature at which the LC50 is the highest as a reference temperature (i.e., LC50ref) and the corresponding LC50 value as 1 reference toxic unit (TUref). The relative difference in the copper toxicity to the same species at a different temperature (T) is expressed as a relative TU value (RTU) which is equal to LC50T/LC50ref. A smaller RTU indicates a higher toxicity. For copper, we detected an average decrease of 0.139 TU/°C increase in temperature and a decrease of 0.076 TU/°C decrease in temperature. The results imply that an assessment factor of 10 will cover up to 6.1°C increase and 11.8°C decrease in temperature for copper, translating into a maximum protective range of 17.9°C temperature variation. Global surface freshwater temperature was found to have an average variation of 11.7±1.4°C. Thus, application of a factor of 10 in this case might be adequately protective to freshwater organisms. In this presentation, we will present more results on other chemicals before drawing a solid conclusion.

TH 265

Evaluation of the combined action of natural stressors and chemical pollutants in algae. Assessment of functional, structural and metabolism alteration

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Nowadays, there is a growing number of studies exploring the combined effect of different stressors on ecosystems. However, the interactions between natural stressors and toxicants are still poorly understood. Among the stressors that might influence ecosystems, toxicants can directly or indirectly affect all relevant ecosystem processes such as primary production. On the other hand, fast and intense changes in the water temperature is one of the major threats for freshwater ecosystems.

The objective of this study was to characterize the physiological responses of cultured algae, *Scenedesmus vacuolatus* in response to physical (temperature) and chemical (anthropogenic contaminants) stressors. The pollutants selected for this study were the priority pollutant diuron (herbicide, PSII inhibitor) and the emerging compounds propranolol (β-blocker) and fluoxetine (antidepressant).

The experiment followed a factorial design with 2 factors (water temperature and toxicant concentration) and their interaction. A previous experiment determined that the optimal temperature was 20°C, while 30°C was used for simulating physical stress. Chemical stress was reproduced at the level of EC30 for each toxicant (5, 850 and 500 µg/L for diuron, propranolol and fluoxetine, respectively). Changes in photosynthetic efficiency (Yeff), photosynthetic capacity (Ymax), photochemical quenching (qP) and non photochemical quenching (NPQ) were studied in control and exposed algal cultures.

The algal toxicity of the three compounds differed considerably. Diuron was the most toxic compound, followed by fluoxetine and propranolol. Effects of diuron occurred immediately after addition, which indicates a very specific toxic action of this compound to algae (inhibition of photosynthesis). Propranolol and fluoxetine toxicity effects appeared later than those detected with diuron exposure (after 30 minutes). Most of the endpoints were affected after 12 hours for all the compounds tested, except in the case of diuron, where NPQ mechanisms were inhibited after 12 hours of exposure, suggesting damage in the pigments where the NPQ takes place.

The increase of temperature reduced the toxic effect of the herbicide diuron and propranolol at the beginning of the exposure as well as at the final time. In the case of fluoxetine, the temperature increase reduced the toxicity at the initial time, but a synergistic effect was observed at final time with an enhanced toxicity.

TH 266

Combined effects of natural (eucalypt leachates) and anthropogenic (copper) stressors on *Echinogammarus meridionalis*: implications for ecosystem functioning in a global warming climate scenario

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The combined effects of natural stressors (eucalypt leachates) and a common environmental contaminant (copper), on the crustacean *Echinogammarus meridionalis* were investigated under a stream water temperature increase scenario. Single and mixtures toxicity bioassays based on mortality and biomarkers were used. In single stressors bioassays, the increase in temperature from 10°C to 20°C was found to significantly increment the toxicity of both copper and eucalypt leachates. In the mixtures bioassays, the increase of temperature by itself resulted in a reduction of glutathione S-transferases activity, increased levels of lipid peroxidation (LPO) indicating oxidative damage and inhibition of acetylcholinesterase activity indicating alterations of cholinergic neurotransmission. Furthermore, at 20°C the toxicity of both stressors was higher, as indicated by a higher mortality and alterations in biomarkers. Toxicological interactions between stressors were also found. These results suggest that the ecological role of *E. desmaresti* populations can be negatively affected in streams contaminated by eucalypt leachates and/or copper and even more at high temperatures.

TH 267

Combined effects of temperature and chemical stress on native and exotic species of the genus *Artemia*

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Global climate changes may have considerable negative impacts on hypersaline environments of the South of Europe which are also facing other pressures such as chemical

contamination and biological invasions. *Artemia* species are key stone species of several hypersaline ecosystems in both marine and continental environments. In the present study, the combined effects of chemical and temperature stress on *A. parthenogenetica*, a native species in continental and marine hypersaline Portuguese ecosystems, and on the exotic species *A. franciscana* were investigated. The heavy metal mercury and the organophosphate insecticide fenitrothion were used as chemical stressors and two temperatures were tested 20°C and 25°C. Laboratory maintained and field collected organisms were used as test organisms in laboratory bioassays. Both chemicals had distinct effects on different populations and their effects were changed by temperature variation. Furthermore, the sensitivity of native and exotic species were different. The implications for hypersaline ecosystem functioning and biodiversity in global warming scenarios are discussed.

This study was carried out in the scope of the project “Chemical wars: the role of chemically mediated interactions in the invasiveness potential of non-native *Artemia*” - Ref. PTDC/MAR/108369/2008 funded by the Portuguese Foundation for the Science and Technology and COMPETE FEDER funds.

TH 268

Acute versus chronic temperature dependence of cadmium toxicity in zebrafish

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Toxicity studies are mainly performed in standardized conditions, most often at the optimal rearing temperature of the test organism. However, environmental temperature has a substantial impact on chemical toxicity. Metal toxicity is generally said to increase with increasing temperature in aquatic organisms, probably due to increased accumulation rates. This study aimed at investigating the temperature effect on cadmium accumulation and toxicity. Adult wild-type zebrafish were acclimated to exposure temperatures for one month prior to experiments. Fish were exposed to nominal concentrations of 0, 38, 52, 77, 115, 183, 284 or 435 µM cadmium at 18, 26, 30 or 34°C for 96 h in an acute experiment. In a chronic experiment zebrafish were exposed to 0 or 5 µM cadmium for 28 days at 12, 18, 26 or 34°C. Mortality was observed and the cadmium total body burden was measured. The temperature dependence of cadmium toxicity differed substantially between the acute and the chronic scenario. The pattern of acute cadmium toxicity was concentration dependent. LC_{50} values were lower at the intermediate temperature (26°C LC_{50} : 102.4 µM) when compared to 18 and 34°C (respectively 19 and 12% increased toxicity). In contrast, the LC_{10} value at 34°C was significantly lower than those at 18 and 26°C, resulting in exactly the opposite order of toxicity at low exposure concentrations: 34°C > 30°C > 18°C > 26°C. In the chronic scenario cadmium caused mortality to increase with increasing temperature and the observed differences were much more profound (57% mortality at 34°C vs. 2% mortality at 12 and 18°C) when compared to the acute scenario. Furthermore, in the chronic scenario increasing cadmium accumulation with increasing temperature played an important part in increasing toxicity, while in the acute scenario other physiological processes weakened this relationship. Apparently in an acute highly toxic exposure scenario prior acclimation to an altered temperature protected zebrafish against future stress, while in a chronic scenario the continuous heat stress eventually aggravated cadmium toxicity. More scientific background is necessary to include the temperature effect on chemical toxicity in regulation. Standardized tests are mostly performed at optimum temperature whereas temperatures in the field are often lower. Based on the present results, both in a peak exposure scenario and in a chronic low exposure scenario this could result in overestimation of toxicity.

TH 269

The effects of thermal anomalies in mussels exposed to Cd at different seasons: cell and tissue level biomarkers

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Marine organisms subjected to pollution may exhibit altered responses to temperature raise associated to global warming. The consequences of temperature raise for biota may be very different depending on the season. Mussels are able to accumulate high Cd burdens in their tissues and are susceptible to the toxic effects of this metal, both bioaccumulation and biological responsiveness being also seasonal dependent. Presently, the seasonal variability in biomarker responses to temperature elevation in Cd exposed mussels *Mytilus galloprovincialis* was investigated. Mussels were collected from Gorliz (Biscay Bay) in summer 2009 and winter 2010 and acclimated to local seawater temperature (18°C summer, 12°C winter) under laboratory conditions for 5d. A set of mussels was exposed to Cd (0.1 mg/l) beyond Day 3 acclimation. A control group (not exposed to Cd) was carried out in parallel. At Day 6, mussels were subjected to heat shock (6hr at +10°C over seasonal local sea water temperature). Then mussels were maintained at initial temperature for the following 3d. Cell and tissue level biomarkers were recorded in the digestive gland just before the heat shock (0), 1, 2, 3, 4, 5, and 6hr along the heat shock and 2-66hr after returning to initial temperature (8, 10, 24 and 72hr after starting the heat shock): lysosomal enlargement and membrane stability, intracellular neutral lipid accumulation, cell type replacement, atrophy of the digestive diverticula, tissue integrity in digestive gland and intralysosomal metal accumulation. Gamete development was determined on histological sections. In summer Cd exposure provoked significant changes in studied biomarkers, corresponding to a typical general stress response however in winter responses were less marked. In summer studied biomarkers exhibit altered responses after a heat shock, although combined effect of thermal stress and Cd exposure provoked higher changes. After the heat shock biomarkers recovered gradually initial values only in mussels subjected to a temperature elevation. In winter no significant responses were observed after a heat shock. On the other hand, marked alterations were observed with a combined effect of heat shock and cadmium exposure where unlike in summer, biomarkers recovered initial values after the heat shock. Organisms subjected to thermal anomalies exhibit different responses depending on the season. When thermal stress was combined with metal pollution the effect of season was less marked

TH 270

Effects of copper on immune performances of *Mytilus edulis* from different salinities

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The ERANET BONUS+ project BEAST (Biological effects of anthropogenic chemical stress) aimed to test and establish biomarkers in the Baltic Sea, with regard to the predominant salinity gradient. Studies of multiple stressors in this environment are still very scarce, thus the present study tackles the question whether *Mytilus edulis* adapted to various salinities do respond differently to copper exposure. Individuals of *Mytilus edulis* were collected in Danish waters at a prevailing salinity of 12 and 20‰, respectively, in September 2011. After an acclimatisation period, mussels were exposed to varying copper concentrations (0, 5 and 15 µg/L Cu) for 1, 7 and 13 days. Copper concentrations were chosen based on environmental measurements to ensure ecological relevance. For each copper concentration, salinity and time point 4 glass tanks (9L) were prepared, each of which contained 3 mussels. Water change and copper re-dosing was conducted daily. Copper concentrations were evaluated in water samples as well as in mussel tissue. Assessed biomarkers encompassed cellular, humoral and functional endpoints. In brief, the total and differential haemocyt count, morphological abnormalities in haemocytes, phagocytic (uptake of Neutral-Red stained zymosan particles) and haemolytic activity (lysis of erythrocytes), apoptosis of haemocytes (caspase 3/7 activity) as well as bacterial clearance of haemocytes (competent killing of *E. coli*) were determined. First results indicate that both environmentally realistic copper concentrations have a negative impact on phagocytic activity of haemocytes in mussels. This decrease was accompanied with a lesser efficiency to kill bacteria. Since the assessment of all data obtained from the experiment is still ongoing, final results including a multivariate statistical analysis will be presented at the meeting. To conclude, phagocytic activity, as an early indicator for changes in molluscan immune responses, is likely to serve as a valuable biomarker in biomonitoring studies on the impact of immunotoxic compounds in brackish water environments. The present study was an attempt to raise awareness to the specific abiotic conditions of the Baltic Sea, with regard to the EU-WFD (2000/60/EC) and the MSFD, as more effort is needed to improve the understanding of such unique environments as well as the impact of multiple stressors.

TH 271

Responses of benthic macroinvertebrate communities to multi-stress conditions in high altitude Andean streams

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High altitude Andes encompass a suite of harsh environmental conditions, including intense ultraviolet radiation (UVR) and acid drainage from metal-rich rocks, which challenge the survival of aquatic biota. The aim of this study was therefore to evaluate the single and combined effects of UVR and metals on benthic macroinvertebrate community composition. Since melanin may play a protective role against both UV and metals, pigments were analysed in field collected animals. Experimental verification of field data was obtained in laboratory experiments exposing mayfly (*Cleon simile*) and midge (*Chironomus riparius*) larvae to Cu and UVR. At 4000 m above sea level (m a.s.l.), UV-B was more than two-fold higher than at 3000 m. The concentrations of metals in the polluted sites ranged from 2 to 588 times those at the reference sites. The number of individuals and taxa did not differ between reference sites at both altitudes, but were much lower at the polluted sites than at the reference sites. Canonical Correspondence Analysis indicated a strong influence of UV-B in shaping communities at reference sites, and of metals at polluted sites. Melanin was strongly correlated with UV-B, being two-fold higher in mayflies at 4000 m than 3000 m. *Chironomus* sp. from polluted sites showed a two-fold higher melanin concentration than those from reference streams at the same altitude. In the laboratory experiments, a decrease in survival in both species was observed in the Cu, UVR and Cu+UVR treatments. Pre-exposure to UVR decreased the sensitivity of only *C. riparius* to UVR and Cu+UVR, which may explain the persistence of chironomids and the absence of mayflies under extreme metal and UV-B levels in high altitude Andes. A significant increase in melanin in *C. riparius* but not in *C. simile* larvae was observed in the Cu toxicity experiment, as seen in chironomids in polluted Andean streams. We conclude that in high altitude Andean streams high UVR and metal leaching create 'life at the edge' multi stress conditions which shape macroinvertebrate assemblages. Melanin may be an adaptive strategy of the few persisting specialized species against the effects of intense UV-B radiation and metals, as confirmed by exposure of chironomids to UV and/or Cu.

TH 272

Joint effects of carbaryl and stress induced by temperature regimes on *Eisenia Andrei* (Annelida: Oligochaeta) survival

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The toxicity of several chemicals in the field can differ depending on the prevailing environmental condition under which species are exposed. Studies have showed that temperature is a key environmental factor governing physiological status, affecting life-trait parameters (survival, growth, reproduction) and can also interact with chemical

pollutants in the field. The aim of this study is to compare the toxicity of single exposure of *E. andrei* to carbaryl and the combined exposure with different air temperature regimes (8°, 12°, 16°, 20°, 22°C, 24°C and 28°C), using survival and biomass loss as endpoints. For this standard acute tests were performed, and adaptations for temperature exposure carried out. To evaluate the joint effect of natural and chemical stressor, observed data was compared with the expected effect predicted by the conceptual model of independent action (IA) and deviations for synergistic/antagonistic interactions, dose-level and dose-ratio dependency were used. From the results we can highlight the changes in toxicity to earthworms depending on the temperature regimes, which provide more realistic information for Risk Assessment procedures.

TH 273

Exposure of *Folsomia candida* to carbaryl and natural stressors: effects on reproduction and genotoxicity

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Terrestrial organisms can be exposed to a great variety of stressors, such as contaminants and/or other physical or biological stressors that affect their life - and genetic traits. Organisms can experience a large range of environmental fluctuations such as temperature changes, drought and flood conditions or even UV radiation increments. The aim of this work is study the effects of carbaryl to a soil-dwelling collembolan *Folsomia candida* at different abiotic conditions, evaluating their reproductive effort and genotoxicity. Single and combined exposures were carried out with carbaryl and three different natural stressors: temperature, moisture and UV radiation. The combined effects were compared to carbaryl exposures under standardized conditions: 20°C, 60% water holding capacity. We observed that carbaryl induces changes on the survival and offspring production. DNA damage was also detected using the comet assay. Results from the combined exposure showed that fluctuations on environmental conditions such as temperature, soil moisture or radiation can induce changes on chemical toxicity.

TH 274

The effects of copper and temperature on interspecific interaction between two collembolan species, *Folsomia candida* and *Sinella curviseta*

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The effect of copper contamination on interactions between two species of springtails (Collembola) at different temperatures (10 and 20°C) has been examined. The instantaneous population growth rates of *Folsomia candida* and *Sinella curviseta* have been compared between monocultures and two-species cultures, influenced by different abiotic factors. Temperature proved to have a more significant impact on interactions between the species than copper contamination. However, the interspecific interactions under suboptimal thermal conditions (10°C) varied depending on copper concentration in soil. The increase in copper concentration caused quantitative changes in the two-species system by increasing the ratio of *S. curviseta*. Therefore, the presence of interspecific competition pressure may have a significant impact on the toxic effects of chemicals. Due to the complexity of factors influencing toxicity, and their possible effects at population and ecosystem levels, it seems necessary to complement the traditional ecotoxicological studies with more complex tests in order to improve the reliability of ecological risk assessment.

TH 275

Combined effects of chlorpyrifos, mancozeb and temperature on the survival and feeding parameters of *Porcellionides pruinosus*

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Environmental contamination and climate changes are two of the most important factors affecting soil ecosystems in agricultural fields. Agriculture regimes are nowadays strongly featured by the use of a wide range of pesticides whose application frequently coincides in time and space. Such mixtures may constitute, *per se*, a serious problem to soil biota but, in the present scenario of global changes in climate, they assume a particular relevance and unpredictability. The main goal of this work was to evaluate how the terrestrial isopod *Porcellionides pruinosus* is affected by the combined action of different temperature regimes and two widely used pesticides, the insecticide chlorpyrifos and the fungicide mancozeb. Endpoints measured included survival and two feeding parameters: the consumption ratio and biomass gain/loss. Preliminary tests were undertaken to assess the toxicity of both pesticides individually, as well as their effects in a mixture. The main experiment was then performed based on these results and using a full factorial design with three concentrations of each pesticide (plus an unexposed control) and three temperature regimes, mimicking real scenarios. Results confirmed that both survival and feeding parameters of *P. pruinosus* seem to be increasingly affected with the interaction of multiple stressors. This highlights the importance of taking climate conditions into account when assessing the impact of pesticides to soil biota, particularly in case of mixtures, and in this particular case that effects on isopods can be transposed to their crucial role in soils as decomposers.

TH 276

Toxicity of copper, zinc and nickel mixtures to fathead minnows (*Pimephales promelas*): implications for Biotic Ligand Model for metal mixtures

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Research on metal toxicity to aquatic organisms has been conducted extensively. However, most research was conducted with individual metals (e.g., Cu, Zn, Ni). This is not always relevant because metals usually present in the natural environment as a mixture of multiple metals. Data on the effects of Cu, Zn, and Ni mixtures to fathead minnows are lacking in the literature. The goals of this research are to characterize the acute toxicity of Cu, Zn, and Ni mixtures to fathead minnows (*Pimephales promelas*) and to generate data that support the development of a Biotic Ligand Model (BLM) for metal mixtures. 96-h acute toxicity tests were conducted with individual and mixtures of Cu and Zn, Cu and Ni, Zn and Ni, and larval *P. Promelas* using moderately hard water. For individual metal exposure, the 96-h LC50s for fathead minnows and Cu, Zn and Ni were 207, 873, and 1750 µg/L, respectively. Results of metal mixture exposures indicated that the toxicity of metal mixtures was more than additive (synergistic). When exposed fathead minnows to either Cu at a concentration of 50 µg/L or to Zn at a concentration of 100 µg/L, no significant mortality was observed. However, 27% mortality were produced when exposed the fathead minnows to a mixture of 50 µg/L Cu and 100 µg/L Zn. When increased exposure concentrations 2 times (100 µg/L Cu and 200 µg/L Zn), total mortality (23%) produced by individual Cu and Zn exposures was less than the total mortality (60%) produced by Cu and Zn mixture exposure. Similarly, mortality (97%) produced by a mixture of 200 µg/L Cu and 400 µg/L Zn was greater the total mortality (40%) produced by individual Cu and Zn. Similar results were also found for Cu and Ni mixtures. At Ni concentrations of ≤ 1000 µg/L, no mortality was observed. However, when exposed fathead minnows to a mixture of 50 µg/L Cu and 500 µg/L Ni, 30% mortality were produced. At 100 µg/L Cu and 1000 µg/L Ni, mortality (97%) produced by mixture exposure were greater than the total mortality produced by individual exposures. For Zn and Ni mixture, at 400 µg/L Zn and 1500 µg/L Ni, 67% mortality were produced by the mixture while total mortality produced by individual Zn and Ni was less than 33%. Using toxic unit (TU) concept, the joint toxicity of Cu and Zn, Cu and Ni, Zn and Ni that produced 50% mortality was 0.567, 0.6219, and 1.154 TU, respectively. Results of the present study are useful for the development of a BLM for metal mixtures.

TH 277

Health of and contaminants in fishes from the Slave/Athabasca River System, Canada, relative to oilsands extraction activities

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The potential environmental impacts of oil extraction activities in the northeastern region of Alberta Canada have recently received significant attention in both the scientific and general media. Chief among the global concerns are the fact that the open pit mining and oil extraction and conversion processes release a variety of environmental contaminants to the local and regional environment. Among the contaminants of concern in these aerial emissions are PAHs and a variety of metals (1) and releases of toxic naphthenic acids to the aquatic environment. The potential for these contaminants to accumulate in fish and wildlife are of particular concern to First Nations consumers of 'wild foods'. Consumption of fish and wildlife from the region are of cultural as well as nutritional and economic significance to First Nations communities. An apparent increase in the occurrence of fishes with apparent deformities and lesions has been reported by local communities as remote from the oilsands operations as the lower Slave River. The aim of this study is to investigate the exposure of fish populations in the Athabasca and Slave rivers to environmental contaminants arising from oil sands activities. In addition to investigating the general health condition of the collected fish and we consider contaminant concentrations in their tissues from both fish and human health perspectives.

TH 278

Influence of environmental factors on the response of *Daphnia magna* population to pesticide applications

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Most of the tests to assess the risk of pesticides are performed using standard toxicity tests on *Daphnia magna* cultured individually. However in the environment organisms experience abiotic and biotic interactions like interspecific and intraspecific competition that are likely to be of crucial importance to obtain a response to toxicants' activity. Moreover input to surface waters from agrochemicals typically occurs in pulses or in repeated exposures due to agricultural runoff and spray drift. However, the literature on the toxicological and physiological responses of populations to fluctuating and repeated pollutant exposure is very limited.

In our multispecies test system we investigated how interspecific competition alters the response of *Daphnia magna* to pulses of pesticide.

Populations of *Daphnia magna* were cultured in the absence and presence of the competitor *Culex pipiens* for 82 days, and exposed to 2 pulse contaminations (24 hours every 4 weeks) of the insecticide Pirimicarb.

Three different concentrations 3, 10, 24 µg/L were tested. *Daphnia magna* in both setups (we will refer to them "with" and "without" competition) showed an acute sensitivity to the pesticide only at the highest concentration tested. *Culex pipiens* larvae were insensitive at all concentrations. Multiple contaminations did not increase the sensitivity of *Daphnia magna* to Pirimicarb. Populations in the setup "without" competition were always able to recover within the 28 days between two pulses. However in the "with competition" system we observed no recovery or extinction.

Moreover we investigated the interaction between the two species: at all concentrations of Pirimicarb and in the control, the abundance of daphnia was negatively affected by

the biomass of *Culex* larvae.

Our experiments revealed how an interspecific interaction can negatively affect the response to pesticide applications of one of the most common test organism in ecotoxicology. We conclude that biotic interactions like competition must be considered when conducting an environmental risk assessment of toxicants.

TH 279

Predicting metal and metalloid partitioning in soils using infrared spectroscopic analysis

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Solid-solution partitioning of a metal (termed the K_d value) is a key property that determines its environmental fate in both terrestrial and aquatic systems. The K_d value can be used to predict exposure pathways and which biota are likely to be exposed once a metal is released into the environment. The aim of this study was to derive partial least-squares (PLS) regression calibration models from mid-infrared (MIR) spectroscopic analysis plus soil pH data of soils and experimental derived K_d values to predict the solid-solution partitioning (log- K_d) values for selected elements in soils of the EuroGeoSurveys geochemical mapping of agricultural and grazing land soils of Europe (GEMAS). The strongest spectral correlations for the partitioning of metals in soils were found to be in the regions for carbonate (2500, 1810, and 1350 cm⁻¹), sand (quartz at 1000-1200 cm⁻¹), clay (mostly kaolinite near 3620-3695 cm⁻¹), aluminium oxide (near 3500 cm⁻¹), particle size (quartz near 1000-1200 cm⁻¹), and organic matter (2850-2950 and 1500-1700 cm⁻¹). The PLS regression plus soil pH models were found to predict experimental log- K_d values for cobalt (Co), lead (Pb), manganese (Mn), nickel (Ni), and zinc (Zn) with good accuracy ($R^2 > 0.8$), antimony (Sb(V)), boron (B), molybdenum (Mo(VI)), tellurium (VI) and vanadium (V(V)) with moderate accuracy ($R^2 > 0.6$ to 0.8), and copper (Cu), selenium (Se(VI)), silver (Ag), and tin (Sn(IV)) with poor accuracy ($R^2 < 0.60$). The findings suggest MIR spectroscopy analysis of soils may provide a rapid and cost effective tool to regulators and industry for assessing the partitioning of many elements in soils.

TH 280

Levels and trends of PCBs and PBDEs in fish (*Rutilus rutilus* and *Cyprinus carpio*) and sediment from South Kazakhstan

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RA21P - Standard vs non-standard methods for hazard and risk assessment

TH 281

Evaluation of carcinogenic potential of perfluorinated compounds using *in vitro* and *in silico* alternative approaches

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Perfluorinated compounds (PFCs) are a family of fluorine-containing chemicals used in different applications to make materials oil and water resistant. Regarding human exposure, PFCs are used in wide array of consumer products and food packaging as surfactants. PFCs persist in the environment due to the strength of the carbon-fluorine bond which prevents degradation by natural processes. Because of their persistence, toxicity and widespread occurrence in the blood of humans and wildlife, they are considered as emerging environmental pollutants. Toxic effects of PFCs in laboratory animals includes a range of pathological changes, such as liver and kidney damage and reproductive dysfunctions. Despite the global attention of scientific community, the toxicity and the mechanisms of action of PFCs are still partially unknown: only limited evidences are reported about the ability of these compounds to work as promoter in carcinogenic process. The aim of this work is to provide an evaluation of the carcinogenicity of these compounds using alternative methods including *in silico* methods and *in vitro* assay. Regarding *in silico* approach, a selection of dozen software from the list of EU project ANTARES were used to qualitatively evaluate the carcinogenic potential of PFCs. Freely available (i.e. VEGA, Toxtree, Lazar) and commercial software were used to obtain multiple responses in order to make a comparison of the predicted results. In addition to the *in silico* evaluation, some compounds were tested using the cell transformation assay (CTA). *In vitro* cell-transformation systems have been developed to model the *in vivo* carcinogenesis process and they are regarded as the only possible *in vitro* alternative to animal testing for the screening of potential genotoxic and non genotoxic carcinogens. Selected compounds were assayed using the CTA based on Balb/c 3T3 clone A31-1 cells. When this established cell line is exposed to carcinogens, cells loose the contact-inhibition property and produce *foci*. Results include the overview of the qualitative prediction from *in silico* software and the experimental value from *in vitro* CTA. The evidences from our study underline the need for experimental data for perfluorinated compounds in order to build more suitable *in silico* models and to confirm results from *in vitro* assays. Moreover they emphasize the complementarity of the selected alternative methods that could help in reducing the animal testing for this critic endpoint.

TH 282

Differences between dossier-based PNECs and EQSs based on all available literature: the case of Imidacloprid

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TH 283

Derivation of water quality standards for plant protection products - the importance of open literature

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The European and national authorisation of plant protection products (PPP) is based on registration dossiers which in most cases only include reports of GLP-studies according to accepted guidelines. For quality standard (QS) derivation according to the methodology of the Water Framework Directive (WFD), all relevant available information should be considered. This means that dossier data submitted for e.g. the European PPP or biocides authorisation process should be used, but access to registration dossiers for QS-derivation is often restricted due to confidentiality claims. Data from the open literature are included in the QS-derivation, provided that the scientific reliability is sufficient. As a result, the datasets for authorisation and for QS-derivation may substantially differ. There are also methodological differences between the two frameworks, with respect to the treatment of data and the choice of safety factors. During the past years, RIVM has derived water QS for a large number of active PPP substances. For these compounds, we investigated whether the open literature resulted in additional endpoints that were not included in the dossier, and how this influenced the outcome of the QS-derivation as compared to the PPP-authorisation. For 65% of the compounds additional acute data were found, additional data on chronic endpoints were available for 40% of the compounds. Acute or chronic endpoints below the most critical dossier value were retrieved from the open literature for 27 and 21% of the compounds, respectively. In 37% of the cases, the chronic QS was lower than the authorisation value, with differences of a factor of 2 to 30. This is due to the lower endpoints from the open literature, but also results from the critical evaluation as to whether the potentially sensitive taxa are represented in the dataset. This applies to insecticides and fungicides in particular, since the standard PPP-dossier does not contain chronic data on insects or fungi. Another difference is that transient effects in mesocosms are generally accepted for PPP authorisation if recovery has been demonstrated. It is concluded that an effects assessment based on the dossier data alone may be useful as a first indication of risks. The inclusion of open literature that is foreseen in the new PPP regulation is expected to improve the dataset and yield a more precise and reliable estimate of risk limits that can be used to set regulatory standards.

TH 284

How to cope with variability in laboratory aquatic gnotobiotic microcosms?

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A protocol of ecotoxicological bioassay in 2-L laboratory microcosms is being developed since 1997 and applied to the study of various pollutants and scenarios of ecotoxicological risk assessment in the field of urban facilities and transport infrastructures (polycyclic aromatic hydrocarbons, municipal solid wastes incineration bottom ashes, road runoff residues, dredged sediments, [3DOTS]). Effects are assessed on five different organisms (micro-algae, duckweeds, daphnids, amphipods, chironomids) using classical endpoints such as growth, emergence (chironomids), reproduction (daphnids), survival, with a duration exposure of 3-4 weeks. This bioassay can be considered at first glance as a multi-species test which provides ecotoxicity data for each species present in the microcosm, however it is actually more since it takes into account interactions between biota and media (e.g. N and P consumption by primary producers, bioturbation of sediment by chironomid larvae, [3DOTS]) and interactions between populations (e.g. daphnid grazing of micro-algae, competition for nutrients between micro-algae and duckweeds, [3DOTS]). This type of approach is generally considered as valuable since it states between single-species tests, which lack ecological relevance and predictivity, and mesocosm assays, which display drawbacks due to their complexity (high variability). However, even in gnotobiotic systems where the main components are known, variability still exists to some extent and limits the statistical power of the test. In this communication we will illustrate what variability can be in microcosm assays and show improvements brought to the protocol in order to reduce this variability : breedings and cultures of test organisms, microcosm setup, improvement of organisms fitness in control conditions, monitoring and sampling. The best results were obtained when the protocol was changed from a static test (no renewal of water) to a dynamic test (flow-through renewal of water column), which allowed to stabilise physico-chemical parameters of water and thus to improve the development of organisms and the repeatability of the bioassay.

TH 285

Detoxification to *Daphnia magna* of 4 pharmaceuticals and 7 surfactants by activated sludge

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Pharmaceuticals are bioactive compounds generally resistant to biodegradation, which can make them problematic when they are released into nature. The use pattern for pharmaceuticals means that they are discharged into water via sewage treatment plants. Also surfactants are discharged through sewage treatment plants, primarily due to their use in detergents and shampoos and other cleaners. In this study the acute toxicity to *Daphnia magna* of 4 pharmaceuticals (ciprofloxacin, ibuprofen, paracetamol and zinc pyrithione) and 7 surfactants (C8 alkyl glucoside, C6 alkyl glucoside, sodium caprylimidiopropionate, tallow-trimethyl-ammonium chloride, potassium decylphosphate, propylheptanol ethoxylate and alkylmonoethanolamide ethoxylate) was determined. The abiotic (without activated sludge bacteria) and biotic (with activated sludge bacteria) detoxification was also determined. The 24-h EC50s ranged from 2 µg l⁻¹ for the most toxic substance (zinc pyrithione) to 2 g l⁻¹ for the least toxic compound (C6 alkyl glucoside). The detoxification rate did not differ between pharmaceuticals and surfactants, but within these groups there were differences in detoxification rate, which should be important in assessments of their environmental hazards and risks. Detoxification rates determined as the ratio between initial EC50 and EC50 after one week in water with activated sludge bacteria ranged from 0.4 (paracetamol) to 13 (zinc pyrithione). For most of these chemicals detoxification rate decreased after one week, but for one (alkylmonoethanolamide ethoxylate) it increased from about 2 to 30 times after 2 weeks. Further studies are needed to determine the degradation products that were precipitated (aggregated) for some of the tested chemicals. Altogether, this study has shown that there are large differences in toxicity among chemicals entering sewage treatment plants, but also that the detoxification of them can differ. Therefore, the detoxification should receive more attention in the hazard and risk assessment of chemicals entering sewage treatment plants.

TH 286

Toxicity of 9 shampoos to *Daphnia magna* and their detoxification by activated sludge

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Shampoos are primarily used for hair cleaning, but also to make hair soft and shiny and to prevent dandruff. To achieve these objectives, shampoos contain, in addition to surfactants, other ingredients like emulsifiers, preservatives, plasticizers, perfumes, thickeners and colour. The anti-dandruff shampoos also contain fungicides. Several of these substances are known to be toxic to aquatic organisms, but unlike other cleaning products, eco-labelling is lacking for most shampoos. The aim of this study was to determine if toxicity and detoxification was different for 3 anti-dandruff, 3 ordinary, and 3 eco-labelled shampoos, and to determine their detoxification with and without activated sludge. The results showed that initial 24-h EC50s for *Daphnia magna* ranged from 0.54 to 163 mg l⁻¹ (302-fold). A comparison between labelled ingredients and reported ingredient toxicity using a TU (Toxic Unit) approach could roughly explain the differences among the studied shampoos. The toxicity and also the variation in product toxicity decreased when the shampoos were detoxified (from 302-fold to 5-fold), but still the anti-dandruff shampoos were more toxic than the ordinary and the eco-labelled shampoos. The major finding of this study was that detoxification can be used as a combined determination of toxicity and biodegradation in order to get an estimate of the combined environmental hazard of complex chemical products like shampoos and detergents.

TH 287

Sediment toxicity testing for prospective risk assessment - a new framework and how to establish it

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The development of ecologically realistic and practical sediment toxicity testing for prospective ecological risk assessment remains a considerable challenge. There is a recognised need to design a new framework for sediment toxicity testing that meets current scientific standards and regulatory requirements, such as reliable assessment of toxicity, which prevents any harmful effects on biodiversity, a strong capability to predict population- and community-level effects, and applicability of the results to decision making. We propose a new framework for prospective sediment toxicity testing, and suggest solutions to the key methodological challenges that hinder establishment of this framework (comparison of sensitivities, design of test batteries, consideration of different exposure routes, extrapolations to population and community levels, use of test results for decision making). The proposed framework consists of the following three units: test-battery system, higher-tier testing systems and additional ecological models, and a decision support system. The key methodologies proposed to establish this framework are compound- tailored test-battery use approach, relative sensitivity distribution analysis, toxicity tests that combine bacteria and arthropods, micro- and mesocosms studies, population and community models, and model-driven decision support systems. The proposed framework, as well as the key methods mentioned above, has the potential to improve not only prospective toxicity testing for sediments, but also ecological risk assessment in general.

TH 288

Improved OECD 105 water solubility test design

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In this poster a simple modification to the OECD 105 water solubility Guideline is described that can improve fitness of data for use in environmental risk assessment of poorly water soluble substances.

The OECD 105 Guideline comprises two methods, one using a shake flask method for water soluble substances (liquids or solids) and the other using a column elution method for poorly soluble solids (despite the fact that liquids are not formally excluded, the column elution is often not technically feasible, due to clogging for example). Poorly soluble liquids therefore pose a problem as the shake flask method can lead to the formation of emulsions and potential overestimation of the solubility. Furthermore, the solubility of certain substances in pure water may differ, sometimes considerably, from their solubility in the more relevant environmental media potentially leading to misconceptions of the toxicity of the substance in certain cases.

The proposed simple modification can be used to improve solubility determinations for poorly soluble liquids and provide reliable indications of true solubility in aqueous media.

It was successfully tested in a first experiment, but needs further improvement.

TH 289

Intense sweeteners in the aquatic environment - is there a bitter (environmental) aftertaste?

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Presence of intense (artificial) sweeteners in the aquatic environment have led to concern about toxic effects in non-target species living in areas receiving discharges from anthropogenic activities. The present work presents the current knowledge about sucralose, as a representative of intense sweetener, on environmental occurrence, fate and effects in the aquatic environment to assess whether there is a reason for environmental concern. Sucralose exhibits high chemical stability, high water solubility, is not metabolized by humans and poorly degraded in sewage treatment works, and is therefore discharged largely unaltered to the aquatic environment where it is found in sewage effluents at low ng/L to moderate µg/L concentrations. As sucralose is not efficiently mineralized in the environment, surface water concentrations are typically 1-2 orders of magnitude lower. No acute or chronic toxicity has been demonstrated at environmentally relevant concentrations in standardised regulatory valid ecotoxicological tests with algae, crustaceans and fish. However, reports of sub-lethal responses in aquatic organism behavior, respiration and global gene expression after exposure to sucralose introduce a need for discussing available data in the context of regulatory and non-regulatory risk/hazard assessment.

TH 290

Model validation in 96h acute aquatic toxicity testing

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The validity of toxicity test results from standard testing protocols is contingent on whether the employed models and assumptions are appropriate and sufficient. A quality control evaluation of conceptual and technical models used in the 96 h acute toxicity test protocol and the ensuing data was carried out. The standard LC50 test design uses exposure concentration as an indirect dose surrogate for the true dose metric - the molar concentration of chemical at site's of toxic action in the body of the organisms. Thus, three key enabling assumptions need to be routinely examined and validated to ensure that LC50 test results represent consistent, comparable measures of relative toxicity. First, steady-state LC50s must occur. Second, steady-state LC50s must occur at a duration that ensures equivalent exposure. Third, protocol design must ensure that all toxicity modifying factors are controlled. A subset of the U.S. EPA-Duluth fathead minnow 96 h toxicity database was analyzed. Approximately 8% of the tests failed assumption 1 validation and should be considered unusable. Although the time to achieve steady-state varied by a factor of about 2000 in the remaining 92% of the data, these data passed validation of assumption 2. However, variance from unquantified effects, ostensibly the result of toxicity modifying factors, such as use of inappropriate exposure times (lack of steady-state), metabolic biodegradation, and body size differences, remain embedded in LC50 estimates rendering them inaccurate measures of relative toxicity, thereby failing assumption 3 validation. Improvements in toxicity test design and interpretation, including explicit specification of models, routine validation of assumptions, and collection of additional information for quantifying the influence of metabolic degradation and other currently uncontrolled toxicity modifying factors, as well as more information on mode/mechanism of toxic action, are necessary. A thorough quality control evaluation of 96 h LC50 methodology and related protocols is warranted to ensure that reliable data are being used for hazard and risk assessment and in the development of QSARs.

TH 291

Assessment of Cadmium effects using standard single-species and non standard microcosm bioassays

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Ecotoxicological assessment of chemical substances is mainly based on standard bioassays, most often single-species tests covering the range of acute and chronic toxicity. In our laboratory we have been developing since 1997 a protocol of ecotoxicological bioassay in 2-L laboratory microcosms and applied it to the study of various pollutants and scenarios of ecotoxicological risk assessment in the field of urban facilities and transport infrastructures. Effects are assessed on five different organisms (micro-algae, duckweeds, daphnids, amphipods, chironomids) using endpoints such as growth, emergence (chironomids), reproduction (daphnids), survival, with a duration exposure of 3-4 weeks. The protocol has mainly been used as a static assay, where water was not renewed for the duration of test. A flow-through microcosm assay (dynamic assay) was developed to improve conditions inside the microcosms, resulting in stabilisation of physico-chemical parameters, increase of organisms fitness and reduction of variability. Since toxicity is closely linked to the conditions of bioassay, which influence the chemical speciation of the substance and the dynamic of exposure, we aimed to compare informations produced by standard single-species tests, multi-species tests with an aqueous phase only, and microcosm bioassays (static and dynamic) on five different species used in both types of tests. Cadmium was chosen as a model toxicant and introduced only at the start of test in single- and multi-specific static assays, and continuously in the dynamic microcosm assay. Cadmium concentrations were monitored in several bioassays, especially in the presence of a sediment phase, due to expected sorption of Cd. Effects on the same organisms in various conditions (duration of test, type of medium, presence or absence of sediment, renewal of water or not) were compared and discussed based on these different conditions. The presence of sediment, leading to decline of water cadmium concentrations and to a multiple exposure of some organisms, complicates the establishment of a link between cadmium concentration and effects, and thus the comparison of simple single-species tests and microcosm assays. However, these ones give a better insight of the fate and effects of cadmium in aquatic environment. Compared to the static microcosm test, the dynamic microcosm test, where cadmium concentrations are kept constant in the aqueous phase, provides less variable data and is more sensitive.

TH 292

Influence of pH, light cycle, and temperature on ecotoxicity of four sulfonylurea herbicides towards *Lemna gibba*

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In chemical risk assessment standard tests are applied to establish the basis for evaluating the hazard of compounds and determination of environmental standards. Using standard tests enables the comparison of the toxicity of different compounds but it may not always describe all aspects of the toxicity of a compound in the environment. The toxicity may be different under different test conditions such as light regime, temperature or pH of the medium. In the environment organisms are rarely exposed to compounds at conditions identical to those of the standard tests, and therefore it is important to study the effect at different test conditions in order to get a more realistic assessment of the potential risk posed by a compound. Therefore, the objective of this study was to investigate what implications changing test conditions may have on the toxicity of a group of model compounds, and thereby obtain a better basis for risk assessment extrapolations. As model compounds were chosen four sulfonylurea herbicides (SUs), which are well-studied compounds in regards to standard tests, although they are somewhat difficult to handle in the lab because they hydrolyse quickly and are in the ionized form depending on pH of the medium. The toxicity of the four SUs towards *L. gibba* was investigated at three pH levels (6, 7.5 and 9), at different temperatures (15 and 24 °C) and under different light regimes (continuous and 12:12 h light:dark). It was shown that test conditions have an effect on the toxicity on the four SUs. Decreasing the pH below the standard test value resulted in a decrease in EC50 values, while increasing the pH caused either no change or an increase in EC50. In general, the EC50 increased between 2.2 and 10 times for the four SUs when pH was increased from 6 to 9. Decreasing the temperature from 24 to 15 °C and introducing a light-cycle did not cause any trends in changes in toxicity. The results indicate that test conditions may have an effect on the toxicity of the compounds studied here and if there is also enough data available to justify a low assessment factor, this should be taken into account when the standard tests are used in determination of environmental standards.

TH 293

New short term toxicity test using single algal species and natural phytoplankton

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Standard algal test using *Pseudokirchneriella subcapitata* normally have a duration of 72 hours. This method might not be feasible for compounds that have fast kinetics of hydrolysis or photolysis, or compounds that have been shown to exhibit properties of sorption or volatilization. Of special interest are also Engineered Nanoparticles (engNPs) which have been shown to agglomerate or adhere to the surface of test organisms. These physico-chemical characteristics, as well as test exposure/duration can therefore influence test results. An alternative to better control these confounding factors in the experiment is to shorten the duration of exposure.

Currently, there is a need to develop tests using natural algal communities to increase the environmental relevance. Thus, the objective of this study was to develop a short term test with natural community algae and elucidate on advantages and limitations, as well as compare sensitivity with standard algal test. The test principle is to expose the algae to a toxicant gradient in the presence of 14C-labelled bicarbonate for 2-3 hours and measure 14C uptake by scintillation counting. Both natural algae communities from Lake Fure (collected at different times of the year), and a laboratory culture of *P. subcapitata* were used. The algae were exposed to a range of different toxicants such as photosynthesis inhibiting herbicides, acetolactate synthase (ALS) inhibiting herbicides, titanium dioxide and silver nanoparticles, and standard reference compounds such as potassium dichromate and 3,5-dichlorophenol. Preliminary results show no toxicity of ALS inhibiting herbicides neither for the natural algal community nor the laboratory culture at the concentrations tested.

TH 294

Quality evaluation of existing critical body residue data for baseline neutral narcosis

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The Environmental Residue Effects Database (ERED) developed by the U.S. Army Corps of Engineers and the U.S. Environmental Protection Agency is the most extensive environmental CBR compilation available. ERED was employed for a data quality evaluation of available CBR-effect relationships for baseline neutral narcosis. Evaluation began with an ERED subset consisting of 7574 records for 318 organic chemicals from 651 references for fish, invertebrates, and algae. The first quality screening used 14 different criteria (e.g., no adverse effects reported, testing of mixtures, difficulties interpreting exposure and responses metrics) to remove inappropriate data. This resulted in an initial working database of 2271 records for 182 chemicals from 294 references that met minimum data quality requirements. A second screening removed data where a specific mode of toxic action was suspected (all or most of the reported CBRs were below about 0.1 mmol/kg). A third screening removed data based on a paper by paper review of experimental results. All data removal and screening results and rationales were documented. The penultimate database for baseline neutral narcosis consisted of 278 records for 39 chemicals from 37 references. Three chemical subgroups were noted: neutral organics, polar organics (polar, ionizing, and nitro-containing compounds and substituted phenols), and PAHs. Narcosis quality evaluation proceeded by rating data either as poor (4 rules) or rejected (11 rules). The rules were based on experimental issues (e.g., absent, low or difficult to interpret responses, experimental problems, specific toxicity according to the Verhaar classification scheme). The final evaluation eliminated some of the neutral organics, all of the polar organics group, and 1 PAH. The remaining high quality CBR data was for 27 chemicals in four general classes: substituted benzenes, other organics, PCBs, and PAHs.

These chemicals are considered to be baseline narcotics, based on this data quality evaluation. This is not to say that these are the only narcotics in the database, or that they always produce toxicity by the baseline narcosis mode of toxic action, only that these data have passed the specified data quality evaluation process. These evaluated data will form the basis for toxicokinetic model development and evaluation.

TH 295

Validity criteria from standard method: the need for assessor common understanding

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Standard data are generated according to recommended and internationally agreed test methods (e.g. OECD Test Guideline, EU Testing Methods). Test guidelines are well described providing technical information on principle of the test, description of test method and usually validity criteria. For the purpose of the evaluation of data quality under Chemical Regulatory program, scientific study reports are primarily checked by assessors to see whether validity criteria are met or not. Within the context of harmonization of data evaluation worldwide, a common understanding of validity criteria from standard method by assessor is a key component for conducting an appropriate reliability assessment. In this presentation, we describe lack of common interpretation regarding one of the three validity criteria from the recommended guideline on Freshwater Alga and Cyanobacteria, Growth Inhibition Test. We explore the implications for reliability assessment and the possible ways to increase assessor common understanding of validity criteria on Algae toxicity testing.

TH 296

Endpoints and dose-response relationships of low-dose and standardized studies of Bisphenol A

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Bisphenol A (BPA) is used in large volumes to manufacture polycarbonate plastics and epoxy resins, which are materials used in numerous consumer products. Diffuse emissions of endocrine-disrupting chemicals (EDCs), including BPA, from consumer products to both indoor and outdoor environments have been identified as an emerging issue. In risk assessment and risk management of BPA, the evaluation and interpretation of the low-dose studies (where effects are reported below 50 µg/kg/day) has been extensively debated.

This study aims to provide an overview of endpoints used in the low-dose toxicity studies of BPA, at what levels the studied effects are reported (NOAEL and/or LOAEL), if the dose-response relationships are monotonic or not, and if exposure is prenatal, postnatal or both. These aspects were compared to the studies of the effects of BPA performed according to standardized test methods.

Data were compiled from a comprehensive review of studies concerning the effects of BPA in the open scientific literature. Endpoints were categorized, e.g. into reproductive

effects, developmental neurotoxic effects and immunological effects, and comparisons were made within and between the endpoint categories. The differences identified between the low-dose studies and the standardized studies are important to consider in the development of appropriate and sensitive criteria for EDC identification and in the refinement of risk assessment methods for BPA, as well as other EDCs.

TH 297

Use of public literature and dossier data in WFD EQS derivation compared to risk limit derivation in other regulatory frameworks: the case of silver

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Recently in the Netherlands a revised Environmental Quality Standard (EQS) for silver was derived. For EQS derivation according to the methodology of the Water Framework Directive (WFD), all relevant available information should be considered. This means that dossier data submitted under REACH should be used, but also data from open literature should be included, provided that the scientific reliability is sufficient and endpoints are relevant.

For the derivation of a chronic EQS for silver in freshwater, 71 unique data sources were found in public literature and the REACH dossier, including reviews. These data sources were scored for reliability using the Klimisch scores (1=reliable; 2=reliable with restrictions; 3=unreliable; 4=unassessable). Studies with a validity score of 1 or 2 were used for final EQS derivation.

In the REACH dossier, for chronic toxicity in freshwater 52 records are available. Within these records, there is a substantial number of duplicates, and the way the records are described varies greatly. Publication sources are often only mentioned as 'publication', 'study report', or 'grey literature'. Thus, public literature is included in the dossier, but it may be difficult to determine the original source. Industry has the obligation to conduct literature searches, thereby disclosing all relevant data, but there is no mechanism in REACH that evaluates this aspect. The only formal requirement, however, is at a minimum fulfilling the basic requirement according to the Annexes VII-X. Within REACH, Klimisch scores may be applied differently than for EQS derivation. For instance, studies in the dossier for silver which were performed using a slightly shorter or longer exposure time than what is described in the OECD guideline, received a reliability score of 3 ('unreliable') while in the EQS derivation they could still be seen as reliable.

Records in the REACH dossier are divided into key studies and supporting studies. In the silver dossier, there are a number of supporting studies with a reliability of 1 and a number of key studies which refer to 'grey literature' without further details. The rationale behind this division is not described in the publicly available part of the dossier. Often, there is no publicly available explanation as to how PNEC values are derived using the available data. This makes a comparison between the PNEC and the EQS difficult.

TH 298

Comparison of methods of application on the toxicity of a test substance to the carabid beetle *Poecilus cupreus*

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For soil incorporated substances it is important that the method of application used in evaluation of the effects on the soil dwelling non-target species *Poecilus cupreus* represents a more realistic situation in regard to exposure and bioavailability than the standard sprayed application in which the carabid beetles, food and substrate are contaminated.

Direct spray application of an insecticide product was compared to simulated shank-chisel and drip irrigation applications with the objective of demonstrating that the method of application has a direct influence on the survival, behaviour and predation rate of adult carabid beetles. For the simulated shank-chisel and drip irrigation applications, the test substance was applied at rates equivalent to a field application rate and volume. Beetles were introduced into the test chambers after application. The comparative spray applications were made at the same rate using a compressed air single lance sprayer calibrated to deliver a spray volume equivalent to 400 L/ha, with the beetles in-situ during application (standard test technique). Control treatments were applied using the same methods. Post application one *Musca domestica* fly pupa per beetle was introduced into each test chamber to provide a food source.

Survival, abnormalities (affected or moribund beetles) and food consumption were assessed and recorded at intervals for 21 days after application. The results showed that there were significant differences in the survival of the adult beetles between the methods of application. Investigation into the relevant application method may be a contributing factor in the design of future studies to ensure the presentation of appropriate data.

TH 299

Modification of the OECD 117 Partition coefficient using the HPLC method for high log Kow substances

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Estimation of bioaccumulation potential of an organic substance may be obtained through measurement of its partition coefficient. The current OECD/EU method based on HPLC method has been validated up to 6.5/6.2, respectively, based on the highest reference substance (DDT) recommended, and extrapolation is expected to lead to high uncertainty. The slow-stir method is not either designed to go higher than log Kow = 8.

However, a low log Kow <4 or extremely high value >9 for Canada or >10 for the EU are considered strong indicators of low potential for bioaccumulation. BCF is expected to increase with log Kow, increasing up to a maximum at approximately 7.5, but then decreasing thereafter. Substances with very high log Kow (>9) are not expected to bioconcentrate greater than 5000 (for Canada) and with log Kow >10, BCF is expected to be <2000, these values being the thresholds for the B criterion in PBT/vPvB assessment under REACH.

Therefore, for test substances beyond the application range of guidelines into force, the limit value does not allow a definitive conclusion on bioaccumulation potential and no method has yet been validated to demonstrate log Kow >9.

This poster illustrates an attempt to select new reference substances for the HPLC method, with higher log Kow values (7 to 10), in order to obtain adequate result for bioaccumulation assessment. The KowWin database was screened for homologous compounds, with linear increase of their log Kow as a function of carbon number, and with structural similarity compared to a given test substance. A total of 18 candidates between 6.97 and 10.89 was selected, among which 9 of analytical grade are commercially available. However, the HPLC method may be subject to limitations in this working range, and suitability of this new set of reference substances for high range log Kow determination have to be confirmed by experimentation before any update proposal of the guideline.

TH 300

Poorly soluble cosmetic compounds - ecotoxicological assessment and substitution' (ECOSM)

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In order to meet different product requirements, 'Personal Care Products' contain a set of various substances. Each substance is meant to fulfil a specific scope in the final product and therefore the substances may show a great variability in physico-chemical properties. 'Poorly soluble substances' are characterised by low water solubility (< 1 mg/l) and represent important ingredients of many cosmetic products. Due to their large use quantities a reliable assessment of their environmental behaviour and their toxicity to aquatic organisms becomes necessary, e.g. as requested by the European REACH-Regulation. However, at the threshold of the water solubility of such substances, ecotoxicity tests are very difficult to conduct. High lipophilicity and thus extensive adsorption of the substance to surfaces, e.g. to test vessels and organisms, complicates the test performance. The majority of existing standard tests provide inadequate test results that can hardly be used for an appropriate risk assessment. The 'poorly solubles approach' is one promising method to solve this issue. The basic assumption is that the concentration of highly lipophilic and poorly soluble substances and, thus, their exposure to aquatic organisms is very low in the aqueous phase of water bodies. As a result of an extensive analysis of ecotoxicological data, the so-called aquatic exposure threshold of no ecotoxicological concern (ETN_{Caqu}) could be defined for inert substances with a narcotic mode of action. Neither acute nor long-term adverse effects are anticipated on aquatic organisms when concentration of such substances is below ETN_{Caqu} = 1,9 µg/l. For the screening of potential ecotoxicological substances with PBT-properties (persistent + bioaccumulative + toxic), the 'poorly solubles approach' also seems to be pertinent. Due to its potential relevance for assessing the environmental risks of highly lipophilic substances, the scope of the project ECOSM is to develop the 'poorly solubles approach' to a pragmatic and reliable evaluation tool. In this context, existing aquatic ecotoxicological test methods have already been adapted to passive dosing techniques and several lipophilic compounds are being tested to validate the 'poorly solubles approach'.

TH 301

A divergence of paths? Novel endpoints in hazard and risk assessment

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Apical responses on survival, growth, and reproduction have long been the mainstay of ecotoxicology research, integrating effects at the organism level. Yet, as science progresses, and there is increasing pressure to accelerate risk decisions and reduce animal use, biomarker endpoints and in vitro data use in early hazard assessment are taking us down a new path. The challenge is in relating these 'omic', cellular, and biochemical markers to adverse effects in a relevant and reproducible manner. Bisphenol A (BPA), is an important high production volume compound used in the production of epoxy resins and polycarbonate plastics. It is also one of the most studied, particularly for potential endocrine interactions. The database of ecotoxicological results for BPA includes traditional guideline acute and chronic data addressing population level effects of survival, growth and reproduction, as well as a variety of studies with secondary, often unique sublethal or biomarker-type endpoints, and a growing library of 'omic', cellular,

and biochemical responses. For example, a number of highly replicated fish life-cycle and multi-generational tests have examined growth, development and reproductive success in combination with biomarker endpoints such as vitellogenin, GSI, and gonad histology. Analyses of sperm quality, gonad development, VTG, genomic markers, and other non-traditional endpoints have also been reported in the literature, often in isolation of a link with more traditional endpoints. In determining the utility and relevance of these studies and their novel endpoints in a risk assessment framework, a clear understanding of the relationship between biomarker and sublethal findings, which may or may not be adverse, to population relevant assessment endpoints must be established and data quality and reliability must be determined. The evaluation of data, including both traditional and non-traditional endpoints should be done in a weight of evidence approach in order to reach a scientifically defensible risk determination. In so doing, potential adverse impacts of the compound can be defined and used as measures of effects that are directly related to assessment endpoints for a sound and robust risk assessment. A case study describing this approach for the evaluation and use of both traditional and non-traditional endpoints will be presented using examples from the extensive aquatic database for BPA.

RA23P - Wastewater effluent discharges: chemical characterisation and understanding potential risks in receiving waters

TH 303

Risk assessment of WWTPs water effluents using fuzzy logic

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This paper presents a new methodology to assess the risk of water effluents based on fuzzy logic, a very well-known theory to treat uncertainty and vagueness, especially in the environmental field where data many times are not fully available. The method has been tested using the effluent's pollution data coming from 22 waste-water treatment plants (WWTPs) located in Catalonia (NE Spain).

The driving force to develop this study has been the increasing need of prioritizing pollutants appointed by different water regulations such as the Regulation 166/2006 [1] concerning the establishment of a European Pollutant Release and Transfer Register. It aims at establishing a Community level register of integrated pollutant release and transfer (known as 'the European PRTR' or 'E-PRTR'). Its application domain affects certain types of manufacturing and production facilities, among them waste-water treatment plants (WWTPs) with a capacity of more than 100,000 equivalent inhabitants. Data gathered under the E-PRTR regulation provide a valuable source of information regarding the emission of pollutants to air, water and waste from the assessed installations.

The Catalan Water Agency (ACA) conducted three different campaigns with this purpose in 2008, 2009 and 2010. A total of 41 micropollutants belonging to different families (e.g. heavy metals, volatile organochlorine compounds (VOX), Polycyclic Aromatic Hydrocarbons (PAHs)), were analysed in the sampling campaigns and their potential risk for the environment and human health has been assessed using the here presented method. Appropriate referenced analytical methods were used to obtain concentration levels of these pollutants.

In order to ensure the appropriateness and consistence of this new method, the results have been compared with a well-trusted method used for chemicals prioritization, the COMMPS procedure developed by the Fraunhofer Institut [2] in 1999.

From the results gathered using the fuzzy model, it has been possible to characterize and compare the different sites (WWTPs) according to their associated risk as well as prioritize the compounds according to their relative risk. Finally, the fuzzy model has been compared with the COMMPS methodology, obtaining more conservative results with the first one due to the inclusion of persistence and variable's weight and the consideration of uncertainty. However, the behaviour of the two models is quite similar, fact that gives consistency to the fuzzy logic model.

TH 304

Simple equation allows assessment of biodegradation of cationic surfactants in activated sludge reactors (OECD 303)

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The Continuously-fed Activated Sludge (CAS) test is suitable to predict the removal of organic compounds from wastewater in activated sludge plants. However, a method to distinct between removal by adsorption and biodegradation is not provided in the Guidelines. Accurate knowledge on the relative role of the different mechanisms in the overall removal of surfactants in activated sludge systems is therefore not available.

The main operating parameters i.e. hydraulic retention time (HRT) and sludge retention time (SRT) are strictly maintained during operation of a CAS unit. The following equation to calculate the maximum concentration of surfactant adsorbed on the sludge was derived from a mass balance for the CAS unit:

$C_{\text{surfactant sludge max}} = \text{SRT/HRT} * (C_{\text{surfactant influent}} - C_{\text{surfactant effluent}})$. (Bio)degradation was assumed not to occur. This maximum concentration of surfactant adsorbed on the sludge and the measured surfactant concentrations in the mixed liquid suspended solids allow calculation of biodegradation percentages. The removal of five cationic surfactants with varying adsorption capacities (decylamine, tetradecylamine, octadecylamine, dioctadecylmethylamine and dioctadecyldimethylammonium chloride) was assessed in CAS tests. The removal from the wastewater ranged from 98.8% (dioctadecylmethylamine) to >99.9% (decylamine). Removal by biodegradation was 69% (dioctadecyldimethylammonium salt) to >99.9% (decylamine). These removal percentages were compared with modelling results obtained with SimpleTreat. Simple Treat not only underestimates the potential of biological treatment systems to remove surfactants from wastewater but also the fraction removed by biodegradation. Especially, biodegradation of surfactants with a high potential to adsorb is not enough appreciated by SimpleTreat. The expression should be included in an update of the test guideline.

TH 305

Predictive modelling of steroid oestrogens in sewage effluent demonstrates the potential for endocrine disruptive effects in wild fish populations in South Australia

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Endocrine disruption (ED) has been observed in wild fish populations worldwide and is particularly well characterised in the UK, where the presence of oocytes in the male gonad and high levels of the female specific protein vitellogenin has demonstrated a feminisation response in wild roach (*Rutilus rutilus*). These phenotypes have been linked to sewage effluent containing the natural oestrogens 17 β -oestradiol (E2) and oestrone (E1) as well the pharmaceutical 17 α -ethinylestradiol (EE2) originating from human excretion. In contrast, little is known about the presence and effects of these chemicals in Australia, where there is a greater strain on water resources. Oestrogens have been measured in sewage effluents at levels comparable with the UK and there is some evidence of ED effects in fish downstream of sewage treatment works (STW's). It is therefore advisable to determine the potential levels of oestrogens entering Australian rivers and to assess the risk to wild fish populations. This study demonstrates the first use of predictive modelling of oestrogens in Australian STW effluent, creating predicted environmental concentrations (PEC's) for a set of STW's in South Australia and comparing them to the UK using a modified version of a model previously for risk assessment in both Europe and Japan. The PEC's were accumulated into an E2 equivalent concentration (EEQ) and were corroborated against measured concentrations from one UK and one Australian STW to determine their accuracy. These were then compared with the UK Environment Agency's predicted no-effect concentration (PNEC) of 1ng/L E2. Predictive modelling was shown to provide a good method for first tier risk assessment estimating the concentrations of oestrogens in effluents. The model overestimated the measured EEQ by an average factor of 1.4 when corroborated with the more robust UK STW data, however the effluent EEQ PEC's for all STW's were 3-31 times higher than the 1ng/L PNEC. The model demonstrated that although Australian STW's serve a lower population, their EEQ's are not significantly different to the UK's, which is likely to be due to the lower flow through Australian STW's which lowers the dilution factor. The results predict that effluents discharged in both the UK and Australia both contain oestrogens at concentrations exceeding the Environment Agency PNEC, suggesting that without sufficient dilution in rivers there is a risk of ED effects occurring in wild fish.

TH 306

Inputs of pollutants by urban wet and dry weather pollution in combined sewer systems: pau urban catchment (CDAPP, France)

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Urban inputs that include household effluents, drainage water, business effluents (e.g. car washes, dental uses, other enterprises, etc.), atmospheric deposition and traffic-related emissions (vehicle exhaust, brake linings, tires, asphalt wear, gasoline/oil leakage, etc.) transported either to wastewater treatment plants or directly to the aquatic environment, are expected to represent today important sources of pollution to the aquatic environment. Wastewater treatment plants represent a major threat to the aquatic environment since they are collecting a wide variety of sources from both domestic and industrial activities and they are continuously discharging various polluted effluents. Urban storm water runoff which is considered as transient source may also contain high levels of pollutants discharged directly in receiving streams by Combined Sewer Overflow (CSO). Implementation of the European Water Framework Directive and its affiliated directives requires Member States to improve their understanding of priority pollutants in urban areas and obviously within wastewater systems.

We studied the urban catchment of Pau urban area (south west France) which is about 50 km² with about 150 000 inhabitants. 4 rain gauges and about 40 flow meters were installed in all the study area to monitor the combined sewer network. Effluents were followed during about 10 campaigns including wet and dry periods for Suspended Solids (SS), Chemical Oxygen Demand (COD), Total Nitrogen (TN) and trace metals (Cd, Cu, Pb, Zn, Al, Fe, Cr, As, Sn). Emphasis is given to the characteristic, the partitioning and the contribution of pollutants from wastewater inputs during dry periods and wet periods in the combined sewer system of Pau. Concentrations of pollutants were combined with flow rate to assess fluxes that were directly discharge into receiving stream by CSO or carry on to the Waste Water Treatment Plant (WWTP). Following a storm event of about 2 hours we showed that this storm event induces an increase of fluxes by a factor of 1.5 to 5 compared to dry conditions. Half to two thirds of pollutants fluxes were treated by the WWTP during this period. The remaining part of the effluent was discharged in the receiving stream the river Gave de Pau. Fluxes of trace metals followed generally the same percentage than SS, suggesting that metal load is mainly associated to SS.

TH 307

Demography, economic activities and health equipments as potential sources of pharmaceutical compounds in wastewater and performance of wastewater treatment in two

French catchments

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Presence of pharmaceuticals in wastewater is attested by numerous studies. Although important research work is conducted on wastewater treatment plant (WWTP) performances, less is known on the contaminants sources. In fact, this information coupled with the knowledge of treatment efficiency could be very useful to manage WWTPs. In this perspective, the objectives of this work were to assess if pharmaceuticals concentrations in wastewaters could be explained by urban parameters in two urban catchments (A and B) and to compare their treatment performance.

Only 40 km separate both catchments, which have similar population density and distribution. However, catchment A has a higher number of inhabitants, health equipments and economic activities. Both WWTPs use the same technology (membrane bioreactor) to treat urban wastewater, but have different inflows (catchment A 10 times higher than B). Influent and effluent streams were monitored during 3 campaigns over 3 days with 24 hours composite samples. Pharmaceuticals and hormones were analyzed using LC-MS-MS. Results are shown for 6/32 studied compounds: acetaminophen (ACT), diclofenac (DIC), carbamazepine (CBZ), sulfamethoxazole (SMZ), iomeprol (IMP) and 17 β -estradiol (E2).

Fluxes were 3 to 9 times higher in WWTP-A for ACT, E2, SMZ and DIC, 80 times higher for CBZ and 1350 times higher for IMP. The differences for CBZ and IMP were not only explained by the cities sizes but also by the presence of more potential sources in catchment A than B. Concentrations of DIC, SMZ and E2 were similar in both WWTPs. CBZ and IMP were respectively 10 and 183 times higher in WWTP-A. In contrast, ACT was 2 times higher in WWTP-B. Those concentrations could be explained by a dilution effect due to the higher discharge in WWTP-A. Then, in both WWTPs, concentrations of CBZ and DIC in effluents were respectively 0% and 6-42% lower than in influents. In contrast, IMP, ACT and E2 showed higher elimination between 76% and 99% in both WWTPs. Therefore, removal efficiencies SMZ was quite stable in WWTP-A (74% to 85 %) and greatly varied in WWTP-B (0% to 96%).

Results showed the presence of potential pharmaceuticals sources: some were strongly suspected, others needed further investigation. The main perspective could be to develop a methodology to predict the fluxes of compounds entering the WWTP based on urban characteristics to define suitable technologies for the best treatment efficiencies.

TH 308

Wastewater effluents and river waters: sources of organic micropollutants to NW Mediterranean coastal waters. Their characterization, fluxes and potential risks

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This study provides a first estimation on the risk associated with the inputs of organic micropollutants (OMPs) to coastal waters from NW Mediterranean Sea. Pollution sources evaluated were wastewater treatment plants (WWTP) effluents and river waters discharged to the sea. Polycyclic aromatic hydrocarbons, polychlorinated biphenyls, organochlorinated pesticides, polybrominated diphenyl ethers, phthalates and alkylphenols were analyzed by solid phase extraction and gas chromatography coupled to tandem mass spectrometry (SPE-GC-EI-MS/MS). Eight WWTP effluents and six rivers discharge an estimated amount of around of 25800 g d⁻¹. The concentration of Σ OMP in coastal areas ranged from 17.4 to 8442 ng L⁻¹. A summarized overview of the patterns and sources of OMPs contamination on the investigated coastal sea waters of NW Mediterranean Sea, as well as of their geographical distribution was obtained by Principal Component Analysis of the complete data set after its adequate pretreatment. Alkylphenols, bisphenol A and phthalates were the main contributors to Σ OMP and produced a significant risk for fish, algae and the sensitive mysid shrimp species in seawater samples. The combination of GC-MS, chemometrics and risk analysis proves of use for a better management and control of OMPs discharges.

TH 309

Chemical characterization and treatment by enhanced coagulation of dissolved organic matter in biochemical-treated effluent of textile wastewater

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Performed with regular physicochemical-biochemical combined treatment process, textile wastewater effluent was treated by enhanced coagulation with a new kind of coagulant. Dissolved organic matter (DOM) in biochemical-treated effluent of textile wastewater as well as effluent treated by enhanced coagulation were isolated and enriched by extraction. Both DOM isolates were characterized by Gas Chromatography-Mass Spectrum (GC-MS) and both effluents by Excitation Emission Matrix (EEM). Results showed that biochemical-treated textile wastewater effluent mainly contained hydrophilic organic matters: triethylene glycol, tributyl phosphate and phthalates, and hydrophobic organic matters: amino acids and humic substances. And after enhanced coagulation treatment, most of the dissolved organic matter was removed from the biochemical-treated textile wastewater effluent.

TH 310

Emerging substances in aquatic phase of landfill leachate

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The potential environmental impacts related to landfill leachate are pollution of groundwater and surface waters. The risk of groundwater pollution is probably the most severe environmental impact from landfills because historically most landfills were built without engineered liners and leachate collection systems. Emerging pollutants are used in large quantities in everyday life and include a diverse group of myriad compounds (e.g., pharmaceutical compounds from different therapeutic classes, personal-care products (PCPs), flame retardants, industrial additives, anticorrosive compounds and others emerging species). The emerging substances are contaminants that have been recently discovered in the surface water due to their long-term, pseudo resistance and increased use in all industrial and human activities. Existing data show high leachate concentrations of all inorganic and organic compounds in the early acid phase due to strong decomposition and leaching. This paper presented the list of verified and detected EmS and various processes which take place in the aqueous layer of the landfill body as the typical heterogeneous system. It will be reported qualitative and quantitative data on the occurrence of emerging compounds with increasing environmental and public health concern. Products from chemical reactions and waste decomposition of emerging substances from aquatic phase of unsanitary landfills could contaminate the surface water, groundwater and aquifer. The preliminary results of Danube surface water in the vicinity of Novi Sad show presence of benzotriazole and caffeine. In some samples of groundwater the most frequently detected were diclofenac, ibuprofen, carbamazepine and metamilzol metabolites 4-AAA and 4-FAA, within 19 pharmaceuticals that were analyzed in Serbia. This research was supported by Ministry of Education and Science, Republic of Serbia (Project number III46009 and 37014) and Program Science for Peace and Security (NATO), Project No ESP.EAP.SFP 984087.

TH 311

Removal of contaminants of emerging concern by a sewage lagoon

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Sewage lagoons are used widely in small communities in North America to treat municipal wastewater prior to discharge into surface waters. There has been little work to evaluate the efficacy of removal of contaminants of emerging concern by these small treatment systems. In this study, a sewage treatment lagoon for the village of Lakefield, ON in Canada was investigated to determine removals during the summer, fall and winter of selected PPCPs, including carbamazepine, trimethoprim, sulfamethoxazole, ibuprofen, gemfibrozil, triclosan, and HHCb and AHTN synthetic musks. POCIS and SPMD passive samplers were deployed at three locations in the sewage treatment lagoon, as well as at several points downstream of the wastewater discharge in the Otonabee River. Extracts from the POCIS and SPMDs were analyzed by LC-MS/MS or by GC-MS. Among the pharmaceutical target compounds, the highest concentrations in the sewage lagoon were measured in the fall for ibuprofen at an estimated concentration of 60 ng/L. Removal rates for ibuprofen and sulfamethoxazole were >75%, but carbamazepine was not removed effectively in the lagoon. Gemfibrozil showed a significant increase in concentrations as it passed through the sewage lagoon, which can be attributed to de-conjugation of metabolites during treatment. Trimethoprim was removed with intermediate efficiency (14-71%). There was a seasonal trend for removals of pharmaceuticals as they declined in the order of the sampling periods in the summer>fall>winter. Estimated concentrations of the target compounds in the river were very low (<1 ng/L) or below detection limits. The small scale sewage lagoon monitored in this study was effective at removing PPCPs at rates that were comparable to removals in conventional WWTPs.

TH 312

Removal efficiency of organic micropollutants in a constructed wetland assessed by passive sampling: results from the WIPE project

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Despite advanced treatment techniques, STP's still are a major source of input of nutrients and micro pollutants into aquatic ecosystems. The WFD requires an ongoing emission reduction in order to meet its targets. On several locations in the Netherlands, wetlands are constructed, adjacent to the conventional STP. While retaining in the wetland, nutrients and pathogens concentrations decrease while oxygen content and biodiversity increase in the effluent. However, little is known about the fate of micro pollutants in the wetland. The aim of the WIPE project was to investigate if wetlands also contribute to a decrease of the environmental risk by removal of micropollutants from STP effluents. Therefore, we deployed passive samplers (silicon sheets) on 4 positions in 3 different constructed wetlands in 7 consecutive rounds from July 2009 till December 2010. Passive sampling enables the detection of very low concentrations of substances, because of their enhanced sampling volume compared to grab sampling. By its continuous sampling, passive samplers detect most substances passing the sampler, depending on the chemical properties of the compound and the sampling rate of the sampler. By this, a more detailed picture of the potential risk can be calculated. After deployment, passive samplers were analyzed for different groups of substances, and the risk of these substances was assessed using a Toxic Unit approach. In total around 250 substances were analysed on the sheets, and large differences existed between locations, but also between rounds. Surprisingly the largest group of substances, in terms of concentrations, were the pesticides, followed by flame retardants and personal care products (musks). Pharmaceuticals and estrogens only formed a small part of the total concentration. Hardly any removal of micropollutants was observed within the three constructed wetlands. In terms of risks large differences were observed between the locations, with one of the 3 locations having a risk approximately 2 magnitudes higher than the other

two locations. This was largely caused by a small number of pesticides with very low PNEC values being present at this location. Although not significant, all three wetlands showed a slight decrease in environmental risk from location 1 to 4. In terms of removal of organic micro pollutants, constructed wetlands only show a minor efficiency, and seem to be more efficient in biologically vitalizing the effluent.

TH 313

Assessing the potential of constructed wetlands in reducing environmental impact of STP effluents: the WIPE project

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At some pilot locations in the Netherlands, the effluent of sewage treatment plants (STPs) is lead through a constructed wetland (so called 'waterharmonica') before being discharged in surface water. This has been shown to revive the treated water, as oxygen demand is reduced and the biodiversity increases. The WIPE (Waterharmonica Improving Purification Effectiveness) project studied the potential of such wetlands to reduce the environmental impact of STP effluents on the receiving water, with special emphasis on ecotoxicological aspects. The (changes in) effluent quality of three STPs was monitored during the passage through constructed wetlands. This monitoring program consisted of passive sampling, chemical analyses, in-vivo and in-vitro bioassays, microbiology and biological and gene-expression responses of chronically exposed stickleback (*Gasterosteus aculeatus*). The monitoring was performed between 2008 and 2011 and generated an extensive dataset. In this presentation we will give an overview of how different environmental relevant end points were affected during the passage through the constructed wetlands.

In time large variations in the characteristics of the STP effluents were found. A wide range of chemicals was detected on the passive samplers, but in general these were not affected in a structural way by the passage through the wetlands. Occasional peaks in toxicity were observed, that indicate a potential risk for the receiving surface water. The intensity of these toxicity peaks was reduced after passage through the wetland. No indications were found that the effluents in general affect the reproductive success of the sticklebacks, although temporal poor effluent quality can affect survival. Gene expression indicated estrogenic effects on the male fish. This endocrine disruption was less at the end of the wetland where the effluent is about to be discharged on the surface water. This observation is in line with the results from the in-vitro-assays for estrogenic potential, and with the observation that the microbial community in the wetland sediments have a high potential to reduce estrogenic activity. Finally, it was found that densities of pathogens in the effluent were substantially reduced during passage through the wetlands. Constructed wetlands can thus help to improve the environmental quality of STP-effluents. Especially by levelling off high peaks in the toxicity and by reducing the density of pathogens.

TH 314

Inventory of emissions of priority hazardous substances in the surface waters in France

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Introduction

According to Article 5 of the Directive 2008/105/EC on Environmental Quality Standards in the Field of Water Policy, Member States have to establish an inventory of emissions, discharges and losses of all priority substances and pollutants listed in Part A of Annex I to this Directive. This paper aims at describing the french approach used to review of the impact of human activity on the status of surface waters through:

- the general working shem of the methodology and few definitions;
- the input data needed for the french approach;
- some exemples of calculations carried out on French river basins;
- the data compilation process.

Methodology guidelines

Methodology definition was guided by the following key ideas:

- A state-of-the-art methodology in agrement with reglementary requirments, and EU guidance.
- A non substance specific methodology. The approach is based on effluent discharges data; in their absence some explicit estimation formulas are proposed.
- A single methodology applicable at different spatial scales.
- A methodology that will work despite scarcity of information and data, and uncertainties.
- Future use of emission inventory as a management tool for designing strategies for the reduction of the impacts of chemical pollution in watersheds.
- A methodology which can easily deals with data from different years. All available information which are representative of a given year to draw a coherent picture of the micro pollutants releases in surface waters during this year must be used.

After a bibliographical study, a pragmatic approach has also presided over the construction of the methodology developed in this paper in terms of work efficiency.

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TH 315

Corbicula fluminea: just an invasive species or also a freshwater bioremediator?

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Much attention has been drawn to the industrial and ecologic impacts of *Corbicula fluminea*, an invasive freshwater bivalve. This species has also been extensively studied as a sentinel for both potentially toxic metals and organic compounds. Such an attribute lies on the ability of these clams to bioaccumulate chemicals in body tissues together with a fairly large tolerance to the effects resulting from exposure. Additionally, the Asian clam seems to alternate filter-feeding at very high rates with pedal-feeding and deposit feeding, and hence an integrated view concerning different ecosystem compartments can be considered. As to our knowledge no attention has been devoted so far to the benefits that these features of the Asian clam may provide if applied to the treatment of contaminated waters.

The main aim of the present study was to generate preliminary results on the evaluation of *C. fluminea* bioremediation potential in the treatment of several effluents with industrial and commercial interest. This study is part of a broader assessment that considers a battery of wastewater effluents such as acid mine drainage, and pulp and oil mill effluents. The toxicity of both the initial clam-feeding solution and the post-filtration effluent to non target organisms (the bacteria *Vibrio fischeri*, the microalgae *Pseudokirchneriella subcapitata* and the cladoceran *Daphnia* sp.) was assessed through laboratorial bioassays. These bioassays were supported by the analytical characterisation of the exposure media before and after the clam filtration. Although the Asian clam is an invasive species and problems may arise from the intentional introduction in the discharge environments, controlled systems that allow us to capitalise on their filtration and bioaccumulation capacities may be assembled. Such a wastewater treatment method could bring huge benefits (e.g. output improvement and costs reduction) in the industrial context.

TH 316

Acute toxicity analysis of urban septic tank sludge

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The objective of this work was to evaluate possible toxic effects of sludge samples from urban septic tanks and upflow anaerobic sludge blanket (UASB) from a wastewater treatment plant located in the University of Espirito Santo, in Vitoria, Brazil. The method employed was the acute toxicity test Microtox (Azur Environmental) based on the bioluminescence inhibition of the marine photobacterium *Vibrio fischeri*. It is a test with many advantages such as ecological relevance, sensitivity, reproducibility, standardization and simple execution. A series of physical-chemical parameters were also analyzed based on Standard Methods for Examination of Water and Wastewater 20th (APHA - 1998). Sludge from four septic tanks was used in the study. From each tank four samples were collected, each aliquot representing a distinct depth, classified as: 1. scum; 2. liquid waste; 3. superficial sludge; 4. bottom sludge and 5. mixture from all the previous. From the UASB equivalent fractions were also sampled. The tests were executed according to the Microtox User's Guide Manual (AZUR Environmental, 1996) and results were expressed in EC50 (Effective Concentration), a value that represents the sample concentration in which 50% of the bacterial luminescence is inhibited. Only in one of the septic tanks, located in a public building toxic effects could be verified in the upper layers "scum" and "liquid waste". All samples from the bottom fractions presented higher toxic effects (EC50= 9.4%) when compared to the fractions above. The average EC50 for fraction 3 was 12.6% and 17% for the mixture, meaning even lower toxicity, possibly due to the influence of upper fractions that are stored in great volumes inside the tanks. The sludge from UASB presented higher toxic effects on topside (EC50= 5.5%) in comparison to the bottom (EC50= 8%), but lower toxicity when compared to tanks samples, since it was analyzed in a dilution of 50 parts in 200, while septic tank samples were diluted in one part in 200. All samples presented elevated levels of TKN, P and COD and were considered as stabilized sludge, since the relation VS/TS (volatile solids/total solids) was below 65%. Although many studies have applied the *Vibrio fischeri* test to a range of environmental samples, the analysis of sludge from septic tank represents a new approach, essential to evaluate the risk of releasing this residue in the environment or even ensure the quality required for its application in other activities.

TH 317

Comparing chemical analysis with literature studies to identify micropollutants to be treated or upstream source controlled in a catchment of Copenhagen (DK)

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The European Water Framework Directive aims at obtaining good ecological and chemical status of European water bodies by the year 2015 (EU, 2000). The directive also requires establishing pollution source inventories, to design and perform monitoring programs as well as to outline strategies to reduce emissions if the environmental quality standards are exceeded (EU, 2008). The aim of this study was to compare two different approaches to identify micropollutants in stormwater from a specified catchment; a literature inventory of potential pollution sources and chemical analysis of urban stormwater runoff. The selected catchment covers an area with roads, a shopping centre, a parking lot, office buildings, an upper secondary school and restaurants in Copenhagen (Denmark). The literature approach is limited to the range of included micropollutants and to how and which information is compiled, whereas the analytical chemical approach is limited to and affected by the sampling procedure, the selection of analysed

substances, available analysis techniques as well as analytical sensitivity and precision. Comparing the two approaches to identify contaminants reveals a discrepancy in the exact overlap of identical micropollutants, though the overall findings are similar.

TH 318

Toxicity identification evaluation for wastewater treatment plant working at different industrial and municipal scales

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A toxicity identification evaluation according international procedure was performed taking in consideration the system used for depuration of wastewater. Process control in the activated sludge plants were correlated with the toxicities of their liquid effluents. Mean cellular retention time (MCRT), hydraulic retention time (HRT), presence of anaerobic and anoxic selectors, sludge microbiology, and operation index such as Sludge Volume Index (SVI), diluted Sludge Volume Index (dSVI), sludge volume (Sv), base and spike Oxygen uptake rate (bOUR and sOUR), Mixed Liquor Volatile Suspend Solids (MLVSS), Mixed Liquor Volatile Suspend Solids (MLSS) and biofloculation index (Bi) were measured for each plant. A set of toxicity tests were carried out, together with the Solid Phase Microextraction (SPME) and Gas Chromatography-Mass Spectrometry (GC-MS) analysis, for chemical characterization of each effluent. Data were interpreted by Principal Component Analysis (PCA) which allowed to classify for the first time, in Argentina, to rank in relation to type of industry, production volume and / or number of inhabitants. From the data interpretation arise that the high production volume chemicals (HPVC) are related with the higher download as toxic unit discharged into the aquatic environments of Buenos Aires province. It is need an urgent update of the parameters that govern the discharge of wastewater and the inclusion of their ecotoxicity assessment to allow the download.

TH 319

Application of delayed fluorescence to estimate the influence of waste water effluent on alga

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Waste water effluent is major source of chemicals discharge in to aquatic ecosystems. Since algae are important primarily producers in aquatic ecosystems, it is necessary to evaluate the influence of effluent not only on animals but also the algae. We are investigating a new method of for evaluating the influence of chemicals on alga by using delayed fluorescence (DF) from algae. The DF is a special type of luminescence; it detects the growth of only those cells that have photosynthetic capability. Since the DF originates from re-excitation of chlorophyll by a reverse reaction of photosynthetic electron transfer, the DF inhibition reflects inhibition of photosynthetic activity that is necessary for growth. Therefore DF is a potential endpoint for the estimation of the influence of chemical substance of algal growth in a shorter time than the conventional 72 hours growth inhibition test (e.g. OECD test guideline 201). The intensity of DF also shows a time decay curve. Chemical exposure changes the decay curve to patterns that have the potential to assist in the identification of contaminants in effluent sample. We have developed a rapid and simple DF test protocol using the green alga *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*). The test algae are prepared by thawing specially prepared -80°C frozen algae in a 1 hour pre-incubation. The prepared test algae can then be immediately exposed to the test sample prepared in a range of effluent concentration (0, 5, 10, 20, 40, 80 %) in small culture tubes (10 ml volume). The DF from algae in the culture tube is directly measured by the ultra-sensitive luminometer at 1 h, 4 h, and 24 h after exposure. DF is inhibited through the influence of the effluent. Further, effluent exposure changes the time decay curve of the DF intensity. From the relationship between the exposure concentration and the DF inhibition, we can estimate the dose-response of DF inhibition for each effluent or mixture. In addition, the pattern of the DF decay curve has potential for application to the identification of contaminants in the effluent. We will discuss results of a feasibility study evaluating treatments of industrial and agricultural effluents.

TH 320

Ecological health assessment and stressor identification using multi-response bioindicators of fish

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This study was evaluated the effect of environmental stressors using various parameters such as chemical water quality, habitat evaluation, and chemical exposure biomarkers in the Gap stream, South Korea during May-June 2009. We collected the pale chub (*Zacco platypus*) as sentinel species to evaluate biomarkers and indicators at four sampling sites in the Gap stream. The major changes observed were increased detoxification enzymes (EROD), DNA damage, physiological parameters, % of DELT anomalies, impaired reproduction, and reduced fish population structure in the downstream site (GS 7.2) of municipal wastewater treatment plant (MWTP). By the stressor identification (SI) process, five candidate causes of fish population degradation were winnowed including habitat alteration, low dissolved oxygen, nutrient enrichment, ammonia toxicity, and chemical pollution. The results of causal analysis suggest that the principal probable causes of fish population impairment in the downstream site below the MWTP were a combination of effects associated with recruitment failure and size-selective mortality caused by nutrient enrichment, ammonia toxicity, and habitat alteration such as degradation in spawning and nursery space.

TH 321

Monitoring potential ecotoxicological effects in the effluent of an urban wastewater treatment plant

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Organic micropollutants cannot completely be removed by traditional waste water treatment technologies. Therefore it is being discussed to add additional treatment steps such as ozonation, active coal filtration, UV-treatment etc. However, there is a lack of long-term studies to clearly prove a direct relationship between micropollutants in waste water and ecotoxicological effects on the macrozoobenthos fauna in the recipient stream to justify future expensive installations. Online biomonitoring of the ecotoxicity of waste water effluents provides a quantitative relation between chemical composition of the waste water and fitness (behavior, survival, biomarkers) of the indicator species. The indicator species is chosen according to pollution-sensitivity, abundance and key-role in the stream ecosystem, in our case gammarids. Gammarids were exposed in the Multispecies Freshwater Biomonitor[TRADEMARK] (MFB) placed in the UniPass[TRADEMARK] flow through system, receiving treated waste water just before release into the river Limmat. Potential short term responses to episodic pollution pulses as well as long-term effects of the potentially toxic cocktail of the waste water were monitored on real-time basis over several weeks. Different species of gammarids were applied (*D. villosus*, *G. pulex*) and their sensitivity compared. These studies establish the baseline toxicity of the waste water in its current treatment before additional treatment steps might be installed in the future in order to record the expected removal of the toxic potential. On the other hand, if during long-term monitoring with gammarids no toxic effects can be seen, combined with sporadic in situ benthos assessment, the need of additional treatment steps should be reevaluated on a case-by-case basis for each waste water treatment plant and its recipient river ecosystem.

TH 322

Implications of municipal wastewater on macroinvertebrate community structure and leaf litter breakdown

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European streams receive huge amounts of municipal wastewater, which, due to the limitations of conventional secondary treatment (i.e. mechanical and biological), may contain a wide range of micropollutants, like pharmaceuticals and personal care products. As such micropollutants are continuously released into aquatic environments, they may pose a potential risk for the integrity of ecosystems. Hence, the present study assessed adverse structural and functional implications of wastewater released by the municipal wastewater treatment plant (WWTP) in Landau, Germany, on its receiving stream, the Queich. For this purpose several endpoints related to the ecosystem function of leaf litter breakdown (microbial and invertebrate mediated leaf litter breakdown, in situ bioassays with *Gammarus fossarum*) and the structure of the aquatic invertebrate community were investigated for six and three weeks during winter and summer 2011, respectively. Macroinvertebrate related leaf litter breakdown in terms of leaf mass loss was by up to 65% significantly reduced at sites situated up to 500 m downstream of the WWTP outlet compared to the upstream site during both seasons. This effect may be explained by a meaningfully reduced abundance of leaf shredding macroinvertebrates such as *Gammarus*. Moreover, gammarids exposed in situ exhibited a significantly (up to 80% 100 m downstream during both seasons) reduced feeding rate at downstream sites, indicating lower energy availability and reduced reproductive output. These implications in leaf shredding macroinvertebrates and the ecosystem function of leaf litter breakdown may be on the one hand caused by direct ecotoxicological effects of the released wastewater and the containing micropollutants, which is supported by the results of the in situ bioassays. On the other hand, indirect pathways, driven by a lower nutritious quality of leaf material, indicated by a significantly reduced fungal biomass (up to 75%) at the downstream site, may have influenced the physiological fitness of shredders. Finally, the present study urges the evaluation of the current procedure in wastewater treatment by considering advanced treatment methods (e.g. ozonation), which may help to meet the requirements of the EU Water Framework Directive.

TH 323

Water quality investigations in the river Lea downstream a sewage treatment works: preliminary results of a case study

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In the north-east of London the Lea Navigation, a canalised reach of the river Lea, is affected by episodes of very low levels of dissolved oxygen. The problem was detected by the Environment Agency in the stretch from the confluence with Pymmes Brook (which receives the final effluent of Deephams sewage treatment works) to the Olympic area (Marshgate Lane, Stratford). In this study the river water ecotoxicity to the freshwater alga *Pseudokirchneriella subcapitata* was tested by algal growth inhibition test according to modified OECD guidelines (Organization for Economic Co-operation and Development, 2006). Results showed inhibited growth rate after 24 hours that was most marked when algal populations were exposed to the polar compounds fraction of the river water samples. Rapid acute toxicity tests were carried out using mediated amperometric whole cell biosensors (CellSense). Results indicated no evidence of acute toxicity in the river water, as no significant differences were detected between pre and post exposure responses to river water samples, with an exposure time of 30 minutes. These results indicate chronic pollution of the river, affecting photosynthetic algae during long-term exposure.

TH 324

Enrichment free LC-HRMS screening method of anthropogenic sewage pollutants in waste water, receiving water, ground water and drinking water samples

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In last decade, there has been growing public concern of potential contamination of water and environment with anthropogenic compounds and their degradation products and possible negative influence to the nature and public health. As a response to this fact, there is an increased interest in more efficient screening techniques of larger number of possible pollutants compared to that traditionally carried out by triple quadrupole mass spectrometers. The high resolution and accurate mass does not require optimization of compound specific parameters and has the ability to properly separate matrix from compounds of interest.

Fast and highly selective methods are necessary to screen, confirm and quantify different classes of contaminants in complex matrix and at low the analyte concentrations.

High resolution mass spectrometry - using full scan analysis mode - has been described as the preferred screening tool due to the possibility to look for a large number of compounds and also because it enables retrospective analysis [1]. Furthermore, the use of a resolving power $\geq 50,000$ FWHM is reported as being as selective as 2 SRM transitions when using triple quadrupole instruments [2].

In this work, the large volume direct injection has been applied to the screening of anthropogenic sewage pollutants in different matrices using a quadrupole-Orbitrap analyser. The experiment consisted on combining full scan mode at a resolving power of 70,000 FWHM with data dependent MS/MS spectra acquired at a resolving power of 17,500 FWHM. The MS/MS spectra were generated by using a high energy collision induced dissociation cell (HCD). This experiment was tested and evaluated in terms of quantification and confirmatory capabilities for the analyses of anthropogenic sewage pollutants. Different sample types were tested, including waste water, receiving water, ground water and drinking water representing different steps within the water cycle of densely populated and intensively agriculturally used areas.

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TH 325

Whole effluent assessment as an alternative to in situ ecological impact measurement? An experimental approach

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There is increasing recognition by regulators that there are limitations to the substance-specific approach for assessing and controlling the environmental fate and effects of effluents. Consequently, many regulators are seeking more holistic techniques such as whole effluent assessment (WEA) to supplement existing approaches. However, to ensure that these approaches are capable of indicating potential environmental effects, it is important to test scientifically robust WEA protocols. In general, WEA methodology assesses toxicity to aquatic organisms. WEA has relevance for the protection of ecosystems although the relevance and interpretation of results ultimately depends on the tests used. In the context of the Water Framework Directive (WFD), European surface water should reach good ecological and chemical status in 2015. The ecological status is generally assessed with ecological indicators. In some particular cases (sampling difficulties because of access, type of substrate, several industrial waste in the area[3DOTS]), this approach is difficult to be applied. In this case, WEA methodology might represent an alternative to ecological indicators.

The main objective of the project presented in this paper, financed by TOTAL and the CONCAWE, is to show the real distance between WEA methodology which is conservative and in situ impact measurement for risk assessment. In this project, the difference between WEA and in situ impact measurement is assessed using dynamic outdoor mesocosms. This project has been designed and realized in three successive steps: (1) Preliminary experiments in Laboratory to assess the best solution to store one effluent in order to limit its variation (how to minimize volatilization, oxidation and crystallization how to resist to outdoor climatic variation[3DOTS]); (2) Feasibility experiments in mesocosms with one effluent (how to select effluent, to sample the effluent in industrial site, to transport, to store and to inject large volume of effluent; How to measure the ecological impact in mesocosms); (3) Final experiment in dynamic mesocosms with several effluents sampled in different sites and comparison with WEA methodology.

In this presentation the design of the three steps of this project will be presented and discussed.

TH 326

Multi-bioassay approach for assessing the impact of industrial discharges on the water quality in Wallonia, Belgium

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Bioassays offer an opportunity for a more holistic and meaningful way of assessing effects of environmental samples and wastes on ecosystems than what is possible by using chemical-based monitoring alone. They can provide predictions of environmental impacts whereas ecological community measures only determine impacts after they have occurred. Therefore, bioassays are useful in helping to implement the Water Framework Directive (WFD). In Wallonia (Belgium), an effect directed active monitoring using bioassays is being carried out for many years. It combines ecotoxicological measurements at emission and immission and physico-chemical measurements. We use a battery of short term and chronic bioassays with the bacteria *Vibrio fischeri*, the alga *Pseudokirchneriella subcapitata*, the rotifer *Brachionus calyciflorus* and the microcrustacea *Daphnia magna*. Moreover, a yeast estrogen screen (YES) assay was conducted as an assessment tool to detect the presence of endocrine disrupting compounds. During 2011, 14 major industrial discharges and 26 sampling points in the receiving waters (upstream and downstream of the effluent) were monitored. The effluents were sampled 6 times a year whereas receiving waters were sampled 4 times a year. Priority List substances of the WFD and other pollutants discharged in significant quantities were also measured. More detailed studies were carried out with complementary tools: upstream and downstream of a discharge of a metallurgical industry, bioassays were conducted on both water samples and sediments and bioaccumulation of metals was assessed using encaged bryophytes; upstream and downstream of a pharmaceutical industry, YES assay was conducted as potential assessment tools in combination with passive samplers (POCIS). The results of these monitorings show that bioassays are good diagnostic tools to determine the causes of poor ecological quality and to trace back to the source of contamination. They are an important "tool in the toolbox" for environmental management. They add value and provide complementary information to that supplied by the chemical and ecological community measures and could help to design appropriate management measures.

TH 327

Combination effects of pharmaceuticals and industrial chemicals in waste water effluents

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We will present our currently starting research activities in testing combination effects of pharmaceuticals and industrial chemicals known for their (co)-occurrence in WWTP effluents.

Mixtures of industrial chemicals and human pharmaceuticals can be found in surface waters as so called micropollutants. Effluents of waste water treatment plants (WWTP) are the main exposure route for both kinds of substances. The emission of these chemicals into WWTP originates from consumer usage of products, articles and drugs.

It is commonly known from literature and discussed in different scientific and regulatory communities that effects of chemical mixtures are significantly larger than single substance effects. At the moment neither in the environmental risk assessment of chemicals (REACH) nor of human pharmaceuticals (EMA/CHMP/SWP/4447/00) possible combination effects in the environment is considered. But taking into account realistic exposure scenarios we assume that combination effects are the rule and not the exemption. Consequently an underestimation of environmental risks is likely.

The study focuses on the question if combination effects of pollutants in WWTP effluents require further assessment. Therefore ecotoxicity tests with algae and daphnids for 6 environmentally relevant substances shall be conducted individually and in different combinations respectively. These substances, (i.e. nonylphenol and ibuprofen) are known for their occurrence in WWTP effluents and their toxic effects to the aquatic compartment. Main objectives are (i) the quantification of the hypothesized underestimation of the risk by referring to single toxicity NOEC/EC10 values and (ii) the possibility to apply Concentration Addition (CA) as a default concept for the chosen scenario. The introduction of a potential mixture assessment factor (MAF) and its magnitude shall be discussed on the basis of the results gained.

TH 328

Illicit drugs as emerging pollutants in surface waters: acute toxicity test

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Today, illicit drugs are considered as emerging pollutants and several studies have been carried out to evaluate their concentrations in sewage waters and in effluent from WWTPs. Most of these compounds are not completely removed during treatment and are found in surface water at concentration around ten ng/L. Thus, it appears important to determine the ecotoxicological impact of illicit drugs.

In order to develop this knowledge, we carried out acute toxicity tests according to ISO11348. Four illicit drug families have been tested: cocaine, heroin, amphetamine-like and cannabis. In a first time, compounds were studied alone in tap water. Then, they were studied alone or associated in sewage water. Results will be presented and discussed according to the environmental concentrations of these compounds.

TH 329

Ecotoxicological characterisation of climbazole, an anti-dandruff agent contained in shampoo

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Effluents of municipal wastewater treatment plants are the most important sources for the entry of emerging pollutants like personal care products into the environment. Only recently, the anti-dandruff agent climbazole has been detected for the first time in the effluent of a German wastewater treatment plant with concentrations around 0.5 µg L⁻¹. In raw wastewater and in activated sludge it was found with concentrations as high as 1.4 µg L⁻¹ and 1.2 µg g⁻¹ TSS, respectively. Climbazole is used as an anti-dandruff agent in hair care formulations acting as fungicide by inhibiting ergosterol biosynthesis. Despite its high efficacy against moulds and fungi, the possible impact of climbazole on the environment has only fairly been studied. The aim of this work was therefore to characterise the ecotoxicological effects of climbazole on aquatic and terrestrial organisms of different trophic levels. Assessing the terrestrial toxicity is particularly relevant when treated wastewater or sewage sludge is to be applied in irrigation or as organic fertilizer on arable land.

In single-species tests the toxicity of climbazole was determined towards the aquatic organisms water lentils, diatoms, green algae, fish embryos, daphnias and rotatoria and towards the terrestrial organisms bacteria, collembolas, enchytraeids and higher plants. All tested aquatic organisms were affected by climbazole concentrations below 15 mg L⁻¹ with the water lentil *Lemna minor* having the lowest EC₅₀ in the µg per litre range. Among the terrestrial organisms the lowest EC₅₀ was determined for the plants (*Avena sativa* and *Brassica napus*) with about 10 mg kg⁻¹ soil dry weight for inhibition of shoot growth. Test results reveal that among the different trophic levels, climbazole is most toxic towards primary producers in the aquatic as well as in the terrestrial habitat. Displayed effects were a reduction of shoot length in higher plants and of leaf (frond) size in water lentil, which suggests an interference with the steroid metabolism of the plants. Assessing the terrestrial toxicity of climbazole is particularly relevant when treated wastewater or sewage sludge is to be applied in irrigation or as organic fertilizer on arable land.

TH 330

Environmental impact of current reserve-antibiotics with increasing incidence of severe infections using modern waste water treatment

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Antibiotic resistance is increasing since 30 years. Antibiotics previously used as reserve antibiotics, e.g. ciprofloxacin, are found in guidelines for treatment of slight infections. Due to uncritical prescription of broad spectrum antibiotics the amount of antibiotics used rises constantly. Many environmental studies proved the existence of different types of antibiotics in hospital sewage, municipal waste water, sewage disposal facilities and surface water or soil. For some substances critical concentrations with effects on algae, bacteria, daphnia and higher life forms were observed.

At the present Rifaximin, Piperacillin, Doripenem as well as the current reserve-antibiotics Tigecycline, Linezolid and Daptomycin are not classified as relevant to the environment because of their minor application. Hence environmental data for these substances are missing, as well as future schemes of use. Nowadays most environmental scientists are focusing on older frequently prescribed substances. As a result the risk assessment lags behind the modern pharmacotherapy and the achievements of the pharmacology.

Our objective is to study potential effects on the environment of above antibiotics. Ecotoxicological testing is performed in standardised bioassays using 24- and 96-well microplates for algae and bacterial growth inhibition tests. Ecotoxicological testing with algae growth inhibition test of new antibiotics showed increasing toxicity for Daptomycin and Tigecycline with EC₅₀-values of 14.4 mg/L and 1.76 mg/L. There is no toxic effect for Doripenem and Linezolid at maximum test concentration (EC₅₀ > 100 mg/L).

Estimating PEC:PNEC-relations of these new substances has to take into consideration the increasing incidence of infections, change in demographics as well as biodegradability, compartment-distribution-tendencies and additional waste-water treatment techniques e.g. ultraviolet light, ozone and membrane filtration.

TH 331

Comparative study of the ecotoxicity of dishwasher detergents

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This study aimed at assessing the ecotoxicity of dishwasher detergents. Indeed, despite the high consumption of such products, available information regarding their environmental impact is still limited. Thus, twenty five of the most representative products available on the French market were selected, covering the different dishwasher detergent categories: "multibenefit" tablets, "classical" products (tablets and powder) and "gel" (both "classical" products and "gel" need the use of rinse aid).

Our approach was taken into account the ecotoxicity of solutions simulating a wash cycle in a dishwasher in typical use conditions, as recommended by the manufacturer. The ecotoxicity of these "washing solutions" was determined by performing a battery of aquatic bioassays from two trophic levels (algae and micro-invertebrates) covering both acute and chronic endpoints. In addition, the assessment of the ecotoxicity of two hand-dishwashing detergents, ten rinse aids (alone) and four dishwashing detergents (without addition of rinse aid in the "washing solution") was performed.

Both approach and selected battery of bioassays were relevant and allowed the classification of the different dishwasher detergents. Mostly, it was shown that the reproduction of *C. dubia* was the most sensitive endpoint compared with the inhibition of the mobility of *D. magna* and the growth of the algae *P. subcapitata*. The "washing solutions" prepared with hand dishwashing detergents were found to be more toxic than those prepared with dishwasher detergents, except for the gel products. Regarding the automatic dishwasher products, it was shown that the ecotoxicity of "washing solutions" from the "gel" products was higher than the ecotoxicity recorded for "classical" products and "multibenefit" tablets. This study also revealed that the ecotoxicity of "classical" tablets was, most of the time, higher than the ecotoxicity of "multibenefit" tablets. Additional studies confirmed that the ecotoxicity recorded for "classical" products was mostly due to the addition of rinse aid in the "washing solution". These results suggested that the ecotoxicity recorded for ecolabelled products should be weighted regarding to the intrinsic ecotoxicity of the rinse aids which are currently not covered by "European Ecolabel".

TH 332

Characterisation of dissolved organic carbon in effluents and the assessment of its overall benefits in mitigating environmental impacts from metals

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Dissolved organic carbon (DOC) is known to form complexes with metals which can markedly reduce the free ion concentration of the metal and mitigate toxicity. Biotic Ligand Models (BLMs) for metals include speciation calculations of DOC with the metal ions, with increasing DOC concentrations usually providing a significant reduction in the bioavailability of the metal.

BLMs assume a composition for the DOC in terms of the ratio of humic and fulvic acids. These default DOC characteristics work well for natural waters with generally good agreement between predicted and observed no effect concentrations. Sewage effluent contains high concentrations of DOC but a significant fraction of this organic matter will not be humic and fulvic acids but proteins, other natural acids, chelating agents such as EDTA, sugars etc. which may bind metals more strongly than predicted by BLMs and so provide greater protection against toxic effects of metals. About 50% of effluent DOC has been shown to be relatively quickly degraded and therefore not likely to be humic or fulvic acids. The DOC in effluents will, therefore, almost certainly interact with metals in a different manner to that predicted by BLMs.

Also, any advanced treatment options used to destroy or remove organic chemicals of concern such as oestrogens or pharmaceuticals are likely to either reduce the concentrations of effluent DOC or affect its composition. Therefore, it may be that infrastructure investment intended to remove some organic chemicals may make the receiving waters more sensitive to metals by reducing protective effects of DOC.

This poster presents two practical elements designed to investigate the effects of advanced effluent treatment on DOC characterisation and metal speciation; the direct measurement of free metal ions in effluents over time using Donnan Membrane Technique (DMT) and Diffuse Gradient in Thin film (DGT) samplers, and the characterization of the DOC using UV spectrophotometry, Nuclear Magnetic Resonance (NMR), Size exclusion chromatography and pyrolysis GCMS - all carried out on the same sewage effluents.

TH 334

Integrating sewage treatment plant monitoring and modelling results to prioritise chemicals of concern and assess environmental risks

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Among the vast range of organic chemicals released via sewage treatment plant (STP), prioritising pollutants of concern and assessing their risks for the aquatic ecosystem entail key scientific challenges for regulators. From the EU perspective, such challenges are common to the chemical legislation, (e.g. REACH regulation, Biocidal Products Directive) and the water legislation (e.g. Urban Water Treatment and Water Framework Directives). In chemical risk assessment, the exposure of the aquatic ecosystem to organic chemicals released via STPs is calculated in two steps: first, local and regional scale emissions to sewage are estimated from consumer use data; then, the fate in a generic STP is calculated using the multimedia box model SimpleTreat. Default calculations are based on conservative (worst-case) assumptions.

A validation study was carried out for two chemicals of concern, LAS and triclosan, to evaluate the existing modelling framework applied to regulatory chemical risk assessment in the EU and to explore synergies with the monitoring and risk assessment activities under the Water Framework Directive.

Concentrations in raw sewage were estimated from product usage and chemical inclusion levels. The distribution and elimination in a conventional activated sludge sewage treatment plant with primary and secondary sedimentation was calculated with SimpleTreat 3.11, from the physicochemical properties and the biodegradation rate derived from STP simulation tests (OECD 303A). Probabilistic model simulations were run to account for the uncertainty of model inputs and the variability of STP parameters across the EU.

Calculated STP influent and effluent concentrations were in good agreement with measured concentrations collected from the literature and the probabilistic simulations captured, though not completely, the observed spread in observed values. Such modelling predictions can complement existing monitoring data and fill gaps for data-poor chemicals, thus supporting the prioritisation and the risk assessment of chemicals of concern. Moving from worst-case, default to more realistic, probabilistic exposure assessments facilitates the comparison of data generated and the harmonization of methods applied across regulations.

Anaerobic biodegradation of PCBs in a grass cut batch reactor

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Due to properties such as high K_{ow} values chlorinated compounds including PCBs, dioxins or chlorinated pesticides in vegetation can lead to bioaccumulation in livestock and in the food chain. Hence, the remediation of these compounds in the environment is an important factor for human health. The degradation of the persistent Polychlorinated Biphenyls (PCBs) under anaerobic conditions in sediments and sewage sludge is described in the literature [1,2]. The potential for degradation in an anaerobic grass cut batch reactor has not been performed before and is investigated in this work. Therefore two series of batch experiments in laboratory scale (0.5 L to 1.5 L of volume) were performed under mesophilic conditions.

The first experimental series was performed at low natural, i.e. not spiked concentration level of Elbe riverside grass and sludge samples at 27 °C for 315 days. This first, explorative experiment showed ambiguous results for the degradation of the 6 indicator PCBs (PCB No.: 28, 52, 101, 138, 153, 180). Concentrations of the lower chlorinated PCBs decreased slightly, whereas the concentrations of the higher chlorinated PCBs were found to be stable at 5 20 µg/kg dry weight sewage sludge.

For the second experimental series 6 PCBs (No.: 28, 52, 101, 169, 138, 189), a technical mixture of PCBs (Aroclor 1260) and the pesticide Endrin were added in high concentration to the batch experiments (33 µg per compound, respectively and 100 µg of Aroclor 1260). In this second experiment different initial weights of grass cut from the Elbe riverside were mixed with anaerobic sewage sludge to determine optimal dry mass rates. For the determination of degradation rates experimental times for these batches vary between 30 and 120 days. Compound concentrations were measured by GC-ECD. Methane and carbon dioxide concentrations (measured by FTIR) in the gaseous phase of batch experiments were used as an indicator for biological activity. Results from the second batch experiment, concerning PCB-degradation rates and their correlation to gas production kinetics due to different dry masses, are discussed on the poster.

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Removal of nutrients by immobilized microalgal beads in a continuous flow system

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Using immobilized microalgal beads in contaminant removal is an emergent method in recent environmental studies. As is well known, phytoplanktons use nitrate (N) and ortho-phosphate (P) during the photosynthesis process for production. In this study, a diatom "*Phaeodactylum tricornutum*" was immobilized in 3.5% sodium alginate solution and hardened with 4% calcium chloride (cation solution) and introduced into a continuous system to remove nitrate and ortho-phosphate. Daily samples were taken from the system and analysed by using UV-Vis spectrophotometer (Chebios Optimum-one) to determine the removal efficiency of the nutrients by the algal beads. The cell numbers in the beads were also determined by counting in a Coulter Counter system during the experimental period. The blank beads were also run in the system and subtracted from the total removal of the nutrients to detect the net removal by the algae. The system was operated as six separate runs with usage of the same algal beads by starvation of three days at each time to increase the removal efficiency of the system. It was observed that the algal beads remove approximately 50% and 40% (average values of net removal by the algae) of the P and N respectively, considering the inflow concentrations of 1000 mg/L of P and 3000 mg/L of N. Disadvantages of this method are indicated as the release of the microalgae out of the beads during the experiment and disintegration of the alginate beads over a long term run.

LC03P - Increasing scientific and policy understanding through meta-analysis of life cycle assessments

Meta-analysis of diesel production in terms of life cycle GHG emissions and energy consumption: comparison and case study of Spanish refineries

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When performing the life cycle assessment (LCA) of petroleum products, life cycle inventory data are generally global compilations and they are not partitioned into sub-processes, but handled as a black box. Several products with different characteristics and uses are usually obtained from oil refining process (olefins, propane, butane, gasoline, kerosene, diesel, paraffin, asphalt, etc.). Allocation of proportional impacts of energy consumption and emissions for each product is a difficult task because many of them are correlated. Therefore, energy consumption and emissions are allocated according to the final product distribution. The most allocation methods used for petroleum products are often based on relationships of energy content, according to ISO standards.

This study compile the results of several studies of diesel production in European and American processing plants in terms of GHG emissions and energy consumption. Furthermore, this study is completed by recent studies of diesel production in Spanish refineries. Results highlight the broad range of values that depend on variables such as site-dependency or product specialization. Spanish refineries values are within the range of average European plants. Conclusions draw attention to the need of real data from refineries in order to obtain more adjusted and accurate results, as well as the possible partial subdivision of the system as a solution, according to ILCD handbook recommendations for allocation procedures.

Towards transparent and relevant use of energy use indicators in LCA studies of biofuels

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The use of energy has led to resource crises during the history of mankind, such as the deforestation of the Mediterranean during antiquity, and of Great Britain before the 19th century, and the oil crisis in the 20th century and continuing. Considering this, the frequent use of the impact category 'energy use' in the environmental assessment tool life cycle assessment (LCA) is not surprising. However, in a previous study, some of the authors noted that the term 'energy use' was not applied in a transparent and consistent way in LCA studies of biofuels. In this work we investigate how energy use indicators are applied in a set of life cycle assessment (LCA) studies of biofuels. In the examined reports and articles, the choice of indicator was seldom motivated or discussed and we observed five inherently different energy use indicators: (1) fossil energy, (2) secondary energy, (3) cumulative energy demand, (4) net energy balance, and (5) total extracted energy. These five energy use indicators were applied to the same cradle-to-gate production system of palm oil methyl ester (PME), giving considerably different output results. This is in itself not unexpected, but indicates the importance of clearly identifying, describing and motivating the choice of energy use indicator. All five indicators can all be useful in specific situations, depending on the goal and scope of the individual study, but the choice of indicators need to be better reported and motivated than what is generally done today. Authors of LCA studies should first define the purpose of their energy use indicator (fossil scarcity, energy scarcity, energy efficiency, cost/benefit comparison) and may then make a motivated choice of the energy use indicator.

A meta analysis review: 'Drop-in' Biofuels - Life Cycle Assessments, substance flow analysis, material flow analysis and relevant information on an advanced biofuel

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The biofuel research and production sector is enjoying an unprecedented amount of public and private effort in order to remove various financial and technical barriers. One of these very important barriers is the challenge of developing biofuels that are compatible with existing gasoline, jet fuel and diesel infrastructure. The United States Department of Energy has been championing efforts that ensure that the next generation of biofuels will be regarded as "drop-in" biofuels. According to the Former Undersecretary of the United States Department of Energy, Kristina Johnson, "drop in" biofuels can be defined as fuels produced from various biomasses which are compatible with the over \$9 trillion energy refinery and gas station infrastructure currently available in the United States. According to definitions used in reviewed literature, drop-in biofuels are described by National Advanced Biofuel Consortium of the United States as infrastructure compatible - they can either be used directly or blended with their petroleum-derived counterparts. The European Commission on the other hand defines drop-in biofuels on the basis of quality specifications (standards) developed by the American Society for Testing and Materials. Numerous LCA works have been conducted concerning what is described as drop-in biofuels. Although this topic of drop-in biofuels is relatively new, literatures regarding LCA and drop-in biofuels based on the definitions provided were available for analysis. A comprehensive study was undertaken with the aim of providing a clear overview of the previously conducted LCA studies in this new field of advanced biofuels. Also, important topics concerning this new chapter in the biofuels revolution were delved into. The results of this meta-analysis review will be presented at the SETAC world congress.

How to assess the data quality of LCI studies - a systematic approach

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The field of energy from biomass is rapidly growing, increasing the need for reliable data on the environmental burdens inflicted by the use of the different conversion technologies. A huge number of LCI studies on these topics can be found, however, most of these studies are not directly comparable to each other and their results may vary considerably depending on different data origins, time and geographical background, technologies and system boundaries.

Therefore it is often unclear how to decide which study is "best" suited for a given case.

The objective of this work is to present a guideline that helps compare different LCI studies. In this guideline a systematic approach on the assessment of literature quality is done. In a first step the current "best practice" for modelling an LCI is summarized. By this a reference for the assessment of the data is defined. This reference will identify a

set of indicators.

In a second step on the base of the indicators developed in the first step the available studies will be analyzed. In the last step, the actual states of the data are compared and assessed with the reference. This approach makes it easy to spot and report any shortcomings the studies may have and eventually decide which study should be used for a given problem.

The guideline consists of four parts:

- Description of the technology of interest and of available literature
- Description of the indicators, the reference values and the available data
- Evaluation of the available literature
- Recommendation

The guideline was tested on an analysis of LCI studies and data sets of an Organic Rankine Cycle plant for German framework conditions.

Using the guidelines data gaps were identified and quantified resulting in a better overall understanding where these gaps came from and how to deal with them. The guidelines allowed for a good comparison on the data quality of the literature. With their use a good overview of the different LCI studies could be achieved and a clear recommendation for data set generation could be given.

TH 343

Finding LCA research direction with the aid of meta-analysis

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Rapid growth in LCA methodological developments has generated a large body of work in the last ten years. New impact categories, indicators and characterization factors have been developed with regard to the LCIA. Moreover, an increased sophistication of the existing methods of characterization has been also proposed, for example with the introduction of spatial and temporal differentiation. On the side of the inventory analysis, the need to assess policies, technological and structural choices characterized by scarce reversibility, long term effects, trade-off between environment, economy and society, etc. has been driving an expansion of the scope of LCA, both in terms of level of analysis (from products to systems, from micro to macro) and coverage of indicators (including also economic and social aspects). Consequential LCA, hybrid approaches combining LCA and input-output analysis, scenario modeling, and new efforts for developing social and economic assessment methods, are only some examples of the developments LCA methodology has been going through. Overall, it may appear that LCA lacks direction on how to further develop.

In this paper the authors developed and applied a structured approach, inspired by the meta-analysis concept, to examine literature and identify research thrusts on how to further develop LCA. The procedure consists of four-steps: i) definition of the research question; ii) carrying out a literature review concerning more than 280 papers - selected from about 2000 articles according to pre-defined criteria - which resulted in the identification of some 60 main methodological topics; iii) research gap analysis, in which the methodological topics identified in the previous step were compared with the research priorities identified through a users' needs survey; iv) interpretation of results, in which the results of both the previous steps were evaluated and organized into coherent research thrusts.

Overall the analysis delivered two main research thrusts: one devoted to increase practicability of LCA, the other to increase model fidelity. The former is aimed at making knowledge available in easily usable way, while the latter focuses on better describing the complexity of the systems analysed and those interrelations that are really meaningful. Specific research topics were identified for each thrust, which suggests that sophistication and practicability can and should coexist in the same method.

TH 344

A web-based approach to handling divergence in LCA

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The last two decades have seen a profusion of studies in the Life Cycle Assessment (LCA) field, as well as growing interest in this work by parties in business and government. Furthermore, the rapid growth of detailed and constantly updated Life Cycle Inventory (LCI) and Environmentally Extended Input Output (EEIO) databases has led to an impressive set of studies and published information, from which it is often very difficult to elicit conclusive assessments due to conflicting results presented by the authors, although ostensibly the same product is being analysed.

At present, a widely discussed approach to handling this situation is through some form of meta-analysis. However, we think it is useful to explore other ideas and approaches, in particular, those that afford solutions that may be available sooner, and also available across a wider range of cases and for a wider range of users.

Our general strategy is to take a more qualitative approach to the problem of divergence or discrepancy in LCA. We think about a web-based toolkit that tries to resolve discrepancies by opening up the information using distributed data. An option for accessing distributed data sources and linking them semantically is the emerging technology of Linked Data (LD). LD technology aims at allowing meaningful interlinks between all sorts of resources within the World Wide Web. It can be seen as a further development of the usage of hyperlinks, extending the simple link (which can be seen as the sentence 'has to do with') to meaningful relations (such as 'isObservedOn' [observedEntity]). While the primary units of the hypertext Web are HTML (HyperText Markup Language) documents connected by hyperlinks, Linked Data relies on documents containing data in RDF (Resource Description Framework) format and residing in the *Web of Data*.

Essentially, our web-based toolkit is supported by a Data Access Layer which will provide search capability over LCA information available in RDF format. We envision that a form-based search interface will allow a user to specify details about the desired quality criteria and on a specific subject and run a *structured query* over the *Web of Data*. In this way the divergencies will be directly resolved at the level of the Data Access Layer. The (free-to-use) basic system we envision will support simple assessments and will help LCA users to produce more robust and policy-relevant results.

TH 345

Environmental impacts of palm oil biodiesel: a Meta analysis

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Environmental impacts associated with the life cycle of palm oil biodiesel is reviewed in this study using meta-analytic method. Most studies found palm oil biodiesel would produce positive energy balance with an average net energy ratio of 3.27, which exhibit the strong potential of palm oil biodiesel as renewable fuel. However, it was found that palm oil biodiesel is a net emitter of GHG to the atmosphere. The origin of oil palm plantation is the foremost determinant of GHG emissions. Converting peatland forest results in GHG emissions up to 60 t CO₂eq ha⁻¹ yr⁻¹. In contrast, converting degraded land or grassland for plantation can positively offset the system to become a net sequester of 5 CO₂eq ha⁻¹ yr⁻¹. Some other cradle-to-grave environmental impacts associated with palm oil biodiesel are acidification, eutrophication, toxicity and biodiversity.

TH 346

Greenhouse gas emissions from wind power: a critical meta-analysis

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In this paper a range of historic lifecycle studies of greenhouse gas-equivalent emissions for on and offshore wind farms are reviewed to identify a definitive set of the most current, original, and transparent studies. It begins by briefly detailing the separate components of the wind farm life cycle before explaining the methodology of the meta-analysis and exploring the range of lifecycle estimates. The paper highlights that ranges of emissions estimates are present for wind power. The paper also suggests that meta-analyses of historic lifecycle studies of this nature help to provide greater advice ensuring comparability, consistency and accuracy for given technologies. The paper then explains some of the factors responsible for the disparity in lifecycle estimates, in particular identifying errors in both the lowest estimates and the highest estimates. It is noted that wind power is not directly emitting greenhouse gas emissions, but rather that lifecycle emissions occur through farm construction, operation and farm decommissioning.

TH 347

The renewable energy directive and cereal residues

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The Renewable Energy Directive provides a methodological framework for a streamlined LCA that calculates the greenhouse gas (GHG) balance of biofuels in order to determine whether they reach the required 35% GHG reduction threshold. It describes which sources of emissions should be included and describes how co-products and residues or wastes should be allocated, however a clear definition of these is lacking. Currently, in order to incentivise biofuel production from second generation sources such as cereal residues and wastes, double credits are to be awarded to these biofuels, and the feedstocks are considered to be available at zero GHG 'cost'. This presentation will consider if cereal residues removal should be accounted for in GHG reporting for biofuels, as there are potentially sustainability implications with removing residues from agricultural land or from markets, and combusting them. The RED does not consider these potential impacts, which questions the ability of streamlined LCAs to account for the sustainability of these biomass resources. This presentation will compare results from streamlined LCAs and a full environmental impact assessment of cereal residue removal and combustion. The results will indicate the importance of inclusion of direct and indirect LCA impacts in policy analysis.

TH 348

Meta-Analysis of LCA studies for bio-based polymers: assessing the environmental performance of polyhydroxyalcanoates (PHA) and polylactide (PLA) in contrast to their petroleum-based counterparts

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The depletion of non-renewable resources plays an important role in present political debates: On the one hand, the depletion of non-renewable resources results in increasing commodity prices and economic dependence. On the other hand, the emission of greenhouse gases causes severe effects on the climate with unpredictable impacts on the human environment. That is why energy from renewable resources as well as bio-based chemicals and materials have attracted great interest.

Polyhydroxycanoates (PHA) and polylactide (PLA) are appropriate bio-based substitutes for a variety of previously petroleum-based products. Life Cycle Assessments (LCAs) according to ISO 14040 and 14044 have been applied to compare the environmental performance of these bio-based polymers in contrast to their petroleum-based counterparts. Numerous cradle-to-gate studies have been carried out to analyse the environmental impacts of the production of polymer resins with a preliminary focus on the impact categories climate change and non-renewable energy use.

Previous studies indicate a wide range of results and contradictory conclusions due to the consideration of different production processes, methodological choices (e.g. system boundaries, co-product allocation, impact assessment methods) and underlying assumptions. To produce more robust and policy-relevant results than individual LCAs, an approach for a Meta-Analysis is developed and undertaken to analyse the outcomes of 25 LCA studies. The underlying studies are quantitatively assessed taking into account the impact categories climate change (measured in metric tons of carbon dioxide equivalents) and non-renewable energy use (measured in megajoules). Relative environmental impacts per metric ton of PHA and PLA are compared with corresponding values for the petroleum-based plastics polypropylene (PP), polyethylene (PE), polyethyleneterephthalat (PET) and polystyrene (PS). Furthermore, differences between the environmental impacts of bio-based and petroleum-based polymers as well as standard deviations are calculated to derive methodological consistent figures.

Results of the Meta-Analysis indicate environmental advantages and disadvantages in both impact categories depending of the selected pair for comparison between bio-based polymers (PHA, PLA) and petroleum-based polymers (PP, PE, PET, PS). Furthermore, the Meta-Analysis reveals that bio-based polymers can help to mitigate climate change and save non-renewable energy.

TH 349

The effect of system boundary and weight of emission factors along the life cycle on the results of a life cycle assessment (LCA) - Electricity generations as example systems
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LCA is a widely used tool for determination of strength and weaknesses regarding ecological aspects of any product or service. It is expected from the scientific-based analysis to deliver robust information, but in fact in literature the results differs significantly for the same product or services. Due to the recognized uncertainty this study addresses the possible deviance of results from literature for electric energy production from different energy sources. The potential deviation could arise from the consideration of different system boundaries (SBs) and the relevance of emission sources along the life cycle. Both could be mainly responsible for the recognized diversity beside others like allocation, cut-off criteria, actuality of literature, etc.

The electric power generation gives a good instance to analyze the impact on the results of the different SBs. For example: the variation between different plant construction demands is often over 80%, but the overall results don't necessary correlate with this deviation. The change of cement demand for construction by 230%, in the case of electricity generation from coal, induces only 1.7-2% difference in GWP of the total system. This is an effect of different proportion of emission sources between the life cycle stages.

The contribution will point out where uncertainties and dependency of the results could arise through the examples of electricity generation from coal(lignite), natural gas, hydro energy, nuclear energy and from wind energy. The whole life cycle of power plant (with fuel upstream), the following system parts and impact categories have been taken into account by the understanding the results of LCAs:

- Construction, fuel upstream, transport, production, operation, decommissioning as life cycle phases
- Construction demand variables (basic construction materials: cement, steel, etc.)
- Fuel demand variables (efficiency factors: fuel purity, thermal efficiency, wind yield, transport, etc.)
- Impact assessment methods (GWP as main LCIA method, eutrophication and acidification potentials)

The chosen system boundaries and other variables contribute to the varying results of LCA studies for electric energy production. Data from the literature should only be used from reliable sources and after thoroughly investigation, if the SBs and information of the used variables is well described. Otherwise the help to mitigate climate change and save non-renewable energy might lead to false results and recommendations.

LC06P - Life cycle management (LCM): Success factors and barriers

TH 350

Using LCA in the preparation of the new waste management plan of Regione Lombardia

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Regione Lombardia has among its tasks the development of management strategies for municipal waste produced in its territory, in collaboration with provincial governments, the control bodies and public and private entities that operate on the collection, recovery and disposal cycle.

In order to pursue sustainability also in this field, Regione Lombardia has decided to consider "environmental performance" as a criterion for supporting future planning decisions, and therefore stressed the need for specific assessments of environmental impacts associated with the planning in the waste field.

Life cycle assessment (LCA) has thus been chosen by Regione Lombardia as a strategic support decision tool in the preparation of its new waste management plan. The goal is to use the life cycle thinking approach to assess the current regional situation and thus to give useful strategic indications for the future waste management. The project (called GERLA: GEStione Rifiuti in Lombardia - Analisi del ciclo di vita) started in November 2010 and will be completed by the end of 2011.

The first phase of the study consisted in the analysis of the present management of municipal waste in Lombardia Region (baseline scenario - reference year 2009). This has involved the identification of all the fluxes of materials (both the source-separated fractions and the unsorted residual waste) in terms of quantity, composition and destination, together with the characterisation of the most important treatment plants, in terms of their capacity, energy and materials consumption, emissions in the environment, energy and materials recovery. After that, the LCA methodology was applied to evaluate the energetic and environmental impacts associated with this baseline scenario.

The critical analysis of the results (Life Cycle Interpretation) of the baseline scenario has allowed the definition of a number of future scenarios for the year 2020, with the final goal of improving the environmental performances of the regional system.

This study represents thus an important application of LCA methodology since, for the first time in Italy, the outcomes of a detailed LCA of a current waste management system are actually utilised in the waste management planning on a regional scale.

TH 351

Life Cycle Assessment for end of life computers in Mexico

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The recovery of waste electrical and electronic equipment (WEEE) is an activity that becomes more important every day in Mexico. In 2010 electronic waste generation reached 307.000 tons annually. Of these, 10% is recycled or valorized, 40% remains stored in houses and 50% is sent to final disposal in landfills and open dumps. The aim of this project was to conduct a life cycle analysis using the program Umberto for electronic waste management in Mexico and compared its environmental impacts with those for three proposals for change in the end of life management pattern in order to determine the best option for a national policy. The policy proposals were modeled as follows: 1) Stage with 25% of recovery system, 2) Stage 35% to recovery system, and 3) 0% for disposal in open dumps. As conclusion it was identified to eliminate the use of open dumps for waste disposal in first place, followed by an increase in 35% of the recycling/valorization system.

TH 352

Implementation of an integrated technological-LCA modelling tool within the water industry - a pragmatic contribution to decision-making

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Nowadays, the water industry is expected to better take into consideration environmental issues. But it still has to deal with operating costs, sanitary risks, technical feasibility and reliability of designed plants. Therefore, the water industry managers need handy tools and practical methodologies that can provide them a complete set of information for projects under study. A good implementation of such decision supporting tool is obviously a condition to its success.

Within the EVALEAU research project, an integrated technological-Life Cycle Assessment modelling tool has been developed with the software Umberto®. The tool is based on an exhaustive library of unit process models. These models are highly detailed and parameterized, thus precisely describing the energy and mass balances depending on a specific project context. Complementary software tools are directly linked to the tool, which makes it benefit from previous modelling efforts and specific industry knowledge. For example, the tool is linked to the software PHREEQC® which simulates chemical reaction.

Every unit process model generates an engineering design report when placed within a modelling scenario. These reports are spreadsheets containing engineering design data (e.g. pumps power). This information is interesting at the design stage since decision can be made knowing how the process will work on the field. Problematic technological solutions can be avoided whereas convenient technologies will be selected.

A code for sensitivity analysis of the process model parameters is also part of the framework of the tool. The mathematical method followed is the Morris method. It aims at detecting the key parameters of the modelled scenario by qualitatively estimating their influence on any of the results (e.g. one selected environmental impact or the operating cost). Decision-makers can better understand how the modelling scenario react to a change on one model parameter. Assuming that the modelling scenario is realistic enough, conclusions can be made on the real plant. This key feature of the tool gives the designers good clues about the future plant functioning and indicates them which are the priority action levers.

The success of the tool implementation relies on its convenience on the field, obtained by previously developing it closely with its future users. Not only environmental issues are taken into account but the tool give the whole picture of an industrial project.

TH 353

LCA on drinking water production from pesticide contaminated ground water

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This poster presents the results from an LCA case study addressing a growing Danish problem on pesticide contaminated ground water. The conventional Danish way of using pesticide free groundwater and treating it by aeration and sand filtration, is compared to using slightly pesticide contaminated groundwater and adding treatment by activated carbon filtration and UV-treatment. The scenario of using slightly pesticide contaminated groundwater is chosen as it is a genuine and relevant alternative in the current Danish situation as groundwater wells are closing due to pesticide contamination and clean ground water is becoming scarcer. Besides the comparison, a hotspot analysis is performed in order to identify the dominating environmental impacts in the life cycle of generating drinking water from ground water. This study is part of the research project DWBiofilters (www.dwbiofilters.dk) aiming at improving sand filters for drinking water production by investigating its inherent microbial processes.

TH 354

Development of a new LCA tool for maintenance at the initiative of the users - a bottom-up approach

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Many practical LCA tools have been developed before a user group was established. The risk of such a top-down approach is a gap between the tool and the way users want to use the tool. This mismatch may result in a tool being less used by the users. In the Netherlands, a group of housing associations and building and maintenance companies were looking for means to calculate the environmental performance of planned preventive maintenance of housing, estates and buildings themselves. There are several theoretical approaches available, but no practical calculation tools. Because of this lack of instruments, they set up a development project for a practical calculation tool, to be developed by the consultancy company W/E and by the Delft University of Technology, who have much experience with LCA in construction. In the development project, generic environmental data on materials were combined with data of maintenance activities gathered by the maintenance companies. The set-up and interface of the calculation tool were designed according to the wishes and practices of the clients. The results are presented according to the three purposes of the tool: optimisation of the environmental performance of maintenance scenarios, comparison of different scenarios, and benchmarking the scenarios with average Dutch maintenance scenarios. The resulting calculation tool is a good example of the effectiveness of this bottom-up approach, aiding to achieve a broad support amongst potential users of this tool.

TH 355

Improving Life Cycle Management (LCM) tools for the food industry: a framework of Product-Oriented Environmental Management System (POEMS)

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The aim of this abstract is to present a LCM tool designed for the agri-food industry, a POEMS framework with a modular structure resulting from the integration of complementary tools: an Integrated Management System (IMS), a simplified Life Cycle Assessment (LCA) and a suitable Environmental Product Label or Declaration (EPLD) (EMAF Project co-funded by the Italian Ministry of Education, University and Research-PRIN 2008TXF8Y). In order to successfully apply this framework in the agri-food firms the main sector-specific barriers (SSB) to LCM implementation have been used as starting points to set the success factors (SF), solutions (S) and tools (T) of the POEMS model. In the following the path to the POEMS framework definition is summarized: the path efficiency is enhanced by the fact that each tool can provide multiple solutions to several barriers.

SSB: resistance to change; dispersion of the environment-related information; in Envtl. Management Systems (EMS) little attention is paid to product performances [ARROWRIGHT]SF: spreading an envtl. cultural change and involvement; structural and organized vision of envtl. aspects; internalize product requirements within the EMS [ARROWRIGHT]S: envtl. training and dissemination; EMS; Integrating EMS with a Quality Management System [ARROWRIGHT]T: Integrated Quality and Envtl. Management System

SSB: main focus on short-term problems; lack of chain management responsibility; lack of awareness of product life cycle envtl. impacts (PLEI); poor access to large amount of life cycle data; lack of in-house expertise; high expensive [ARROWRIGHT]SF: internalization of chain management vision and long-term value creation; identification of PLEI; allow SMEs to perform envtl. assessment by themselves providing an easily understandable tool [ARROWRIGHT]S: LCM and Life Cycle Thinking; LCA; simplified approaches [ARROWRIGHT]T: Simplified LCA

SSB: envtl. commitment is not perceived as an opportunity; problems with results communication and chain involvement; complexity and uncertainty in choosing the most suitable envtl. message [ARROWRIGHT]SF: ability to transform the envtl. measures taken into commercial advantages; spreading envtl. cultural change and involvement with external dissemination; identification of the proper envtl. label/declaration [ARROWRIGHT]S: EPLDs; envtl. product communication; guidelines to support decision making [ARROWRIGHT]T: EPLD guidelines

IMS+Simplified LCA+EPLD guidelines=POEMS

The EMAF project is in progress with applications in the canned vegetables, wine, pasta, olive oil and coffee supply chains.

TH 356

Finnish guidelines on carbon footprinting - supporting practical implementation of LCA in the food industry

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Life cycle thinking is spreading among environmentally responsible companies around the world. Specially carbon footprint guidelines, which have been developed in the last years, have increased the interest of industries to life cycle assessment on company and product level. Growing general knowledge and pressure to act on climate change has given incentives to companies to use carbon footprinting in their consumer communication as well. Consumers show growing interest to, for example, carbon footprints in many Gallup polls.

Many international standards and guidelines on LCA are published but no standard widely approved method on evaluating environmental impacts of food are available.

In the Foodprint - research programme sound national methodology for calculating carbon and other footprints of food has been developed in close collaboration with the Finnish food sector. As published guidelines are too generic to give practical instructions to companies to produce comparable carbon footprints for communication, the project has specified detailed methodology to follow. International standardisation, developments and best practices on evaluating environmental impacts are taken into account when preparing national specific guidelines. Finnish food sector is actively consulted to ensure practical methodology which can be easily applied.

In addition to development of national calculation guidelines, the project is also providing practical calculation tools to facilitate calculations at company level. The project also tested different data collection tools in R&D projects.

The project has been carried out as iterative process between research, companies and other stakeholders. Food industry wide workshops around the guidelines are used to make the guideline more applicable and user friendly for the industry. As many Finnish food companies use carbon footprinting, industry participation is high. The guidelines were also reviewed in a detailed way by other Finnish LCA experts from LCA and from other standards' point of view.

The company R&D projects were crucial for developing practical guidelines for the industry. Motivation of the whole supply chain of the companies in the beginning of the project is fundamental. Additional workshops for the whole industry gave important feedback and shall facilitate larger implementation of the guidelines.

TH 357

Life cycle thinking applied to an immunological product (vaccine) used for boar taint control in male pigs

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In 2009, Pfizer Animal Health (USA) started to apply the Life Cycle Assessment (LCA) methodology to some innovative products, with a first case-study on ImprovacTM, an immunological product (vaccine) for male pigs that, by providing farmers with an alternative way to avoid the problem of boar taint, also allows them to increase the efficiency of male pig production, which may in turn provide considerable life-cycle environmental and social aspects benefits: to avoid physical castration is in fact perceived as an animal welfare issue by many in the public sector.

In 2010, after an initial project which included a consistent data collection from the vaccine production plants as well as from farms and slaughterhouses at global level, the first Environmental Product Declaration or EPD on a veterinary product was published by the International EPD System, a well recognised ISO 14025 Program Operator (www.environdec.com); the results of this project were presented for the first time at the 2011 Annual European SETAC Conference in Milan.

In late 2011, the sample of farms participating in the global survey was extended for the development of the renewed and updated EPD to be published by the end of January 2012. The new data collection work led to the inclusion of a considerable number of representative farms from other countries worldwide.

This analysis provided LCA data to assess possible environmental benefits of the vaccine's application: the reduction of the carbon footprint is a strong perceived advantage for the agricultural and food sectors and for this reason, it is the major impact category of interest; other LCA relevant impact categories are presented and discussed to provide a comprehensive view of the life-cycle impacts of the product. The calculated carbon footprint for the Improvac pig system demonstrates a reduction vs. the physically castrated pig system, mostly depending on country specific feed production and slurry management practices. Starting from the feed components, a detailed examination was conducted on agricultural practices by country; the same holds for different slurry management procedures and technologies.

For this reason, the information collected and analyzed in the LCA process provides an important opportunity for swine producers to understand the magnitude of their

carbon footprint and how to potentially reduce it not only through the use of Improvac.

TH 358

Pilot test in food sector of product environmental footprint (PEF) Guide developed by European Commission

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In 2011, the Directorate General for the Environment (DG ENV) started to work with the Commission's Joint Research Centre (JRC) Institute for Environment and Sustainability (IES) and other European Commission services towards the development of a harmonised methodology for the calculation of the environmental footprint of products.

The Product Environmental Footprint (PEF) Guide developed by JRC IES has been tested using a limited number of pilot studies representative of a wide variety of goods and services. Pilot tests have been conducted on five Nestlé food and beverage product systems: Nespresso, Purina Gourmet Pearl Chicken and Vittel products have been quantitatively assessed whereas Nescafé and Kit Kat products have been qualitatively assessed.

The results of Nespresso case study according to PEF Guide are presented and they are compared to the results of the full life cycle assessment compliant with ISO 14040 and ISO 14044 published in 2011. The comparison highlights some differences related to specific modelling issues as Life Cycle Inventory (LCI) choices (e.g. electricity mix), LCI databases (e.g. ecoinvent or European Reference Life Cycle Database (ELCD)) and Life Cycle Impact Assessment (LCIA) methods (e.g. land use, terrestrial ecotox and turbined water as well as endpoint were assessed in the original study whereas they lack in the draft PEF Guide published in November 2011). The results will be used to discuss the degree of alignment of the PEF Guide with ISO 14040 and ISO 14044 as well as the practical implications of following the PEF Guide as compared to current LCA practice.

The PEF Guide aims at providing detailed technical guidance on how to conduct a PEF study identifying single requirement for each decision point with the objective to increase the consistency and the comparability of results whereas recognized methodological guidelines provide several alternatives, ranging from very general in the case of ISO 14040 and ISO 14044 to high specificity in the case of the French standard BPX30-323.

The Nespresso pilot test will be presented and shows an example of PEF Guide application and used to provide feedbacks about the PEF Guide.

TH 359

SETAC's influence on LCAs growth and direction

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The launch of Google labs' Books Ngram Viewer, which allows any user to graph the frequency of occurrence of words or phrases in Google's database of 500 billion words from digitized books, allowed for an investigation into the relationships of a number of LCA-related words over time. One combination, "SETAC" (Society of Environmental Toxicology and Chemistry) and "life cycle assessment", yielded a very interesting relationship for the years 1980-2008. The "SETAC" acronym first appeared in books in the 1980s. Its relative frequency of appearance grew steadily from 1990 through 2004, showing a tenfold increase. In 1990, SETAC sponsored an international workshop at which the term "life cycle assessment" was coined. Before then, a few practitioners in the U.S. and Europe used different terms such as "Resource and Environmental Profile Analysis" ("REPA"). SETAC established the accepted name (and framework) for life cycle assessment. The occurrence of the phrase "life cycle assessment" in books grew very similarly to the occurrence of "SETAC" from 1990 through 2004. Was this a coincidence or were there activities within SETAC that contributed to this parallel growth? This paper will provide a perspective of the role of SETAC in both NA and Europe had on the development of LCA.

THPC1-1

Occurrence of pharmaceuticals in several wastewater effluents and evaluation of a non-conventional biodegradation treatment

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Among the vast array of contaminants of anthropogenic origin reaching our water supplies, pharmaceutically active compounds (PhACs) have one of the largest inputs into the environment. The main route of entrance into the aquatic environment is ingestion following excretion and disposal via wastewater, manufacturing and hospital effluents, land applications (e.g. biosolids and water reuse), concentrated animal feeding operations (CAFOs) and direct disposal/introduction into the environment. Many studies have reported on the limited degradability of pharmaceuticals under the conventional treatments applied in WWTPs, concluding that it is necessary to investigate on other possible ways of treatment to achieve high quality effluents. It would be desirable that selected treatment methods would be effective for as many different pharmaceuticals as possible. This goal could be achieved with fungal treatment; in particular ligninolytic fungi have a powerful enzymatic system which has demonstrated its ability to degrade a wide variety of xenobiotics.

In this context, the present work will show the results derived from an extensive characterization of 80 human and veterinary pharmaceuticals in several wastewaters where concentration of pharmaceuticals is expected to be significant such as hospital and urban wastewaters and reverse osmosis concentrate. Results indicated that pharmaceuticals are widespread pollutants in all these matrices. As expected, high levels were found in hospital wastewaters followed by those found in reverse osmosis concentrate and urban wastewaters (concentrations between high ng/L and high µg/L). Among all PhACs investigated, the iodinated X-ray contrast (ICM) agent iopromide was detected in all wastewaters, with especially high concentrations in hospital wastewaters (nearly 200 µg/L), whereas in reverse osmosis concentrate and in urban wastewaters levels were much lower (40 and 70 ng/L, respectively). The persistence of ICM through conventional wastewater treatment is well documented. For this reason, this substance was selected as target compound to study its degradation and to assess the potential of white-rot-fungi to degrade PhACs from real effluents.

THPC1 - Wastewater effluent discharges: chemical characterisation and understanding potential risks in receiving waters

THPC1-2

Possible removal of several contaminants from the wastewaters by a natural biofiltration process

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Many recent studies have revealed the presence of concentrations of hundred pharmaceuticals and illicit drugs in the high ng L⁻¹ to low µg L⁻¹ range both in surface and sewage waters. Since more of them are only partially removed by the sewage plants, they contaminate the receiving surface waters with potential implications for wildlife. Moreover, some sewage effluents are used for agricultural purposes and they can transport these pollutants to soil and cultivated plants also. The aim of this research is the evaluation of possible abatement of these chemicals in sewage waters by the use of a biological process. In detail, we built a pilot-plant (600 L of volume) in the biggest depuration plant (Depuration plant of Nosedo) of Milan (Italy) in which we put several thousand of specimens of the freshwater bivalve *Dreissena polymorpha*. Mussels were attached to Plexiglas panels that force the waste to follow a zigzag pathway. We exploited two natural behaviors of this mussel: the heavy filtration rate (average of 200 mL/mussel/h), which transfer the contaminants from waste to the bottom of tank by the production of faeces and pseudo-faeces, and the bioaccumulation capability of lipophilic chemicals in mussel soft tissues. Thus, by the cyclic elimination of faeces and contaminated mussels from tank, we may depurate the waste. We will measure the possible clearance of several environmental pollutants: pharmaceuticals, illicit drugs, some persistent organic pollutants (POPs), several heavy metals and pathogen bacteria. Our project plan foresees three different steps: 1) the evaluation of the capability of *D. polymorpha* specimens to attach themselves to panels and to live in wastewater 2) to test the possible decrease of pollutants in re-circulation conditions; 3) to check the contaminant abatement from the inlet to the outlet of pilot-plant. In the first phase, we collected from two Italian lakes about 100,000 mussels that were put in two "nursery" tanks filled with tap water in which the Plexiglas panels were horizontally positioned. We evaluated the subsequent attachment and the optimal conditions for the mussel sustenance (type of food, water flux, temperature, mussel density). Secondly, panels with the attached mussels were put vertically into the pilot-plant to carry out the second step, which is in execution at this moment.

THPC1-3

A modified clay material as cost-effective sorbent for waste printing developer decontamination

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The application of moderated fired clay with polymer addition to the purification and treatment of printing wastewater may potentially revolutionize water treatment processes. The efficiency of treatment technologies at removing specific substances or groups of substances, especially those identified as priorities under the WFD is confirmed with Annex II lists of the waste and pollutants covered by the register, which include greenhouse gases, acid rain pollutants, ozone-depleting substances, heavy metals and certain carcinogens such as dioxins. Nanotechnology exploits the novel phenomena and properties of matter at atomic and molecular levels. Because of their particle size they can exhibit an array of novel properties that can be used to develop new water treatment technologies and improve existing ones.

Clay derivative materials with high adsorption capacities are very attractive from an economical point of view. Some naturally occurring clay minerals may serve as cost-effective sorbents for the removal of Zn(II) ions. While their sorption capacity is usually less than those of synthetic sorbents, these materials could provide an inexpensive substitute for the treatment of printing wastewaters. To enhance the sorption capacity, the clays are modified with various polymer additions. However, due to the economic constraints, a development of cost effective and clean processes is desired. Adsorption processes has proved to be the most effective, especially for effluents with moderate and low heavy metal concentrations, as like as in waste printing developer.

The present work was undertaken to investigate the feasibility of the cost effectiveness adsorbents: pure fired clay, newly designed fired clay with the polymer addition (5 mass% PEG 600) and bentonite for Zn(II) ion removal from waste printing developer. The adsorption efficiency and distribution coefficient of used adsorbents were determined as a function of adsorbent amount. The results show that newly designed clay adsorbent with polymer addition has great potential (up to 93.5%) to remove Zn(II) ion from waste printing developer.

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THPC1-4

Remotion of POPs from industrial wastewater using UVC/H2O2 Photolysis

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In this study use of H2O2-assisted TiO2 photocatalytic degradation was applied to remove persistent organic pollutants from industrial wastewater. Experiments were carried out with treated and filtered (0.7 µm) urban wastewater, spiked with AZO Orange II (5mg/l) and 4-Chlorophenol (0.36mg/l).

The experimental equipment employed in the assays consists in a tubular reactor of borosilicate glass, (600ml) operating in a recirculating circuit, irradiated with an UVC lamp (8W). The Hydrogen Peroxide (H2O2) in the process was 200mg/l. The catalyst (TiO2) was immobilized on the external surface of a cylinder of frosted glass (C=40mm). Experiments have been conducted using a total working volume of 1200ml and a recirculation flow rate of 600 (ml/min).

The efficiency of primary degradation was determined by monitoring the process, for AZO Orange II (absorbance 478 nm) and 4-Chlorophenol compounds. Toxicity assays were also carried out with the aim to evaluate the effects by products. An economic study of this process was included in the global analysis.

THPC1-5

Impacts of advanced wastewater treatment on metal speciation and bioavailability

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Increasing pressure to remove priority chemicals from wastewater treatment effluents is leading to pressure to utilise or consider advanced tertiary treatment options such as UV radiation, filtration, GAC, and ozonation as part of the wastewater treatment process. The use of these adsorptive or destructive methods may, however, increase, decrease, or leave largely unchanged the concentrations of ligands in effluents that are important for complexing metal ions and which, consequently, reduce the toxicity potential of metals such as copper and zinc. Since there is not much data on the metal complexing characteristics of effluents before and after tertiary treatment, there is uncertainty in the extent by which tertiary treatment options might impact metal bioavailability. To investigate possible impacts, a chelex column ion exchange method was used to assess the effects of GAC, ozonation, and UV radiation in combination with H2O2 on the ligands in sewage effluent that are responsible for complexing copper and zinc. Preliminary results indicate that tertiary treatment options tend to increase the concentrations of metal complexing ligands relative to the concentration of dissolved organic carbon present. This effect was also of greater significance for copper than for zinc. The ozone treated effluents displayed lower UV absorbances (350nm) in relation to other secondary and tertiary treated effluents, indicating ozonation to have some impact on the concentrations of aromatic substances in the effluents, which is consistent with the elimination of humics; however, this did not impact complexation capacity. These findings indicate that advanced tertiary treatments are unlikely to increase the bioavailability of metals in sewage effluents.

THPC1-6

The influence of terminal electron acceptor on the removal of pharmaceuticals in Anaerobic digester sludge

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Within each stage of the wastewater process pH and redox conditions fluctuate, and the characteristics of each sludge vary. These changes result in differences in the microbial population present, and variations in how strongly micropollutants adsorb to each sludge. The total removal of micropollutants, the rate of removal, and the mechanisms responsible for removal will therefore vary depending on the redox conditions present.

Anaerobic digestion is used for the treatment of 2/3 of municipal biosolids within the UK, and is a growing technology for the treatment of industrial and food waste due to the generation of renewable energy in the form of biogas. Anaerobic digestion is a multi-step microbiological degradation process, and can be performed in a single reactor or in multiple reactors where the hydrolytic fermentation and methanogenic phases are separate. Hydrolysis, acidogenesis, acetogenesis and methanogenesis each occur under different redox conditions, and each is associated with a different terminal electron acceptor (nitrate, sulphate, carbonate and methane respectively).

Separating hydrolysis from methanogenesis has been shown to be more efficient for the generation of biogas, however, biodegradability tests performed to determine whether a test substance will degrade under anaerobic conditions are performed under methanogenic conditions only. A test system whereby the redox potential is controlled through amendment of terminal electron acceptors was validated through the measurement of redox potential (Eh), the reduction of carbonate, nitrate and sulphate, and the removal of dissolved organic carbon. When it was demonstrated that these conditions could be effectively controlled this test system was applied to the removal of test substances under amended conditions.

Conditions were amended in a batch test and in a serum bottle experiment to measure the total removal and mineralisation of two prioritised test substances that have been identified within the biosolids. The results of these experiments will be discussed and compared with data obtained from biodegradability experiments performed under unamended conditions following guideline OECD 311.

EP06P - Perfluorinated compounds: From emission sources to the place of impact

TU 001

Particle size distribution of airborne perfluorinated compounds

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In recent years, perfluorinated compounds (PFCs), particularly perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) have been described as compounds of increasing environmental concern and numerous studies were conducted to investigate the environmental distribution and fate of these compounds. Besides their transport with ocean currents, atmospheric transport was identified as important (long-range) transport pathway. As the particle size is an important parameter characterizing the fate of atmospherically transported compounds, knowledge about the particle size distribution of particle-bound PFCs is of particular interest. Therefore the objective of this study was to investigate the particle size distribution of PFCs.

Particle phase samples were taken in parallel using a cascade impactor with cut-off diameters of 11.4 µm (A4, B4), 3.81 µm (A3, B3), 1.38 µm (A2, B2), 0.46 µm (A1, B1), and 0.14 µm (back-up filter, A0, B0). Each particle size fraction of each sampler was extracted separately using ultrasonication and methanol. Samples were measured by HPLC-ESI-MS/MS.

PFC concentrations in particle phase samples (sum of all fractions) were between 0.5 and 2.5 pg m⁻³. Of 25 analysed PFCs, 18 could be quantified. These were C4, C6-C8 PFSA, C4-C14 PFCA, PFOSA, MeFOSE and EtFOSE. PFOS and PFOA were observed in highest concentrations (0.17-3.5 pg m⁻³, 0.09-4.8 pg m⁻³, respectively), followed by PFBA and PFNA.

The observed particle size distribution of PFCs in parallel samplers were quite similar indicating a good precision of the entire method. However particle size distribution between perfluoroalkyl sulfonates and perfluoroalkyl carboxylates differed. Whereas carboxylates were primarily detected in the finest particle fraction (<0.14 µm) sulfonates were mainly observed in mid-size particle fractions (cut off 3.81; 1.38) of the investigated particle size spectrum.

TU 002

Binding and sequestration of perfluorinated surfactants in soil

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Perfluorinated surfactants (PFCs) produced since the 1950s draw great attention due to their wide application and increasing occurrence in the environment. Sorption coefficients (Kd) and organic carbon normalized soil adsorption coefficients (Koc) are essential for determining soil adsorption behavior and assessing the risk of transfers of these chemicals from contaminated soils to plants and groundwater. We therefore analysed the international literature for concentrations of perfluorinated octanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) in different environmental media (surface water, sewage treatment plants, sediment, and soil) in order to assess the distribution of PFOA and PFOS between these environmental compartments and compared the results with published experimental sorption coefficients. Experimental log Koc values for PFOS for different soils and sediments available in public literature are remarkably similar with a coefficient of variation of only 18%, which should limit the uncertainty of concentrations in drainage water and groundwater calculated from soil concentrations (average log Koc = 2.9). For PFOA however, published sorption coefficients are sparse and the published information regarding the analogy between sorption of carboxylated and fluorinated PFCs is controversial (average log Koc = 2.6). Our review shows that current pattern of environmental PFOA and PFOS concentrations are strongly influenced by local pollution sources. Nevertheless, we calculated "global" Kd/ Koc distribution coefficients based on median concentrations in sediments/surface waters or sewage sludge/sewage. The "global average" Kd calculating from the median concentrations of PFOA in WWTP effluent and sewage sludge equals 1958 l kg⁻¹ (log Kd = 3.3). Assuming an average dry matter organic carbon content of 31% for sewage sludge would give a global average log Koc of 3.8. Dividing the median sediment concentration of 0.6 ng g⁻¹ by the median surface water concentration results in a "global average" Kd value of 275 (log Kd = 2.5). The "global average" log Koc assuming an average sediment Corg concentration of 1.7% equals 4.2. Furthermore, the estimated "global" log Koc were similar to experimental log Koc values found in the literature. The moderate affinity of PFOA and PFOS to soils, sediments and sewage sludge indicates that drinking water must be considered a potential pathway of exposure of animals and humans to these compounds.

TU 003

Investigating the sorption of Perfluorinated Compounds in different types of sludge

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Perfluorinated compounds (PFCs) present significant research interest in recent years due to the global distribution, persistence, bioaccumulation and potential toxicity of these compounds. Despite the fact that PFCs are commonly detected in municipal wastewater treatment plants (WWTPs), so far there are few data for their sorption potential to sludge. The aim of this research was to investigate the sorption potential of different PFCs to three different types of sludge (primary sludge, secondary sludge and digested sludge). For this reason, sorption experiments were performed with eight PFCs belonging to two different classes; perfluorocarboxylic acids (PFCAs) that contain a carboxylic group in their molecule and perfluoroalkyl sulfonates (PFASs) that contain a sulfonate group in their molecule.

At first, sorption experiments were performed to investigate the equilibrium time for each target compound and the role of sludge inactivation on sorption potential of PFCs. For this reason, samples were taken at different time intervals and PFCs were detected in dissolved and particulate phase. PFCs were extracted by solid-phase extraction (dissolved phase) or sonication (particulate phase) and determined using High Performance Liquid Chromatography - Ion Trap Mass Spectrometry (HPLC-IT-MS).

Afterwards, sorption experiments were performed for six different spiking levels of PFCs, ranging between some ng L⁻¹ to few µg L⁻¹ and solid - water distribution coefficient (K_d) values were calculated. Finally, the effect of solution pH on PFCs sorption was investigated in experiments performed at pH values commonly found in WWTPs (6, 7 and 8).

According to the results, K_d values of PFCs were differentiated significantly according to the type of sludge. Moreover, it was shown that both the length of the perfluorocarbon tail and the functionality of the head group can influence the sorption of these compounds to sludge materials.

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TU 004

Presence and behaviour of perfluorinated compounds in sewage sludge

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Perfluorinated compounds (PFCs) are used in industrial and domestic appliances and have emerged as significant global environmental pollutants with persistent, bioaccumulative and toxic properties. After use or disposal of PFC-containing products, these compounds enter Waste Water Treatment Plants (WWTP) and accumulate in sludge because their intrinsic physicochemical properties make them resistant to final mineralization. Among other options, the use of sewage sludge as agricultural fertiliser is common practice all over Europe. The aim of this study was to determine the presence of 5 perfluorinated compounds (PFCs), namely perfluorooctanesulfonate (PFOS), perfluorohexanesulfonate (PFHxS), perfluorobutanesulfonate (PFBS), perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA) in sludge and evaluate their transfer to agricultural soils. PFCs were analyzed in sludge from 15 WWTP from Spain and Germany. These WWTP receive both urban and industrial wastes. PFCs were detected at 0.28 and 5.15 ng/g dw for Spanish sludge and from 14.2 to 56.0 ng/g dw for German Sludge, with differing concentration patterns of the individual compounds. In addition, the degradability of PFCs within the sludge treatments (primary sludge, anaerobic digested and centrifuged sludge) was evaluated and we found that anaerobic digestion and centrifuging did not have an effect on the PFC elimination. Subsequently, the amount of PFCs accumulated per ton of sludge generated was determined for each WWTP. According to the use of sludge in each country, the calculated contribution of PFCs to agricultural soils was estimated between 0.06 and 12.88 g/ha month. Finally, the concentration of PFCs were determined in agricultural soils and sludge amended soils as a way to determine the real impact of PFCs in soils. Although there are no legislated limits for PFCs in sludge (Council Directive 86/278/EEC and Royal Decree 1310/1990, regulating the use of sewage sludge in agriculture), its use in agriculture may lead to diffuse pollution of soils and groundwaters. In Spain, 95% of the sewage sludge is used as fertilizer in agriculture while in Germany, most of the sludge is incinerated or used for landfilling.

TU 005

Occurrence and evaluation of the fate of Perfluorinated Compounds in wastewater treatment plant of Athens (Greece)

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Perfluorinated compounds (PFCs) have been classified as emerging contaminants and they are commonly detected in municipal wastewater treatment plants (WWTPs). In this study, contamination profiles of eighteen target perfluoro analytes (C5 to C14 carboxylic acids, C4, C6-C8 and C10 sulfonic acids and 3 sulfonamides) were detected in wastewater (influent and secondary effluents) and sludge samples, originating from WWTP of Athens (Greece). Two seasonal sampling campaigns were performed and samples were taken in seven consecutive days in each sampling campaign. The analytes were extracted by solid-phase extraction (dissolved phase) or sonication (solid phase). Qualitative and quantitative analyses were performed by LC-MS/MS. Recovery values generally ranged between 80% and 115%, while lower recoveries were obtained for longer perfluorocarboxylic acids (PFCAs) and perfluoroalkyl sulfonates (PFASs) in sludge samples. The limit of quantification of the target compounds varied from 0.29 to 3.0 ng L⁻¹ and from 0.15 to 1.5 ng g⁻¹ for liquid and solid samples, respectively. The seasonal and daily variations of target compounds' concentrations were investigated and the results were compared to those reported in the literature for other WWTPs worldwide. Daily mass flows normalized to inhabitants were also calculated and distribution of target compounds between dissolved and particulate phase was determined for wastewater samples. A mass balance was also performed to investigate the fate of PFCs during wastewater treatment plants and to estimate the role of degradation and sorption on their removal. According to the results, perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) were detected in all samples and they were the major contaminants in wastewater and sludge. Mean concentrations in influents, effluents and dewatered sludge ranged up to 90.4 ng L⁻¹ (PFOA), 103.5 ng L⁻¹ (PFUdA) and 377.9 µg Kg⁻¹ (perfluoroundecanoic acid, PFUdA), respectively. Excepting perfluorooctane sulfonamide (PFOSA), perfluoropentanoic acid (PFPeA), perfluoroheptanoic acid (PFHpA) and perfluorodecanoic acid PFDA that were partially removed during wastewater treatment, concentrations of other PFCs in effluents were similar or higher comparing to influent wastewater. The observed increase in concentrations of some PFCs in effluent wastewater, suggests a possible formation of these compounds within the wastewater stream due to biodegradation of precursor compounds.

TU 006

Biodegradation of proposed PFOA & PFOS precursors in aerobic batch assays

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Perfluorinated compounds (PFC) are a group of emerging environmental pollutants even though no natural sources of PFC have been proposed. Nevertheless they can be detected in the environment throughout the world. Due to their unique water-, fat- and oil-repellent as well as stain-resistant properties, they are widely used in various industries and commercial products. Some of them are classified as persistent, bioaccumulative and toxic compounds. Hence they display a threat of nature. Potential sources of PFC can be consumer and industrial products as well as manufacturing processes.

In some sewage treatment plants worldwide, an increasing mass flow of perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) was observed with higher concentrations in the effluents compared to measurements in the influents. This leads to the assumption that there have to be precursors which are biodegraded during the sewage treatment. Thus the aim of this work was the detection of precursors for PFOA and PFOS by studying their aerobic biodegradability.

Amongst others perfluorooctanesulfonamide (PFOSA)¹, 8:2 telomeric acid (8:2 FTCA), 8:2 telomeric alcohol (8:2 FTOH)^{2,3}, and a technical product containing polyfluoroalkyl phosphates were chosen to study their fate in aerobic batch assays. The test period was 14 days each at a water temperature of 12.7°C. The activated sludge samples were obtained from a municipal sewage treatment plant and the test medium was chosen following the EN ISO 9888 guideline, June 1999, with a 10-times higher phosphate buffer concentration. The water and sludge samples were analyzed using LC-MS/MS and GC-MS.

All in all the above mentioned substances degraded to PFOA or PFOS in ranges from 1 to 8 per cent during 14 days. The lowest metabolic rate was monitored for PFOSA. In addition the technical product degraded to perfluorohexanoic acid (PFHxA) as well. The absence of degradation products in the abiotic controls show that the metabolism has to be attributed to microbial degradation only.

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TU 007

Identification of the sources of polyfluoroalkyl substances (PFASs) in the Italian surface waters

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A survey of the distribution of perfluorinated carboxylates (from C5 to C10) and perfluorinated sulphonates (C4 and C8) in the main Italian river basins have been carried out in 2011 in order to get a reliable picture of the polyfluoroalkyl substances (PFAS) contamination and possibly to identify the main sources for the aquatic environment. Monitoring campaigns on river Po (north of Italy), the major Italian river which flows in the Adriatic Sea, its tributaries, river Adige, river Tevere, river Arno and river Brenta basin have been carried out in different hydrological conditions. A survey has been performed also in transitional coastal areas such as river Po Delta and Lagoon of Venice. Chemical plant discharges and drinking waters present in the same basins were also sampled.

Analyses of PFAS were achieved by using an on-line SPE-HPLC-MS-MS system. Recovery for PFASs for all analytes was above 70% at µg/L levels. Limits of detection (LOD) ranged from 0.2 to 2.5 ng/L.

The monitoring campaigns allowed to identify hot spots in the main Italian basins.

Unlike rivers Tevere and Adige which are not significantly impacted, concentrations determined at the basin closure of the river Po are comparable to those measured in the PFAS heavily impacted areas of northern Europe: PFOS concentrations are low (ranging from < LOD to 2.5 ng/L) while PFOA is the main compound (about 25 ng/L). Fluoropolymer factory, present in the Tanaro/Bormida watershed, was confirmed as the main source of the latter molecule. Plant for the production of fluorochemicals, used as intermediates in the polymer synthesis, is a significant source of PFOA and PFBS in the river Brenta which discharges in the Adriatic sea.

Two important textile industrial districts in Italy (counties of Vicenza and Prato) have been also identified as a significant source of PFPeA and PFHxA which are discharged in the Brenta basin and in river Arno. These shorter chain perfluorocarboxylic acids can be considered as tracers for this kind of industrial pressure. The same compounds were also measured in the river Adda, an important tributary of the river Po, but the source shall be still to be identified.

The diffusion of these substances in the drinking waters in the same areas has been also studied.

TU 008

PFAS sources to groundwater and drinking water: identification and origin

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All humans are exposed to perfluoroalkyl acids, as has become evident from blood analysis. The exposure to PFAS occurs primarily via the dietary intake and more specifically via drinking water. It was estimated that when assuming a tap water concentration of PFOA of 9 ng/L the intake via water would amount to 55 % of the total daily Dutch PFOA intake.

Knowledge about sources of PFAS to drinking water is currently scarce and focuses on surface water based drinking water. Waste water treatment plants have been identified as major sources for PFAS to surface waters and ultimately to the drinking water produced from it. However, groundwater is in many countries the major source for drinking water production (in NL 65%). The extent of contamination of groundwater by PFAS and the contamination pathways to groundwater bodies remain largely uncertain.

In the work presented, we identified different direct sources of PFAS to groundwater within the catchment area of a public supply well field (PSWF) in the Netherlands. It was found that landfill leachate, and urban/military base affected rainwater contaminated the groundwater within the catchment area of the PSWF. Rainwater not affected by the direct sources also infiltrated in this area and provided a background contamination to the groundwater.

Maximum concentrations encountered in the landfill leachate plume were 1758 ng/L perfluorooctanoic acid (PFOA) and 1165 ng/L perfluorobutanoic acid (PFBA).

Maximum concentrations measured in the groundwater halfway the contaminant sources and the PSWF (15 years travel distance) were 29 and 161 ng/L for PFOA and PFBA respectively. Concentrations in the groundwater wells at a travel distance of 25 years were much lower: 0.96 and 3.5 ng/L for PFOA and PFBA respectively. The chemical signature of the groundwater showed that infiltrated rainwater, the landfill leachate and the infiltrated urban/military water contaminated the abstracted water. Based on the hydrological modeling it was shown that 1% of the pumped groundwater was originating from the landfill and military area and 99% from background contamination. This is seen in the relative abundance profile of the PFAS in the pumped groundwater.

TU 009

Seasonal distribution of perfluorinated compounds (PFCs) in surface water from Elbe River and North Sea, Germany

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Since the ubiquitous occurrence and potential toxicity to human beings, the perfluorinated compounds (PFCs) have attracted more concern in the recent years. In 2010, one of the PFCs, perfluorooctane sulfonate (PFOS) was added to Annex B of Stockholm Convention on Persistent Organic Pollutants (POPs). Recently, more substitutes, i.e. perfluorobutane sulfonate (PFBS) and perfluorohexanoic acid (PFHxA), were widely used and the elevated levels have been found all over the world. In this study, four cruises in Elbe River and three cruises in North Sea were conducted throughout the whole year of 2011 to investigate the seasonal distribution of perfluorinated compounds in surface water. Perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonates (PFASs) were determined in both particle and dissolved phases in more than 100 samples. The profile patterns suggested the industrial and urban discharge which were the major source of PFCs in river and coast water.

TU 010

Occurrence of 14 perfluorinated compounds and other priority and emerging organic compounds in fishes from the Rhone River (France)

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The Daughter Directive [‘EQS’ Directive, 2008/105/CE; EC, 2008] details the application of the Water Framework Directive for the monitoring of priority substances and other pollutants in surface waters; in particular, it requires the implementation of temporal and spatial trend monitoring programs. The use of integrative matrices (biota and sediments) is strongly recommended to achieve such objective, especially for compounds having a log K_{ow} > 3.

The main goal of this study was to obtain data on the occurrence and levels of 34 priority and emerging organic compounds in freshwater fish sampled in the Rhone River. The analyses included 14 perfluorinated compounds (PFC), and also 5 alkylphenols (AP), bisphenol A (BPA), 3 hexabromocyclododecanes (HBCD), 9 polybromodiphenylethers (PBDE) and 2 compounds, hexachlorobenzene (HCB) and hexachlorobutadiene (HCBD) for which Environmental Quality Standard (EQS) have been set for biota (10 and 55 µg/kg fresh weight -fw- respectively) [E.C., 2008].

Fishes were captured from August 2008 to January 2009 at 3 sites located upstream and downstream of the Lyon metropolitan (France). The four freshwater fish species studied, namely the barbel (*Barbus barbus*), the common bream (*Abramis brama*), the white bream (*Blicca bjoerkna*) and the chub (*Squalius cephalus*), were chosen because they may reach large size and are long-lived species, but they have different diets and exploit different habitats. Chemical analyses were conducted on pooled fish samples and also on some individual fish samples in order to check the homogeneity of pooled samples. A total of 49 fish samples were analysed (pooled or individual fish samples).

Results on the quantification frequencies, mean, median, minimum and maximum concentrations measured for the 14 AP and the other selected compounds in the 49 fish samples will be discussed according to what has been already found in the literature. Comparison on contamination level according to the fish species and also to the sampling site will be presented and discussed.

References

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TU 011

Perfluorinated compounds (PFCs) in peregrine falcon eggs from southwestern Germany - levels, patterns and temporal concentration variations

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As a top predator, the peregrine falcon (*Falco peregrinus*) is capable of accumulating a wide range of persistent pollutants and therefore a valuable object for observation and chemical analysis. The species attracted not only scientific but also public interest, at the latest since it was at the brink of extinction in Germany and elsewhere through excessive burdens of organochlorine pesticides such as DDT. A network of volunteers in the German state of Baden-Württemberg offered the possibility to collect unfertilized or unhatched peregrine falcon eggs as samples for monitoring environmental contaminants. In the past years, perfluorinated compounds (PFCs), especially PFOS raised scientific and public concern as their POP-like properties became apparent. Therefore, the analysis of PFCs in peregrine falcons' eggs was integrated into the existing monitoring programme.

Samples were collected in Baden-Württemberg from 2008 to 2011. About half of the number of the 20-30 egg samples collected per year were analysed for 11 PFCs (4 sulfonates, 6 carboxylates and the perfluorooctane sulfonamide). The analysis was performed using cold sample extraction and ion exchange-SPE clean-up followed by LC-MS/MS detection at LOQs of about 0.5 ng/g dry weight.

For this poster we aim for presenting data in order to form a baseline for the PFC levels in the eggs samples for the years from 2008 to 2011. This will offer the first possibility to establish the temporal concentration variations for PFC in peregrine falcons from Baden-Württemberg.

For 2009 and 2010, the substance spectrum was clearly dominated by PFOS at average concentrations of about 150-260 ng/g dry weight. Longer chain carboxylates were observed at concentrations of about 1-30 ng/g dry weight, whereas the PFHxA, PFHpA and PFOSA were not detected. The dry matter content of the eggs was at around 20%. PFOS concentration in the highest contaminated eggs approaches the toxicity threshold (LOAEL 5000 ng/g fresh weight; NOAEL 500 ng/g fresh weight) for PFOS established for chicken eggs. Thus Peregrine falcon eggs may be compromised by PFOS if their sensitivity is similar to the chicken. These findings generally confirm results presented earlier (e.g. Holmström et al., 2010, Sweden).

TU 012

PFASs in feathers of white tailed eagles (*Haliaeetus albicilla*) from Greenland and Norway; useful for non-destructive monitoring?

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The white-tailed eagle (*Haliaeetus albicilla*), also referred to as white-tailed sea eagle (WTSE), is a large predatory bird from the Northern part of Eurasia. It is a top predator from the aquatic ecosystem and feeds mainly on marine fish, waterfowl and carrion.¹ Because of its high trophic position, the WTSE is a very interesting biomonitoring species for studying accumulation of persistent organic pollutants (POPs).

In this study, our aim was to increase the knowledge on the analysis of perfluorinated aliphatic substances (PFAS) in biological tissues and specifically develop a reliable method to measure PFAS in feathers of this raptor species. Feathers have already been used successfully for monitoring of heavy metals, and recently also for POPs, but a reliable method for PFAS was not available and needed to be established.^{3,4} The results gained from this research enabled us to monitor endangered raptors without harming the birds and their offspring and to better understand the fate of PFAS in birds.

From Greenland body feathers (n=11) and primary wing feathers (2th, 5th and 8th primary; n=46) and preen oil (n=7) was collected when available in a sufficient amount for analysis. Tissue samples were taken from the Greenland carcasses to analyze for PFAS and POPs⁵. In addition, tail feathers were sampled at active nest sites of WTSE situated in Northern Norway (n=18) in 2009.

For the first time, PFAS were detected in feathers and preen oil of white tailed sea eagles from Greenland and Norway. In the wing feathers from Greenland WTSE, PFOS and PFOSA were detected. In some cases, PFNA, PFUnA and PFTrA were detected in minor concentrations as well. The average concentrations of the primary wing feathers #2, 5 and 8 were similar. In addition, the body feathers showed similar PFOS levels, but lower PFOSA levels.

TU 013

Perfluorinated chemicals in Belgian barn owls (*Tyto alba*): comparison of levels in feathers and tissues

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Feathers have proven very useful regarding non-destructive biomonitoring of contamination with heavy metals and organic pollutants. For this study, we have investigated perfluorinated chemicals (PFCs) in feathers and tissues of barn owls (*Tyto alba*), collected in the province of Antwerp (Belgium). A major PFC plant (3M) is located in the close vicinity of the city of Antwerp and levels of PFCs in biota from this area have been found to be very high in previous studies. We analysed levels of PFCs in tail feathers and tissues (liver, muscle, preen gland, adipose tissue) obtained from barn owl road-kill victims (n=15). We used methods for left-censored data to cope with levels below the limit of detection. We aimed to study the main sources for PFCs in feathers as levels can both originate from internal sources (via the blood) as from external contamination (via e.g. air, dust).

PFOS was detected in all tissues (median concentration: 135 ng/g in muscle, 305 ng/g in liver, 431 ng/g in preen oil, 203 ng/g in adipose tissue), also in feathers (median 14.7 ng/g; < 2.2 - 56.6). PFOS levels in feathers and liver tissue were highly correlated (r=0.79, p<0.001), but this was not the case for PFOS levels between feathers and muscle (r= 0.15, p=0.47) or between muscle and liver (r=0.46, p=0.08). This may be due to the chemical properties of PFCs which are mainly bound to proteins in the blood, thus reducing the suitability of muscle tissue. PFOA was measured at high levels in the feathers (<14.1 - 670 ng/g), but not in the tissues (more than 50% < LOD). Furthermore, PFOA levels in feathers and liver were not significantly correlated (r=-0.17, p=0.57). These results suggest that PFOA may be present on the external surface of the feathers and was not washed off by using distilled water and hexane. External contamination with PFOA is most probably originating from the air due to the close vicinity of potential point sources. PFHxS could only be quantified in liver and preen oil. Sporadically, other PFC compounds were detected as well, mostly in liver.

Overall, these results indicate that PFOS levels in feathers and liver are highly correlated, while PFOA levels in feathers are probably originating from external contamination. Therefore, the feasibility of feathers for PFCs monitoring should be investigated more in depth in the future.

TU 014

Monitoring of perfluorinated compounds

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Because of their persistence and wide ranging application perfluorinated compounds (PFC) are found as ubiquitous contaminants in aquatic and terrestrial organisms as well as in food products. HPLC-MS/MS analysis of animal and non-animal foodstuffs and wild game was performed within the framework of preventative consumer protection.

Fish from regional waters:

Sixteen fish from the Rhine river including eel, perch, roach, tench and catfish were examined for the presence of PFC. Concentrations between 5.2 µg/kg and 143 µg/kg were detected in muscle tissue as the sum of PFC detected (Σ PFBS, PFPeA, PFHxA, PFHxS, PFHpA, PFOA, PFOS, PFNA, PFDA, PFDS). The highest concentration was measured in perch muscle tissue.

Roe deer:

Use of PFOS has been declining since the year 2000. In order to observe the ensuing temporal changes in PFC concentrations in animal matrices a retrospective study of liver tissue from roe deer was undertaken. A total of 110 samples taken between 1998 and 2010 from the German Environmental Specimen Bank were examined. A decline in PFOS contamination since the beginning of this millennium is evident. Average PFOS concentration was 9.2 µg/kg in the year 2000 and 1.9 µg/kg in 2010.

Wild boar:

PFOS and PFOA concentrations were measured in 506 muscle tissue samples and 529 liver samples from wild boar. The arithmetic mean of PFOS concentrations detected in muscle tissue was 1.38 µg/kg whereas the mean PFOA concentration was below the LOQ. In liver tissue the mean PFOS concentration was 4.02 µg/kg (Maximum value 45 µg/kg) (1).

Foodstuffs:

Measurements of 82 samples of French fries showed PFC concentrations above the LOQ in 3 samples. No concentrations above the LOQ were detected in 30 samples of ice cream, 14 samples of whole milk, 19 samples of carrots or 16 samples of grains. A total of 84 samples of ocean fish, farmed fish, seafood, fish in packaged salads and canned tuna were tested for the presence of PFC. No PFC were detected in 82 of the samples. Two carp from fish farms had 2 and 14 µg/kg, respectively in muscle tissue.

(1) T. Stahl, S. Falk, K. Failing, J. Berger, S. Georgii, H. Brunn, Article title: PFOA and PFOS in Liver and Muscle Tissue from Wild Boar in Hesse, Germany, Arch Environ Contam Toxicol, DOI: 10.1007/s00244-011-9726-3

TU 015

Development of a pharmacokinetic model (PBPK) for the assessment of infant exposure to PFOS and PFOA for health risk assessment

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Perfluorinated compounds (PFCs) are very stable compounds with a lot of industrial application, but also very persistent on the biota and the environment. Among them, PFOS (perfluorooctane sulfonate) and PFOA (perfluorooctanoic acid), have a long persistence in the human body (4-6 years). This persistence creates an important concern among the scientific community and the health authorities. In fact, PFOS was listed under the Stockholm Convention in May 2009 as a persistent organic pollutant (POP), while PFOA is a serious candidate to enter that list. The main target organs of accumulation are blood and breast milk. Thus, PFCs can be a risk for adult population and infant. In order to assess the tissue concentration, PBPK models are able to assess the concentration along the time in the main target organs of concern. PBPK models are mathematical representations of the human body where the organs are considered as compartments, and they can be resolved computationally as a set of equations.

The PBPK model presented here is a multi-compartmental model for PFOS and PFOA for adult and childhood exposure during the breastfeeding period. Aside from the blood and breast milk, another tissue such as brain and kidney have been considered. PFCs are well absorbed, non metabolized and eliminated by urine and breast milk. The main sources of exposure for adult population are food and water intake. The data for food and water ingestion have been obtained from a large monitoring study in Catalonia, Spain.

For toxicity, the tissue residue approach (TRA) was used. The TRA is the use of tissue concentrations as the dose metric for characterizing toxicant potency. The PBPK model here presented was validated by comparing theoretical values with experimental data of PFCs chemicals in blood serum and breast milk collected in Catalonia, Spain. Finally, uncertainty and sensibility analyses have been performed. A normalized local sensitivity analysis was performed on the model to examine the influence of each model parameter on the model output. Sensitivity coefficients were calculated for the predicted plasma area under the curve (AUC; total concentration) with the original parameters and for those resulting from a low change in each parameter value.

TU 016

Acute toxicity of Perfluorinated compounds to two kinds of cladocerans

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Due to their unique properties, poly- and perfluorinated compounds (PFCs) have been manufactured and used during the past 50 years in a variety of industrial and commercial products, such as household surface finishes, food packaging, water- and stain-resistant materials, and fire-fighting foams. PFCs were released to the environment during production, usage and disposition. PFCs tend to persist in surface waters. Therefore their toxicity to aquatic organisms is of particular concern. Serving as a food source for amphibians, fish and other aquatic organisms, cladocerans are one of the key trophic elements of aquatic ecosystems. In addition to their ecological significance, cladocerans have the advantages of being useful as test organisms due to their short life cycle, their ease of laboratory culturing, their limited space and water volume requirements, and their sensitivity to chemicals. Cladoceran species are therefore widely used in aquatic toxicology. Among freshwater cladocerans, *Daphnia magna* is probably the most commonly used test organism in ecotoxicological studies. Some toxicity tests have been performed on cladocerans for perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) or their salts.

Because of their global distribution, environmental persistence and potential risk to human beings and aquatic organisms, PFCs are of particular concern for research and regulatory communities. However, insufficient toxicity data are available to accurately assess their possible environmental hazard. Therefore, the acute toxicity of seven poly- and perfluorinated carboxylic acids and alcohols on two cladocerans, *Daphnia magna* and *Chydorus sphaericus*, was evaluated in the present study. The adverse effects of these PFCs on these two cladocerans decreased with increasing fluorinated carbon chain length (nC) and quantitative structure-activity relationships were developed to quantify this observation. As the EC50 values obtained are far above concentrations typically found in surface water, acute harmful effects of these chemicals to *D. magna* and *C. sphaericus* are not expected in the real environment.

TU 017

Perfluorooctanoic acid toxicity in zebrafish (*Danio rerio*)

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Perfluorooctanoic acid (PFOA) is a common surfactant with wide use due to its unique nature, and has been detected as a contaminant in soil, air, water and biota. Previous studies have shown that PFOA has detrimental effects on different life stages in zebrafish (*Danio rerio*).

We have evaluated the effects of PFOA toxicity covering different life stages of zebrafish through three tests that were based on OECD guidelines. In the Fish Embryo toxicity (FET) test, fertilized embryos were used to calculate effective concentrations (LOEC and NOEC) based on early life stage lethal and sub-lethal endpoints. Based on the FET test, a Fish Sexual Development Test (FSDT) was conducted and effects on vitellogenin (VTG), body and liver somatic indices and gonad histopathology including sex ratios were evaluated. A reproduction test (Fish Screening Assay: FSA) was also performed where effects on reproductive parameters like spawning, fecundity and fertilization rate were evaluated, as well as effects on histopathology, liver enzyme activity VTG-levels and key genes expression levels in the adult fish.

TU 018

Structure-activity relationship of four perfluorinated compounds based on early developmental effects in the zebrafish

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Perfluorinated compounds (PFCs) are a group of anthropogenic chemicals containing diverse functional groups and chain lengths. Their chemical structure gives them unique properties which resulted in massive production and the use of these compounds in several familiar products since the 1950s. However, these unique properties also cause them to be persistent and bioaccumulative explaining their presence in wildlife, humans and the environment worldwide. Despite the global occurrence of PFCs, the toxicological information on these chemicals is still incomplete and insufficient to assess their environmental impact and structure-activity relationship. In the present study, the developmental effects of PFOS (perfluorooctane sulfonate, C8), PFOA (perfluorooctanoic acid, C8), PFBS (perfluorobutane sulfonate, C4) and PFBA (perfluorobutanoic acid, C4) were evaluated in zebrafish embryos (*Danio rerio*) during 120 hours post fertilization (hpf). The different chain lengths and functional groups of the selected chemicals made it possible to determine the structure-activity relationship of these compounds. PFCs with longer chain lengths (C8) tend to be more toxic than PFCs with shorter chain lengths (C4). Comparison based on the functional groups of compounds with the same chain length indicates that PFCs with a sulfonate group have a larger toxic potential than the ones with a carboxyl group. This resulted in the following order of toxicity: PFOS>PFOA>PFBS>PFBA. Furthermore, exposure to the different PFCs resulted in some general effects, such as deformations of the tail and an uninflated swim bladder, as well as in more specific effects depending on the structure of the tested chemical. Oedemas and effects on length could only be detected in 8-carbon PFCs while malformations of the head were a more specific action of the sulfonated PFCs. Only PFOA caused significant effects on hatching rate and success. The effects of PFOS, PFOA and PFBS on the heart rate at 48 hpf could be the result of effects on intrinsic processes that control the heart rate, while altered heart rates at 72 hpf can be attributed to the shorter body lengths. In conclusion, PFCs with short chain lengths should be primarily considered throughout the development of new perfluorinated alternatives.

TU 019

Embryotoxic effects of perfluorooctane sulfonate compounds in sea urchin *Paracentrotus lividus*

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Our environment has been affected by increasing concentration of organic and inorganic pollutants from anthropogenic sources. Perfluorinated compounds are important source of pollution and they are major risks for the aquatic ecosystems. Perfluorooctane sulfonate (PFOS) and Perfluorooctane sulfonyl fluoride (POSF) are both industrially synthesised perfluorinated chemicals and they produced in substantial amounts. They are found widely in surface water and aquatic sediments. In this study, the comparative embryotoxicity of increasing concentrations of PFOS (five concentrations ranging from 0.5 to 10 mg/L), and POSF (five concentrations ranging from 0.5 to 10 mg/L) were examined on embryos of the sea urchin *Paracentrotus lividus*. Results were evaluated by observing: larval malformations, developmental arrest and embryonic/larval mortality. The results indicated that, low concentrations of these chemicals generally caused malformations in the skeletal system. It was observed that high concentrations (10 mg-PFOS/L, 10mg-POSF/L) inhibited the growth of embryos in the early life stages by preventing mitosis. As a result, POSF was more toxic than PFOS (EC₅₀: 1.795 mg/L) on sea urchin embryo as the EC₅₀ is 1.074 mg/L and also both of the chemicals were harmful on the embryonic development of sea urchin in the sense of whole embryo and cellular level. In conclusion PFOS and POSF present a major risk to the normal development of *P. lividus* in the environment, so that it has ecological importance due to the hazard at population level considering the cumulative effects of other environmental pollutants.

TU 020

Ecotox findings for ammonium perfluorohexanoate

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Ammonium perfluorooctanoate (APFO) has long been used for polymerization of fluororesins and rubber. There are many reports on the hazards of perfluorooctanoate (C8). Accordingly, its replacement substances have been examined. Perfluorooctanoate (C6) disappears from a rodent's body and perfluorobutyrate (C4) disappears rapidly from rodent's and primate's bodies.

The toxicities of both substances are low. It is considered because they do not accumulate in the body. The accumulation property in the body is considered to have a correlation with the partition coefficients. Accordingly, we examined the correlation between the partition coefficients and effects on *Daphnia magna*. Also, we studied the effects of C6, which is available for industrial use, on development of fertilized eggs of rainbow trout.

The relation between LogPow of perfluorinated carboxylates (PFCAs=C4, C5, C6, C7, C8) and immobility ratio of *Daphnia magna* was examined. As the carbon number increased, the LogPow also increased. For C4 and C5, immobility rate became 100% in 24 hours. While C8 reached 100% in 48 hours, the immobility rates of *Daphnia magna* for C6 and C7 were at the same level. For PFCAs, no correlation between the carbon number and immobility was observed.

In fish early life stage toxicity test of C6, hatching success in the control group was 74%, satisfying the validation criterion for hatching success (>66%). The NOEC and LOEC for hatching success were determined as 10 and >10 mg/L, respectively.

Larval survival until Day 28 post-hatch in the control group was 93% thereby exceeding and satisfying the validity criteria for post-hatch survival (70%). Post-hatch survival across all remaining treatments ranged between 95 and 100%. In terms of concentrations, the NOEC and LOEC for post-hatch larval survival until Day 28 were both considered to be equal to or greater than 10 mg/L.

For fish total lengths, the NOEC and LOEC determined on Day 28 post-hatch were 10 and >10 mg/L respectively. For fish dry weights, the NOEC and LOEC determined on Day 28 post-hatch were 10 and >10 mg/L respectively. All validity criteria were satisfied during the test, therefore the test was considered to be valid. C6 proved to have no impact on fish in an early life stage toxicity test.

Industrially, C6 is easy to produce than C7. It can also be used for polymerization, therefore, it is considered to be a good replacement substance.

TU 021

Determination of perfluorinated compounds in cord blood samples by an on line method : turbulent flow chromatography LC-MS/MS

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The PFCs are persistent compounds that bioaccumulate attached to proteins. Toxicity studies in mammals revealed they can disrupt different metabolic activities. Early exposures have been studied and different authors have reported the transference during breast feeding or during pregnancy. This work presents the preliminary results of a cord blood study in Catalonia (Spain) for 18 PFCs in 165 cord blood samples.

The mixture of PFCs standards used was: MXB (13perfluoroacids and 4perfluorosulfonates) and perfluorooctanesulfonamide (PFOSA) from Wellington Laboratories Inc., Canada. Extraction and separation was achieved using a Thermo Scientific Aria TLX-1 system utilizing TurboFlow[TRADEMARK] technology (Thermo Fisher Scientific, Franklin, MA). For the purification process were used 2 TurboFlow columns Cyclone and C18 XL (Thermo Fisher Scientific).

The analysis consists in, internal standard addition, protein precipitation with acetonitrile and centrifugation followed by the analysis using turbulent flow chromatography coupled to tandem mass spectrometry (LC-(QQQ)-MS/MS). The influent solvent consisted in water pH 3.4 (HFO) at turbulent flow of 1.5 ml/min. The loop elution volume of the optimized method was 250µl. Transference was performed using water pH 3.4 (HFO); methanol (20:80) and water pH 3.4 (HFO); methanol (70:30) at flow of 0.2 ml/min. Separation was carried out in a LC-column Hypersil GOLD PFP (50 x 3) (Thermo Scientific). The mobile phase consisted of (A) aqueous ammonium acetate 20 mM (B) MeOH. The total run time for each injection was 16 min at 0.4 ml/min. Thermo Scientific TSQ Vantage mass spectrometer (Thermo Fisher Scientific, San Jose, CA), coupled to TLX-1, was used for analytical purposes, and equipped with a Turbo Ion Spray source.

The preliminary results of this study presents the profile and concentrations of 18 PFCs in cord blood samples from Catalonia (Spain). In this study we found that the more representative compounds were PFOA and PFHxS in agreement with other studies [3]. The concentration levels were 5361-828 ng/L and 10609-536 ng/L for PFOA and PFHxS, respectively. Other relevant compound was PFBA found in higher concentration 53900 ng/L.

The obtained results are in agreement with the literature, however PFOS has been found in lower concentrations than previous studies. This study can provide valuable preliminary information to study the transplacental exposure to perfluorinated compounds.

TU 022

Plastic debris and toxin releases in the Pacific Ocean

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Plastics comprised the majority of collected waste in worldwide beach cleanups in 2006, 2007 and 2008. In California, Washington, Oregon, and Hawaii the five most common plastic debris items on beaches are cigarette filters, food wrappers and containers, beverage caps and lids, bags, and food service items, e.g., cups, plates, and cutlery. The majority of plastic items are made from four common plastics; polyethylene, polypropylene, polystyrene, and PET, accounting for 75% of the plastic debris. Pre-production plastic pellets also account for significant amounts of plastic in the oceans from storm run-off of industrial areas. The fate of plastics in the oceans can lead to fragmentation and result in slurry of plastic particles that can degrade and release toxic chemicals such as phthalates, flame retardants, BPA, antimony oxide, heavy metal inks, and styrene monomer as the plastics break down. Plastics can accumulate toxins floating in the oceans from persistent organic pollutants (POPs). POPs can include DDT, hexachlorobenzene, polychlorinated biphenyls (PCB), polycyclic aromatic hydrocarbons, among others.

TU 023

Micro-plastics in the marine environment - a global assessmentJ. Kershaw¹, H.A. Leslie²¹CEFAS, Lowestoft, United Kingdom²Institute for Environmental Studies (IVM), VU University, Amsterdam, Nederland

GESAMP has set up a new Working Group entitled 'Sources, fate and effects of micro-plastics in the environment - a global perspective'. With multi-agency support and with a multi-disciplinary membership, it is reviewing a wide range of information about the supply of plastics and microplastics to the ocean, and the biological, chemical and physical processes controlling transport, fate and effects of both micro-plastic particles and the contaminant burden. It will provide an evidence base for use by policy makers, regional bodies a other stakeholders, and help to develop research priorities. The poster will describe the Terms of Reference, work programme, membership and intended outcomes of the group, and will issue an invitation to SETAC Europe participants to become involved in this initiative

TU 024

Marine micro litter under the marine strategy framework directive - science and policyG. Hanke¹, F. Galgani², S.S. Sadri³, R.C. Thompson⁴¹European Commission Joint Research Centre, Ispra (va), Italy²Ifremer, Bastia, France³University of Plymouth, Plymouth, United Kingdom⁴Plymouth University, School of Marine Science and Engineering, Plymouth, United Kingdom

The European Marine Strategy Framework Directive 2008/56/EC (MSFD) recognizes Marine Litter as one of the indicators for the Environmental State of the European Seas. The Commission Decision on criteria and methodological standards on good environmental status (GES) of marine waters (Commission Decision 2010/477/EU), lists specific indicators for assessment of Marine Litter. Indicator 10.1.3 regards marine micro-particles, in particular micro-plastics:

Trends in the amount of litter washed ashore and/or deposited on coastlines, including analysis of its composition, spatial distribution and, where possible, source (10.1.1)

“ Trends in the amount of litter in the water column (including floating at the surface) and deposited on the sea- floor, including analysis of its composition, spatial distribution and, where possible, source (10.1.2)

“ Trends in the amount, distribution and, where possible, composition of micro-particles (in particular micro- plastics) (10.1.3)

10.2. Impacts of litter on marine life

“ Trends in the amount and composition of litter ingested by marine animals (e.g. stomach analysis) (10.2.1).

A technical working group under the MSFD implementation strategy has prepared technical recommendations in support of the implementation of the directive by EU Member States. The group identified options and available tools for the monitoring of Marine Litter and developed a roadmap showing the necessary next steps in preparing the implementation of the directive. The poster is presenting specific information regarding marine microlitter under the MSFD.

TU 025

Using the continuous plankton recorder to determine the abundance of microplastic debris in the subsurface marine watersS.S. Sadri¹, M. Edwards², R.C. Thompson¹¹University of Plymouth, Plymouth, United Kingdom²Sir Alistair Hardy Foundation for Ocean Science (SAHFOS), Plymouth, United Kingdom

Fragmentation of plastics debris into “microplastic” pieces is an emerging issue of concern. Our knowledge of distribution and accumulation of microplastic is patchy and in order to quantify and monitor the distribution and in particular temporal trends there is a need for a broad sampling programme and standardised methods. The aim of this research is to study the spatiotemporal abundance of microplastic debris in ocean surface waters and our preliminary investigation of microplastic contamination in Continuous Plankton Recorder (CPR) samples reported by analysts at the Sir Alistair Hardy Foundation for Ocean Science (SAHFOS) has confirmed the presence of synthetic polymers in several European locations (North Sea, Irish Sea, English Channel and the North Atlantic). We used Fourier Transform Infrared (FT-IR) spectrometry to identify the unknown pieces. The most common plastic types were Polyethylene terephthalate (PET) followed by Nylon and Acrylic but mean abundance was typically less than one item per cubic meter of sea water.

TU 026

Microplastics in personal care products from Brazil: is this source relevant to the aquatic contamination?

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Plastics have been recently incorporate into the group of emerging pollutants due to their wide distribution in marine and coastal environments all over the world, as well as their persistency and hazard to the wildlife. Moreover, they have highly diverse diffuse sources and high capacity of distribution. In addition to other sources, microplastic (< 5mm) have been recently reported in frequently used skin cleansers and others personal care products. As a result, this specific class of plastic has been found contaminating sediments and pelagic zones. Despite the effects related to direct ingestion, microplastic might also transfer adsorbed organic contaminants (OCs) to aquatic organisms. In the current study, particles from 18 of the most frequently used personal care products (soaps and creams) in Brazil were characterized regarding amount, size, form and composition. Analyses were performed by suspension and filtration of plastic particles, followed by microscopic analysis and weight of the particles. Undergoing chemical analyzes by Fourier transform infrared spectroscopy (FTIR) will confirm the apparent predominance of polystyrene among plastics. The amount of plastic ranged from 1.3 to 103.1 mg g⁻¹ of product, whereas the distribution frequency ranged from 100 to 400 microns. Due to methodological limitation s, only 10 products had their size measured. Most of the plastics have irregular shapes, although spherical particles were also seen in some products. The preliminary results pointed out this as a relevant source of microplastic to the environment. However, this physic-chemical characterization of personal care products is essential to assess their potential to adsorb, concentrate and transfer OCs, improving somehow their bioavailability, from the environmental to the organisms.

TU 027

International Pellet Watch : background levels, hot spots, legacy pollution, and temporal trendsH. Takada¹, M. Heskett², R. Yamashita¹, M. Yuyama¹, M. Itoh¹, Y.B. Geok¹¹Tokyo University of Agric. & Technol., Tokyo, Japan²Surfrider Foundation, Oahu, United States of America

International Pellet Watch (IPW) is a volunteer-based global monitoring program designed to monitor the pollution status of the oceans and to understand the risks associated with chemicals in marine plastics. Based on the analysis of resin pellets from remote islands, background levels of persistent organic pollutants (POPs) have been tentatively determined. By comparison of POPs concentrations in pellets from industrially-developing countries (e.g., Ghana) with the background levels, PCB inputs from e-wastes were suggested. Through global comparison, hot spots of PCB pollution were identified, e.g., Le Havre (France), Athens (Greece), Tokyo Bay (Japan), Sydney Harbor (Australia), Los Angeles, San Francisco, Boston Harbor, New York Bight, Lake Erie (USA), Santos Bay (Brazil). Combination of IPW with passive air sampling indicates that legacy pollution is major contributor to most of the hot spots of PCB pollution. In addition to the spatial variation, pellet watch was applied to reveal temporal trend in POPs pollution in California coast and Tokyo Bay.

TU 028

Partitioning between water and plastics of polychlorinated biphenyls in marine animals

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The presence of plastic debris in the oceans is a potential hazard for marine animal species in several ways. It is not only the plastics themselves that form a potential hazard. Chemical additives are added during plastics production in order to catalyze monomers into polymers and give it different properties. Some of these additives have been associated with carcinogenic and endocrine disrupting effects. Moreover, due to its hydrophobic nature, plastic debris can be a hazard to marine animal species because of accumulation of organic contaminants. An important hazard are the persistent organic pollutants (POPs), which do not completely dissolve in water and do not degrade into harmless materials in a short timeframe. Exposure to POPs, e.g. polychlorinated biphenyls (PCBs), has been associated with a wide range of health impacts on both wildlife and humans. This study focuses on the equilibrium partitioning of polychlorinated biphenyls after ingestion by marine animals. In order to get more insight in the bioaccumulation potential of these persistent additives, we compared plastic-water partitioning, a simplification of the partitioning of plastic additives in the gastric fluid, with octanol-water partitioning. The outcomes will be discussed within the framework of the current knowledge gap and we will describe challenges for future research on this topic.

TU 029

Potential for increased exposure to persistent organic pollutants through ingestion of marine debris: the relevance of partitioning coefficients

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An important step in predicting the availability of plastic-sorbed persistent organic pollutants (POPs) to the marine environment is understanding the plastic:water partition coefficient (Kpw) for a great number of chemical and plastic combinations. While plastics have the capacity to absorb chemical substances from their surrounding environment, little is known about the factors effecting sorption and desorption of POPs to plastic debris, the abundance and bioavailability of POPs on plastic debris, and the amount of plastics marine organisms consume. One step in understanding the potential for exposure is an evaluation of relevant partition coefficients.

An extensive literature search was performed for this presentation. This review summarizes articles that calculated and presented plastic to water partitioning coefficients.

Only published, peer reviewed literature was examined. The results of our review identified Kpws for 22 specific types of polymers and 198 individual compounds representing 24 different chemical groups. Analyses of these data showed a wide range of values (e.g., 0.91 to 7.8 log Kpw) among compounds for any given polymer. Of note, our correlation analysis showed a relationship between log Kpw and log Kow (octanol-water partition coefficients) for most polymers. Consequently, log Kow may act as a surrogate for estimating log Kpw, which could allow for assessment of availability based on known properties of chemicals and plastics.

This finding is of particular importance because of the lack of log Kpw data for a wide array of plastics. A logical next step is to use log Kow to generate log Kpws through modeling of a wide range of plastics in order to understand the potential for these plastics to adsorb POPs in their environment. It would also be possible to further substantiate these findings by gathering partition coefficients and related information from other sources such as plastics companies, federal/state agencies, etc.

TU 030

Determination of DEHP in culture media by GC-MS/MS using PCI Ammonia

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This method is a mechanism of elucidating the target phthalate, bis (2-ethylhexyl) phthalate (DEHP), in culture media by gas chromatography and tandem mass spectrometry (GC-MS/MS) using positive chemical ionization (PCI) with ammonia reagent gas. Phthalates are routinely analyzed in electron ionization (EI) with 149 m/z as the principal ion. Since all phthalates have similar spectra, it is difficult to identify which phthalate is present. By using PCI, the molecular ion is formed. Culture media samples with varying concentrations of DEHP were analyzed on a GC-MS system consisting of an ion-trap series mass spectrometer and a gas chromatograph. Structural elucidation was achieved by performing MS/MS on the molecular ion. Next, the fragments formed were compared to those in Mass Frontier software, which predicts fragmentation and the mechanisms of product ion formation by comparing the scan results to existing library data. Ammonia was selected as the chemical ionization reagent gas to increase the yield of the molecular ion.

A calibration curve with points at 0.1, 1, 5, and 10 µM were analyzed in culture media. The correlation coefficient for this calibration curve was 0.9969. Two unknown samples were analyzed and quantitated against the calibration curve. These were found to contain 4.2 µM and 3.1 µM DEHP.

TU 031

Selective uptake of microplastics by a marine bivalve (*Mytilus edulis*)

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Microplastics, the degradation product of larger plastic debris, are accumulating in marine habitats worldwide. Given their small size (< 1 mm) these particles can potentially be ingested by marine animals. Data on the uptake of microplastics in invertebrates is, however, scarce. In this study, we tested the hypothesis that the uptake of microplastics from the gut to tissues is size-selective and has an adverse effect on the energy allocation in the mussel.

The blue mussel (*Mytilus edulis*) was exposed for 14 days to three different sizes of microplastics simultaneously (10, 30 and 90 µm). 10 µm-particles, as well as 30 µm-particles, were added at 50 particles.mL⁻¹ and 90 µm-particles at 10 particles.mL⁻¹, resulting in a total concentration of 110 particles.mL⁻¹. Analysis of the faeces demonstrated that *M. edulis* filtered and ingested all particle sizes. In the faeces, the 30 and 90 µm-particles were detected in the same ratio as they were added to the seawater (i.e. 5/1).

However, the 10 µm-particles were detected in much smaller quantities than expected based on the observations of the other particle sizes, i.e. 8 times less than the 30 µm-particles were observed (even though they were initially present at the same concentration). Although this discrepancy was probably partly due to difficulties in detecting the particles in the faeces, analysis of acid-destructed mussels and hemolymph samples demonstrated that 10 µm-particles were able to translocate to the circulatory system of the mussel. Using a syringe, 300 µL of hemolymph was extracted from the posterior adductor muscle and on average 15.3 ± 3.2 particles (n=15) were found in the hemolymph of the mussels. This uptake accounts for 0.3% of all 10 µm-particles ingested. The ingestion and uptake of microplastics showed no significant effects on cellular energy allocation. Exposed mussels did show a significant increase in energy consumption, indicating a rise in metabolic activity.

Although *M. edulis* was exposed to a very high concentration of microplastics, which was approximately 1000 times the environmentally relevant concentration, no significant short-term adverse effects of ingestion and translocation were detected. Long-term exposure studies could, however, provide more conclusive answers on the effects of ingestion and uptake of microplastics in *Mytilus edulis*.

TU 032

Presence of plastic debris in sea turtles stranded along the Tuscany coasts

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Marine debris is proven to have a widespread negative impact on marine wildlife, including physiology-level effects and reduced fitness, reproductive failure, changes in community structure to direct death following blockage of intestinal tract or entanglement.

The main sources of marine debris are litter from ships, fishing and recreational boats, and garbage carried into the sea from land-based sources in industrialized and highly populated areas. Marine debris is an important threat for sea turtles and the evaluation of the impact of plastics on development, survivorship, health and reproduction of sea turtles is highlighted as one of the global research priorities for these threatened species. The loggerhead turtle (*Caretta caretta*) and leatherback turtle (*Dermochelys coriacea*) are considered two of the sea turtles species ingesting the largest amount of debris, in all their life stages, most likely because of their habitat and feeding behaviour.

In this study we quantified plastic debris ingestion in twenty-nine specimens of loggerhead turtle having a curved carapace length (CCL) of 29.0-71.0 cm. Moreover we had the opportunity of collecting one specimen of leatherback turtle incidentally captured dead by fisheries in Elba Island (CCL of 124.0 cm).

During dissection of animals, biometric parameters were taken and the entire gastrointestinal tract was removed and subdivided into oesophagus, stomach and intestine. In a second step the debris and litter were collected from oesophagus, stomach and intestine and the plastic founded were subdivided in different categories: sheetlike, threadlike, foamed and fragment user plastics. Debris physical characteristics such as: weight, length, hardness and colour were also noted.

The first result obtained from this study was that a large number of plastic was found, prevalently into the intestine, in more than 60% of the animals. The main categories of plastics found in the specimens were soft sheetlike user plastics, that the animals can mistake to jellyfish. This may support the assumption that loggerheads are attracted by floating debris more than by debris on the sea floor. The second type of plastics found in the specimens were fragments; moreover in some specimens we found little amounts of tar in the entire gastrointestinal tract. Another important result obtained was the absence of food in the specimens that show an high number of plastic debris.

EP08P - What is the current state of the science on the fate, exposure and effects of pharmaceuticals in the environment?

TU 033

Occurrence of Triclosan in fresh waters from São Paulo, Brazil - the need for regulatory actions

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Triclosan, 5-chloro-2-(2,4-dichlorophenoxy)phenol, is a broad-spectrum bactericide used in pharmaceuticals and personal care products of daily use. It is a non-volatile compound (5.3 x 10⁻⁴ Pa at 20 °C), relatively soluble in water (10 mg/L at 20 °C) with a log Kow of 4.8. Triclosan is highly toxic to aquatic organisms and some PNECs were proposed in the literature, varying from 1.4 to 1550 ng/L.[1] In Brazil triclosan is not regulated and there aren't reports about the presence of triclosan in surface waters.

In this work, six rivers from the State of São Paulo (Atibaia, Capivari, Sorocaba, Cotia, Tanque Grande and Rio Preto) were monitored for triclosan in a one year study. Caffeine was monitored as a surrogate of domestic source of contamination. One liter of water samples was filtered using 0.45 µm pore size cellulose acetate membranes, extracted by solid-phase extraction and the compounds were determined by liquid chromatography tandem mass spectrometry. The limit of quantification (LOQ) of method applied were 0.7 ng/L for triclosan and 15 ng/L for caffeine. From 100 samples analyzed 43% presented triclosan at concentrations ranging from 2.2 to 66 ng/L, and 98% presented caffeine at concentrations about 1,000 times higher than triclosan, but no correlation was found with triclosan concentrations. No differences were observed in dry or wet seasons. If we compare the results with the lowest PNEC found in the literature the 43% of the samples where triclosan was found the concentrations were above 1.4 ng/L. If we using the PNEC value 4.7 ng/L, 40% of the samples presented concentrations above that limit. And even if we apply PNEC of 50, 3 samples were determined triclosan concentrations above this amount. Our results suggest that triclosan is an ubiquitous substance and should be considered a priority pollutant at least in the State of São Paulo and therefore included in the water regulation in order to protect the water for aquatic life. Further studies in other water bodies in Brazil are needed to determine the occurrence of triclosan nationwide.

TU 034

First pilot study on the occurrence of UV screens and preservatives in a Victorian estuary

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The southern hemisphere's elevated UV light exposure encourages the year-round use of UV protective chemicals (UV filters and UV light stabilizers) in personal care and plastic products. Some UV-filters and preservatives are known to be hormonally active in vitro and/or in vivo, raising concerns of possible endocrine disrupting effects in the environment. There is little published data of occurrence of those compounds in Australian aquatic environments. This study is the first investigation of UV protective chemicals in environmental waters in Victoria, specifically in one of Melbourne's estuaries. Water samples were taken from four sites (A - D). Screened compounds included 11 UV filters, 10 UV stabilizers, 12 preservatives and the metabolite, and one fragrance, including commonly used compounds in Australia such as 4-MBC, EHMC, octocrylene, UV-328, HHCB, 2-phenoxy ethanol, methyl paraben, and propyl paraben. Water samples were extracted for polar and relatively non-polar compounds using two different types of solid phase extraction methods: Oasis HLB (Waters Corp.) for polar compounds, Empore C18FF (3M) for relatively non-polar compounds, then analysed by GC-MS, after derivatization for polar compounds. Samples from Sites A, B, and C had low concentrations of the screened chemicals with similar compound profiles. Site D had a very different contaminant profile, with very high concentrations of many of the screened compounds. Sites A, B, C are both at the mouth of creeks and rivers flowing into Port Phillip Bay; Site D is about 5 km upstream of site C in Melbourne on the second biggest river entering Port Phillip Bay. Although there are several small wastewater treatments located up stream of site D, the levels of HHCB (an indicator of domestic and municipal wastewaters) measured at Site D were similar to the levels observed at Sites A, B and C, suggesting that the chemicals observed at site D do not originate from a wastewater treatment plant but a different source. The detection of common UV filters, such as 4MBC, EHMC, OC and the common preservatives 2-PE, MP, and PB in a Victorian estuary proves that the existence of personal care products in the environment is not just an issue for more densely populated countries in the northern hemisphere, but also potentially of concern in Australia.

TU 035

A comprehensive study of pharmaceuticals sources and fate in a highly urbanized and inhabited area in Italy

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Pharmaceuticals are used in high quantities in our society. They can be excreted unchanged and/or as active metabolites in urine and feces, and directly conveyed to wastewater treatment plants (WWTPs). If they escape degradation during wastewater treatment, they finally end up in surface water. The present study aimed for the first time to study sources and fate of several selected classes of pharmaceuticals in the most inhabited and industrialized area in Italy (urban and suburban area around Milan). The investigation was conducted in waste, surface and ground water. Composite samples were collected, filtered, enriched with internal standards and solid-phase extracted. LC-ESI-MS/MS with multiple-reaction monitoring and stable-isotope dilution assay was used for quantitation. Recoveries were generally higher than 80% and limits of quantifications were in the low ng/L range. Ciprofloxacin, ofloxacin, atenolol, furosemide, hydrochlorothiazide, clarythromycin, ibuprofen, ketoprofene, diclofenac, naproxen, and carbamazepine were the most abundant residual drugs in urban wastewater. Their removal in conventional WWTPs was generally lower than 50%, with no removal for bezafibrate, hydrochlorothiazide, furosemide and carbamazepine. Removal rates were also affected by the type of advanced treatments adopted (i.e. disinfection, UV treatment) in the plants investigated. The amounts of pharmaceutical active substances discharged in the environment daily through treated wastewater ranged between 1.2 and 2 kg. The total amounts of the investigated pharmaceuticals in the rivers flowing through the area investigated ranged between 0.9 and 2.7 kg/day respectively before and after the city of Milan. Traces of some compounds as carbamazepine were found in the low ng/L range also in untreated ground water. Moreover, the pattern of contamination of ground water was higher in sites closer to rivers, suggesting the contribution of surface water to the contamination. This investigation allows the quantification of the mass flow of these substances flowing through a highly urbanized and industrialized area.

TU 036

An evaluation of free water surface wetlands as tertiary sewage water treatment of micro-pollutants

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Increased attention is currently directed towards potential negative effects of pharmaceuticals and other micro-pollutants discharged into the aquatic environment via municipal sewage water. A number of additional treatment technologies, such as ozonation, have therefore been suggested as promising tools for improving the removal efficiency of pharmaceuticals in existing Sewage Treatment Plants (STPs). Constructed wetlands are also capable of removing a variety of micro-pollutants, including some pharmaceuticals, and could hence be a resource efficient complement to more advanced treatment technologies. The purpose of the present study was therefore to increase the knowledge base concerning the potential use of constructed wetlands as a treatment step to reduce emissions of organic micro-pollutants from municipal sewage effluents. Under cold winter conditions, incoming and outgoing waters from four Swedish free water surface wetlands, operated as final treatment steps of sewage effluent from municipal STPs, were sampled and analyzed for levels of a set of 92 pharmaceuticals and 22 inorganic components as well as assessed using subchronic ecotoxicity tests with a macro alga and a crustacean.

Sixty-five pharmaceuticals were detected in the range 1 ng L⁻¹ to 7.6 µg L⁻¹ in incoming and outgoing waters from the four investigated wetlands. Although the sampling design used in the present study lacks the robustness of volume proportional 24 h composite samples, the average estimated removal rates ranged from 42 to 52%, which correlates to previous published values. The effects observed in the ecotoxicity tests with the macro alga (EC50s in the range of 7.5 to 46%) and the crustacean (LOECs in the range of 11.25 to 90%) could not be assigned to either pharmaceutical residues or metals, but in general showed that these treatment facilities release water with a relatively low toxic potential, comparable to water that have been treated with advanced tertiary treatments.

From the present study it can be concluded that constructed wetlands may provide a complementary sewage treatment option, especially where other treatment is lacking today. To fully remove micro-pollutants from sewage effluent however, other more advanced treatment technologies are likely needed.

TU 037

Large screening of pharmaceuticals and hormones in sludge based on LC-ToF-MS

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Ecosystems are potentially contaminated by many chemical substances from domestic activities, through the spreading of treated sewage sludge. Indeed during the last thirty years, the application of municipal sludge from urban to agricultural soils is the option favoured internationally for sludge management as it contributes positively to recycling nutrients, soil properties and fertility. The literature data indicate that the removal efficiencies of organic contaminants in sewage treatment plants vary between 24 and 100% depending on the contaminants and treatment processes, which means that significant percentages of products can be present in the sludge.

Steroid sex hormones and pharmaceuticals are considered emerging environmental contaminants of particular concern, as many of them display biologically active properties (including endocrine-disrupting). At the European level, few studies exist on the characterization of these emerging substances in sludge or on the assessment of health and environmental risks associated with their use.

This is partly explained by the difficulties of detection and quantification of such pollutants in the solid phases. It is, indeed, a real analytic challenge given the diversity of analytes, their presence generally in trace amounts and the complexity of the matrix which makes difficult the extraction step. Thus it appears that the development of analytical methods sensitive, reliable and robust is an essential step for the characterization of emerging substances in sludge.

In this context, the aim of the study was to develop a method for the large screening of pharmaceuticals and hormonal steroids in sludge. Therefore, nearly two hundred substances were analyzed by liquid chromatography-time-of-flight mass spectrometry. The analysis of complex matrices such as sludge needed a rigorous sample preparation to obtain a repeatable and enough sensitive analysis to achieve the detection limits required. For this purpose, an extraction step using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method was set up. This analytical methodology was successfully applied to various kinds of sludge (limed, digested, dried, liquid, composted) collected in several sewage works in France.

TU 038

Adsorption of human pharmaceuticals to activated sludge: how many different sludges should be tested for an environmental risk assessment?

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The potential risk of human pharmaceuticals to the environment has to be determined, e.g. according to the EMEA Guideline EMEA/CHMP/SWP/4447/00. This guideline focus on the risk by the use of the pharmaceutical and the entry into the environment via excretion and wastewater effluent to a sewage treatment plant (STP). It is assumed that a substance with high sorption to activated sludge may reach terrestrial ecosystems by land spreading of sewage sludges. The adsorption to sludge has therefore to be tested according to the EMEA Guideline in a adsorption study. A terrestrial risk assessment must be additionally conducted with the drug if its adsorption coefficient to sludge exceeds a defined trigger value (Koc of 10'000 L/kg or Kd of 3'700 L/kg) and the drug is not readily biodegradable.

The sorption of chemicals to sorbents like soils or sediments typically depends on several parameters of the sorbent (e.g. pH, ion-exchange capacity, redox potential, organic carbon content, clay content, texture). The question arises how different activated sludges are regarding these parameters, how large the adsorption to sludges varies and consequently how many sludges should be tested for a safe risk assessment. The number of sludges to be tested is not clearly defined in the guideline EMEA/CHMP/SWP/4447/00.

In this presentation the results of batch equilibrium sorption tests with different sludges will be shown for several pharmaceuticals. The variability of the adsorption coefficients, obtained for different sludges by the same analytical method and within the same laboratory will be discussed in the context of the trigger values used in the environmental risk assessment.

TU 039

Analysis of antimalarial drugs in water

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Malaria is a general illness in many countries and therefore antimalarial drugs are prescribed in great quantities. There are more than 20 different molecules being used

for treatments. These drugs are excreted mainly via urine. As a result, they may reach the environment after waste water treatment, through yellow water used as a fertilizer or by direct discharge. There is limited information on the fate of antimalarials in the environment. It is believed that some of these drugs are rather persistent due to their organohalogen moieties. Hence, the fate of these drugs in the environment might be a subject of concern. To date, many methods to analyze antimalarial drugs in blood and urine have been published. However, only two methods have been published to analyze a few antimalarial drugs (artemisinin derivatives and chloroquine) in water or soil. For that reason, the first multi-residue method for the analysis of antimalarial drugs and their metabolites in water has been developed. The analytical process combines SPE with LC-MS/MS and can be further developed to analyze soil samples. The presented method can be used to describe environmental fate profiles of antimalarial drugs in the environment.

TU 040

Matching micropollutant loads of influent and effluent for reliable mass balances in WWTPs

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The removal efficiency of pollutants such as COD in a WWTP is usually evaluated by data from long-term time series where variation caused by transient flow and concentration are averaged out. The analyses of micropollutants is however work and cost-intensive and that is why measurement campaigns usually only cover short periods with a small number of samples available to establish a mass balance. Considering that biological reactor tanks are well-mixed systems, a sampling scheme comprising single 24-h composite samples in inlet and outlet is most likely to compare two largely different water volumes. For a completely-mixed system, it was shown that a 24-h effluent load is composed of water volume fractions from several previous days (Majewsky et al., 2011).

In the present study, we designed an individual sampling strategy using hydraulic calibration of a Luxembourg WWTP to validate the proposed model. The elimination efficiencies of 5 pharmaceuticals were calculated by a 3-day influent and 1-effluent campaign on the basis of 2-h composite samples (time-proportional) which allowed to explain >80% of the origin of the effluent load sampled. Results showed that the method was capable of reproducing realistic elimination values while short-term mass balances lead to erroneous values.

The validated model allowed to evaluate the accuracy of different sampling scenarios as a function of the sampling mode and frequency (Ort et al., 2010) and of the number of samples taken in the inlet and outlet of the WWTP. The aim is to maximally reduce the uncertainty and/or to explain the bulk of the effluent load origin. A relationship can be established between the influent proportions that are captured by an effluent sampling period and the mean hydraulic retention time (HRT). Based on these findings, we propose to develop a generic method to design sampling strategies in WWTPs without having to rely on prior hydraulic calibration.

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TU 041

Occurrence and fate of triclosan and triclocarban during wastewater and biosolid treatment

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Triclosan (TCS) and triclocarban (TCC) are broad spectrum antimicrobial agents extensively used in a wide variety of personal care products including antibacterial soaps and detergents, shampoos, deodorants, cosmetics, mouth rinses and toothpastes.

During wastewater treatment, the removal efficiency of triclosan and triclocarban from the aqueous phase can be considerable in activated sludge processes but is dependent on treatment techniques. Triclocarban is recalcitrant during the sewage treatment process and is detected in streams and sediments downstream of WWTPs. Triclosan is amenable to biodegradation during sewage treatment and both TCS and the transformation product methyl-triclosan (MTCS) are detected in WWTP effluents.

TCS, TCC and MTCS have lipophilic properties and hence partition to sludge during wastewater treatment and may be present in concentrations ranging from ng kg⁻¹ to % in the dry solids depending on the initial amounts present and the extent of removal during wastewater and sludge treatment. Moreover, the sludge accumulated in WWTPs is often processed to produce biosolids intended for land application. Thus, the agricultural recycling of biosolids, the favored option for sludge management, may lead to adverse environmental impact.

In this work, TCS, TCC and MTS were monitored in both waters and sludge at different stages within the wastewater treatment process in two WWTPs with different characteristics located in the coastal area of Girona (NE Spain). Moreover, the influence of sludge digestion process on the degradation of target compounds was evaluated by analyzing the resulting digested biosolids under both aerobic and anaerobic conditions. The effect of the temperature on the occurrence of the studied compounds in the final biosolid was also investigated.

TU 042

Dominant time scale of antibiotics concentrations in waste waters through one-year field campaign study

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Among the long list of pharmaceuticals that can be found in the aquatic environment, antibiotics receive important and increasing attention, as they are suspected to foster bacterial resistance. But if many studies have listed their constant presence in waste waters, few of them curiously have focused on the temporal dynamics of their concentrations. In the presented study, we compare the relative importance of monthly, weekly, daily, and hourly time scale when assessing the dynamics of antibiotics in waste waters. For this, a one-year experimental sampling campaign was conducted at the Vidy WWTP of Lausanne in Switzerland. One week a month during one year, hourly samples were taken from WWTP influent. Samples were aggregated together to obtain measured hourly variations, daily variations, monthly variations and seasonal variations. A total of more than 180 samples over year 2011 were analyzed using UPLC-MS/MS, and the concentrations of 9 antibiotics (azithromycin, ciprofloxacin, ofloxacin, clarithromycin, clindamycin, metronidazole, norfloxacin, sulfamethoxazole, trimetoprim) compared. We conclude that even if a seasonal trend for concentrations can be observed for several substances, the hourly time scale totally dominates temporal fluctuations of concentrations. In other words, there is a high hourly fluctuation of antibiotics concentrations, which is superior in magnitude to other (daily or seasonal) time scales. Thus, more efforts in terms of modelling and treatment optimization should be conducted at this temporal resolution (hour) for antibiotics, in order to reduce the risk they can represent for the environment.

TU 043

Quantification of pharmaceuticals and endocrine disruptors in river sediments: development and validation of a QuEChERS based extraction

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Assessing exposure concentrations can be challenging for the analyst as environmental concentrations and toxicological or eco-toxicological effect induced quantities are often not consistent with analysis capacities. To encounter those limitations analysts use pre-concentration and purification steps like Accelerated Solvent Extraction (ASE) and Solid Phase Extraction (SPE) to extract and analyse pharmaceuticals or hormones at the lower or sub ng/g level from solid matrices. A recent extraction method known as Quick Easy Cheap Effective Rugged and Safe has been developed for the analysis of pesticides in food matrices.

This method has already been extended to various matrices like rats' serum and gonads [1] in our laboratory. We developed and validated a multiresidue analytical method with QuEChERS extraction for the screening of multi-families of pharmaceuticals and endocrine disruptors in river sediments. The molecules were chosen among the priority pollutants listed in a prioritisation for French surface waters [2], in the Water Framework Directive and within the potential or confirmed EDCs. This selection of 13 pharmaceuticals includes β -blockers, anti-inflammatories, antibiotics, anxiolytics, anti-depressants and anti-analgesics 24 EDCs includes pesticides, alkylphenols, hormones and phenolic derivatives and. Quantification is performed on a 3200QTrap tandem mass spectrometer (MS/MS) coupled to Liquid Chromatography (LC) Agilent 1200 system with a Kinetex XB-C18 column (100x2.1mm, 1.7 μ m).

Extraction was optimised to get acceptable recoveries (>60%) and the linearity of the method has been verified by statistical means. Acceptable Relative Standard Deviations of both injection and preparation steps (<20 to 30%) were calculated for intraday injections of 3 independent solutions. Repetition of a calibration over three days allowed us to calculate good interday RSD. Limits of quantification were determined by injection of 5 independent standard solutions and were consistent with environmental concentrations.

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TU 044

Developing a miniaturized push-pull test to study the transformation of pharmaceuticals in the hyporheic zone of rivers

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The hyporheic zone is a key compartment for the attenuation of organic micropollutants in rivers. The comparatively slow flow velocity, the continuous import of nutrients and oxygen, and the export of metabolic waste provide ideal conditions for microbial transformation processes. A better characterization of hyporheic processes would substantially improve the quantification of the self-purification capacity of streams. Unfortunately, measuring transformation rates of organic micropollutants directly in the hyporheic zone is difficult due to limited accessibility, but also due to a lack of suitable experimental methods. Therefore, we are developing a miniaturized push-pull methodology that allows measuring transformation rates of pharmaceuticals directly in the hyporheic zone.

Push-pull tests are an established means of testing the transformation capacity of aquifers with respect to organic or inorganic contaminants. There, a solution containing a set of conservative and reactive tracers is injected ("push") into an aquifer, and after a certain time the groundwater is extracted ("pull") through the same well. The difference between conservative and reactive tracer recovery can then be used to estimate in-situ transformation rates. The aim of our study is to downscale this approach so that it can

be used on the spatial scale of centimetres to decimetres.

The experimental concept is validated in a small flume that allows the simulation of different hydraulic conditions in the hyporheic zone. Under abiotic conditions, we applied the approach successfully to measure retention of several pharmaceuticals at residence times of up to 16 hours; longer residence times will be evaluated. Currently, we are testing the approach under biotic conditions with the same set of pharmaceuticals and, in addition, with some reference compounds. Our contribution will summarize the experimental concept and the major finding of this method development study, and it will highlight the potential of the method for improving our understanding of hyporheic processes and thus of the environmental fate of organic micropollutants.

TU 045

Comparative analysis of contaminant of emerging concern discharges from a centralized municipal treatment plant and on-site wastewater treatment systems receiving common wastewater influent

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Though over 25% of the United States population employs decentralized on-site technologies for wastewater treatment, a comparative understanding of treatment efficacies of these systems remain less understood than those from centralized municipal wastewater treatment plants. In fact, it is estimated that 10 to 20 percent of these systems malfunction annually, releasing untreated wastewater to groundwater and surface water, which can degrade water quality and result in impacts on surface waters in regions experiencing high groundwater - surface water exchange. Even less understood is a comparative understanding of contaminants of emerging concern (CECs) associated with these systems and subsequent loadings to the environment. The primary objective of this study was to assess the occurrence and removal efficiencies of select CECs among several different wastewater treatment systems. Our secondary objective was to evaluate the influences of seasons and influent dosing scenarios on-site aerobic treatment systems. The current study further examined the occurrence of CECs, including drug of abuse metabolites, between weekend and weekday sampling events. The studied CECs covered a variety of common pharmaceutical classes, including analgesic, anti-hypertension, antibiotic, psychostimulant metabolites, antihistamine, anti-seizure, benzodiazepine, anticoagulant, anti-inflammatory, antilipemic, caffeine, and the artificial sweetener sucralose, which appears to represent a robust tracer of anthropogenic activities. An isotope dilution liquid chromatography tandem mass spectrometry (LC-MS/MS) method that employed independent isotopically-labeled standards for quantitation of each compound was applied to quantitate target analytes for all samples. ANOVA was performed to test differences of treatment type, and season on CEC concentrations and other routine water quality parameters. Results showed that concentrations of CECs are typically in the range of <1-100 ng/L in the effluent discharges, which are generally consistent with peer-reviewed literature reports for these CECs. CEC concentrations from advanced on-site systems were reduced relative to septic systems. The present study, which included CECs with varied physicochemical properties, provides an initial understanding of the range of environmental loadings from common on-site wastewater treatment systems.

TU 046

Water-sediment biodegradation: challenges in modeling and screening for pharmaceutical transformation products

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The current OECD 308 test is a water-sediment simulation test that is typically conducted over a 100 - 200 day period. It is intended to estimate the parent half life in the water phase, sediment phase and collectively in the total water-sediment system; determine the distribution and mass balance of the residues in the test system; as well as characterize the potential transformation products of the parent substance over the study period. Given the length of the test period, the analytical expertise and costs associated with conducting the test, it is often desired that a more rapid screen for assessing the potential transformation of pharmaceuticals would be available. Such information would be helpful in developing the ERA testing strategy especially when the identification of a key transformation product would be helpful early on in the risk assessment; as well as optimizing sampling intervals and conditions for the OECD 308. This presentation looks at the observed transformation products identified in the OECD 308 study and compares those to what may be predicted in an expert system to understand how selective the predictions may be and what additional information might be helpful in making a predicted pathway more realistic. Challenges in screening for water-sediment transformations in a laboratory test are also discussed to assess how such a test might be performed. For both situations, case studies will be presented to highlight some of the challenges present in advancing these further.

TU 047

A comparison of fish bioconcentration factors for several pharmaceutical compounds obtained following the standard OECD 305 Protocol and the estimated BCFs obtained using the reduced sampling method

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Understanding whether an active pharmaceutical ingredient is likely to be taken up from the aquatic environment by fish and bioaccumulate has triggered the need to conduct bioconcentration studies in fish. Such studies are typically considered appropriate for pharmaceuticals having a logD value > 3 and required, as per the EMA Guideline, for pharmaceuticals having logD values > 4.5.

The standard OECD Guideline 305 includes an exposure (uptake) phase followed by a post-exposure (depuration) phase typically equal to ½ the duration of the uptake phase. During the uptake phase, ranging between 14 and 60 days, water and fish samples are analyzed periodically until steady state has been established and the depuration phase may be initiated. During the depuration phase, analysis of water and fish samples continues until a plateau has been reached. Based on the sampling schedule outlined in the guideline, >100 fish per study may be required to determine a kinetic bioconcentration factor (BCF). In an effort to reduce the number of fish required to obtain a BCF, an approach to estimating the aquatic bioconcentration factor using reduced sampling has been developed (Springer, et al.2008). This reduced sampling approach aims to estimate BCFs using a minimum number of sampling time points and therefore a reduced number of fish.

The goal of this project is compare the BCFs of several active pharmaceuticals obtained through standard OECD 305 test methods with those BCFs determined using the approach of estimating aquatic bioconcentration factors using the reduced sampling method.

TU 048

Waterborne beclomethasone dipropionate affects fish while beclomethasone is not taken up

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Glucocorticoids are steroid hormones, playing important roles in several aspects of vertebrate physiology. The synthetic corticosteroid beclomethasone has anti-inflammatory actions and is commonly used in the treatment of asthma. It is administered as the more lipophilic prodrug beclomethasone dipropionate (BDP) which is metabolized to the monopropionate (BMP) and free beclomethasone in humans. A recent publication demonstrated effects of waterborn BDP (1 µg/L nominal concentration) on plasma glucose levels in fish. Although levels in of beclomethasone and its lipid esters in effluents or surface waters are not known, it can be assumed that most of the consumed prodrug BDP has been metabolized into the less lipophilic forms before reaching the environment. We have therefore compared the potential of beclomethasone and BDP to bioconcentrate and to affect the physiology of exposed fish. Controlled flow-through studies with rainbow trout over two weeks to up to 0.65 µg of beclomethasone/L did not result in measurable plasma levels. Exposure to 0.65 µg/L BDP, on the other hand, led to accumulation of both beclomethasone, BMP and BDP in plasma, indicating a considerably more efficient uptake of the more lipophilic prodrug as well as metabolism within the fish. Accordingly, exposure to 0.65 µg/L of BDP significantly increased blood glucose levels, in agreement with previously published results. Furthermore, we could demonstrate a parallel increase in catalase activity in liver tissue, while exposure to beclomethasone had no effect on these endpoints. We are currently screening for beclomethasone, BDP and BMP in sewage effluents and surface water.

TU 049

The degradation of antidepressant pharmaceuticals in aerobic sludge

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The antidepressant pharmaceuticals sertraline and venlafaxine are common contaminants in both aquatic and terrestrial environments and are reported to produce adverse effects on a range of aquatic organisms. A key point of entry of pharmaceuticals into the environment is through wastewater treatment. To learn whether microbial communities in wastewater sludge have the ability to degrade sertraline and venlafaxine and, thus, consequently reduce their environmental loads, microcosms containing aerobic sludge were spiked with deuterated sertraline and venlafaxine. Samples collected over 36 days were analyzed for the depletion of the added pharmaceuticals by microscale solvent extraction followed by liquid chromatography tandem mass spectrometry (LC/MS/MS). Municipal aerobic wastewater sludge was found to degrade sertraline and venlafaxine by over 90% over the course of 36 days, whereas no change in the added sertraline and venlafaxine concentrations were observed in abiotic control microcosms.

TU 050

Time trend of cyclic volatile methyl siloxanes in Baltic herring

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Cyclic volatile methyl siloxanes (cVMS) were studied in herring from the Swedish environmental sample bank. Fish from a site from the Baltic Proper southeast of Stockholm (Landsort) and from the northern part of the Bothnian Bay (Harufjärden) were analysed. Skinless dorsal muscle samples from six individuals were pooled. Two pooled samples were analysed from each site for every second year from the period 1989 - 2009. Octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6) were analyzed. The D5 concentrations were of the 50 ng/g lipid weight, while the concentrations of D4 and D6 were lower and frequently below the MDL. The concentrations were generally lower at the beginning of the period and highest around 2005-2007. There were no marked differences in concentration between the two locations.

TU 051

Degradation studies of the ionophores lasalocid, monensin, narasin, and salinomycin

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Coccidiostats are antimicrobial feed additives used prophylactic in poultry production to prevent the parasitic disease coccidiosis. The most heavily used sub-group of coccidiostats are the antibiotic ionophores. The excretion rate of coccidiostats from chickens is high and typically the chicken dung is applied onto agricultural fields. Hence, the aim of this study is to describe the environmental fate of ionophores and to identify new and ecotoxicological relevant transformation products.

Abiotic and biotic transformation studies were conducted on four ionophoric anticoccidials; lasalocid, monensin, narasin, and salinomycin. Abiotic studies focusing on hydrolysis and photolysis were conducted following international OECD guidelines. Hydrolysis was observed under acidic conditions for narasin, monensin and salinomycin, while lasalocid seemed stable under all conditions. Only lasalocid absorbs environmentally realistic wavelengths, so that photo-degradation of monensin, narasin and salinomycin can be discarded. Biotic degradation studies were conducted in lab-scale bioreactors inoculated with Nycodenz extracted soil bacterial communities. In addition, during the work with these very lipophilic anticoccidial agents, it was observed that adsorption to analytical equipment was highly relevant to assess under acidic conditions.

After abiotic transformation of the ionophores, the soil bacterium *Nitrosomonas europaea* was used as a screening tool for transformation product toxicity. This effect-directed study was conducted by exposing bacteria to the mixture of ionophore degradates.

TU 052

Formation and structure elucidation of stable transformation products of pharmaceuticals in the water cycle

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Pharmaceutically active substances undergo transformations starting from human metabolism to degradation in advanced effluent treatment, in environmental processes and finally during drinking water treatment. Often degradation in sewage and water treatment and the environment is incomplete, resulting in the formation of stable transformation products. The formation and presence of such stable transformation products in the effluent of sewage works, surface water, and drinking water treatment is reported in the scientific literature with increasing frequency. In only a few cases, full mineralization of the parent compounds is achieved. This is even more of importance as advanced oxidation techniques employing e.g. ozone, hydrogen peroxide, light or electro-coagulation are increasingly under discussion for the removal of pharmaceuticals and other micro pollutants in effluent treatment and drinking water treatment. Treatments using these techniques may even lead to the formation of transformation products that are more toxic than the parent compound.

In the past years, comprehensive information on the degree of contamination of our environment with pharmaceuticals has been collected, but there are still some important knowledge gaps regarding the ecological and human health impact of two groups of pharmaceuticals, which will be addressed in the Pharms project (EU grant agreement no. 265346). On the one hand, for anti-cancer drugs only little information is available on environmental concentrations, while for the antibiotics, concern is raised regarding their occurrence and the potential role of these compounds in spreading resistance against these valuable pharmaceuticals.

For a selection of pharmaceuticals of both classes, the formation of stable transformation products in various stages of the water cycle (both drinking and sewage) will be investigated in laboratory and pilot scale studies. Treatment processes include mainly chlorination, ozonation and UV-disinfection for drinking water treatment and advanced oxidation, photolysis/photocatalysis/photo-fenton for sewage treatment. For structure elucidation of the transformation products formed, different LC-MS/MS approaches as well as high resolution MS techniques will be implemented. Characterization of the stable transformation products in terms of (eco)toxicological behavior will be done using a battery of in vitro bioassays.

TU 053

Predicting environmental concentrations of carbamazepine and oxcarbazepine and their main metabolites in a coastal system

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Predicting environmental concentrations of carbamazepine and oxcarbazepine and their main metabolites in a coastal system

Predicting of environmental concentrations of pharmaceuticals is commonly done for risk assessment at a regional level. The difficulties appear for the prediction at a local level, where data available is scarce. This work developed a model to predict the behavior of carbamazepine (CBZ), oxcarbazepine (OxCz) and their main metabolites in a regional environmental system : a coastal zone with a WWTP submarine outfall. The model takes into account the initial CBZ and OxCz prescriptions, metabolisms and partial transformations of CBZ and OxCz into their metabolites, their fate in wastewater treatment plants and their flow to the coastal zone through the submarine outfall. Regional data on CBZ and OxCz prescription were obtained from the medical care system. CBZ and OxCz prescribed amounts have been studied over 6 months in view to calculate concentrations in wastewater treatment plant effluents and so allow for Predicted Environmental Concentrations (PECs) estimation. The human metabolic pathways of CBZ and OxCz required to include in the model the contribution of the major urinary metabolites: carbamazepine-10,11-epoxide, 10,11-dihydro-10,11-trans-dihydroxy-carbamazepine, 10-hydroxy-carbamazepine and other hydroxylated derivatives. PECs lie in the ng/L level in the wastewater effluents and were compared to the measured concentrations (MECs). The diffusion in the coastal zone was estimated with an hydrodynamic numeric model (MARS 3D). The behavior in the coastal zone was qualitatively compared to measured concentrations obtained with POCIS passive samplers. Data on pharmaceuticals in the coastal zone are scarce and PECs and diffusion model might therefore be useful for studying pharmaceutical transfer and fate in the coastal environment.

TU 054

Fate of anti-diabetic drugs: gliclazide, glibenclamide and glimepiride in soil environment

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It is assumed that about 3-5% of human population suffers from diabetes mellitus type 2. Since large quantities of anti-diabetic drugs are used for treatment, it is possible that huge amounts of these pharmaceuticals and their metabolites are deposited in the environment due to excretion from humans and because of the improper utilization of off-date drugs. The most common way to enter the natural environment by these drugs is by sewage. Therefore, the most endangered are natural waters, soils and sediments. Considering the composition of these matrices it is obvious that the most dominant interaction are sorption and biodegradation processes. Transport of the compound in porous media, such as soils, sediments and aquifers, is strongly influenced by its tendency to sorb on to the various components of the solid matrix. Furthermore, since molecular transfer is a prerequisite for the uptake of organic pollutants by organisms, the bioavailability of a given compound and thus its rate of biotransformation, is affected by sorption as well.

Sorption process of three sulfonylurea based anti-diabetic drugs were tested on three various soil. Selected soils differed in their physical and chemical properties and thus, it was possible to assess the influence on sorption of: organic matter content; pH; moisture; fine fraction content and cation/ anion exchange capacities. Other external factors like: change of pH, ionic strength of solution and temperature were also tested. Various of sorption and kinetics models were used to describe obtained results.

TU 055

Evaluation of pharmaceuticals and personal care products focused on anthelmintics in human-waste, sewage, hospital wastewater, livestock wastewater and receiving water

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We investigated 33 PPCPs focused on anthelmintics in the human-waste treatment plants (HTPs), sewage treatment plants (STPs), hospital wastewater treatment plants (HWTs), rivers and seawater. Additionally, the livestock wastewater treatment plants (LWTs) were surveyed to assess the concentrations of anthelmintics. The LWTs and HTPs had relatively high levels, indicating that livestock wastewater and human-waste are principal sources of anthelmintics. In the sources except for the LWTs, human anthelmintics, albendazole and flubendazole, were the most dominant. In some receiving water samples, veterinary anthelmintics showed higher proportion than human drugs, suggesting the possibility of other sources flowing into the water environment. Overall, anthelmintics showed insignificant removal efficiencies in wastewater treatment plants. Compared with NSAIDs, antibiotics and β -Blockers, anthelmintics showed relatively lower levels. In the future, additional studies need to be performed to understand the pathway of anthelmintics and their metabolites in the water system.

TU 056

Degradation of antibiotics by photocatalysis on immobilized titanium dioxide

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Antibiotics are micropollutants which release in the aquatic environment has been a subject of concern for several years as they can give rise to two problems: i) they can have a direct effect on bacteria, either those involved in wastewater treatment (activated sludge, biofilms) or those present in natural aquatic environment where they contribute to river self-cleaning, ii) they can promote the development of resistance to antibiotics and the transfer of antibiotic resistance genes between bacterial species, including those pathogenic to man and animal. Most antibiotic are non biodegradable and an advanced physicochemical process should be applied for their elimination. There are several such processes but photocatalysis is often proposed as it can use part of the solar spectrum as an energy source and is reagent free. However most of the works described in literature are based on titanium dioxide slurries. After treatment these nanoparticles should be recover which is not easy. One solution to this problem is photocatalyst immobilization. However immobilized photocatalysts are generally less efficient than suspended photocatalysts as less surface can be activated. Within this framework tests

of photodegradation of two antibiotics often found in the environment: a veterinary drug, tylosin (916 g/mole, pKa = 7.1) and a human drug sulfamethoxazole (253 g/mole, pKa = 5.7). Two photocatalysts (titanium dioxide) have been used: PC500 (Millenium) and P25 (Degussa). Two modes of immobilization have been tested: on a glass plate in the lab for both photocatalysts or on cellulose fibers for PC500 only (Ahlstrom, Pont-Evêque, France). Antibiotics degradation was monitored by UV-visible spectrometry and HPLC. End products (organic carbon and nitrogen species) were also quantified. Degradation was observed, P25 being more efficient than PC500. Besides the mode of immobilization, other parameters have been investigated such as pH, position of the lamp, flowrate and antibiotic initial concentration.

TU 057

Direct and indirect photolysis of human metabolites of antibiotic sulfamethoxazole

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Both direct and indirect photodegradation processes have been reported to be important removal mechanisms of sulfamethoxazole (SMX) in the environment. However, most of SMX enters the environment as human metabolites, yet their environmental fate is not known. In this study, we assessed the direct and indirect photodegradation kinetics at 3 different pHs of 5 human metabolites of SMX, namely, nitro-SMX, nitroso-SMX, acetyl-SMX, hydroxyl-SMX and SMX-glucuronide. Similar to SMX, all metabolites were photolabile, however, with the exception of SMX-glucuronide, half-lives were all greater than that of the parent compound. The protonation state affects absorbance of the compound and degradation rate. Slowest direct photolysis rates were observed at environmentally relevant pH 8.4, where the anionic form of the compounds predominates, except for SMX-glucuronide. The faster degradation of the latter at basic pH was attributed to SMX-glucuronide missing an acidic functionality; therefore, in contrast to SMX and other metabolites, it remains in its neutral and more photoreactive form at environmental pH.

In presence of sensitizers, humic acid and nitrate, which produce respectively reactive ¹O₂ and/or triplet excited state dissolved organic matter (DOM) and hydroxyl radicals, SMX metabolites degraded faster. The contribution of indirect photolysis to the overall photolysis was found to be greatest for nitro-SMX (70% in presence of 10 mg/L humic acid). Experiments conducted in lake water (1.19 mg-L⁻¹ NO₃⁻; 2.43 mg-L⁻¹ TOC) showed degradation rates comparable to humic acid experiments. Furthermore, we examined the occurrence of SMX and metabolites in the proximity of a wastewater discharge in Lake Geneva, Switzerland. Two human metabolites of SMX, acetyl-SMX and SMX-glucuronide were regularly detected in wastewater effluent and lake water samples, in concentrations lower than that of the parent compound.

TU 058

Ubiquitous distribution of sulfamethoxazole in tropical Asian and African waters

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Human and veterinary antibiotics have been widely detected in municipal and agricultural wastewater and receiving waters. Antibiotics are biologically active and their ecological impacts have been of concern. Especially, induction of antibiotics resistance is of great concern. Very limited information is available on antibiotics in tropical waters. Infectious disease is more spreading and more percentage of people suffer the disease in tropical area. This is mainly due to climate conditions (hot and wet) favorable for the incubation of vector microorganisms and insufficient sewer and water supply systems. More infectious disease may cause more usage of antibiotics, leading higher concentrations of antibiotics in the tropical waters. In addition to human medicine, large amounts of veterinary antibiotics are expected to be used in tropical countries due to intensive husbandry and aquaculture activities. However, only very limited information is available on the types and abundance of antibiotics in tropical Asian and African countries. Seven sulfonamides, trimethoprim, five macrolides, lincomycin, and three tetracyclines were measured in sewage, livestock and aquaculture wastewater, and river and coastal waters in 7 tropical Asian countries. Sum of the concentrations of the target antibiotics were sub to low ppb levels in sewage and heavily sewage-impacted waters. Most abundant antibiotic was sulfamethoxazole (SMX) followed by lincomycin or sulfathiazole. Average concentrations of SMX in sewage or heavily sewage-impacted waters were 11500 ng/L in Kenya (Nairobi; n = 7), 2930 ng/L in Ghana (Accra; n=7), 1750 ng/L in Vietnam (Hanoi, Ho Chi Minh, Can Tho, n=15), 650 ng/L in Philippines (Manila; n=6), 538 ng/L in India (Kolkata; n=4), 282 ng/L in Indonesia (Jakarta, n=10), 76 ng/L in Malaysia (Kuala Lumpur; n=6). These concentrations were comparable or higher than those in Japan, China, Europe, US, and Canada. Predominance of sulfonamides, especially SMX, is unique in these tropical countries. The higher concentrations and dominance of SMX can be ascribed to lower price of the medicine. Concentrations and compositions of livestock and aquaculture wastewater were highly variable. In many cases, sulfamethazine, oxytetracycline, lincomycin, SMX was predominant among the target antibiotics.

TU 060

Environmental fate, occurrence and toxicity of the antiparasitic pharmaceutical toltrazuril

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Toltrazuril is an organohalogenic endoparasitic pharmaceutical used in the acute and prophylactic treatment of livestock such as poultry, piglets, sheep and cattle against coccidiosis. It is metabolized through stepwise sulfoxidation resulting in pharmacologically active metabolites. Hence toltrazuril is excreted as the parent compound and as two active metabolites; toltrazuril sulfoxide and toltrazuril sulfone, mainly via feces. The massive use of other anticoccidial drugs has resulted in increasing resistance of the parasites [1] as a result the use of toltrazuril is expected to be escalating.

Already in 2003, Boxall et al. [2] expressed concern about this compound and considered, it as a high-priority drug with respect to especially environmental and toxicological concerns. However, today still little is known about its environmental occurrence, fate and toxicity in the open literature. In 2008, concerns were raised by Germany during the Marketing Authorization approval of a toltrazuril product [3]. Toltrazuril and its metabolites may present a risk to the environment due to toxic effects on terrestrial plants and due to a risk for ground water contamination caused by manure application on agricultural fields.

Analytical methods utilizing LC-MS/MS have been developed to quantify toltrazuril and metabolites in aqueous matrices using solid-phase extraction (SPE) and in solid matrices using pressurized liquid extraction (PLE). These methods are applied in this work to determine the occurrence of toltrazuril and its metabolites in drainage water, upper ground water and soil from agricultural fields amended with manure containing toltrazuril. Furthermore, the developed methods have been applied to determine the fate of toltrazuril and metabolites in different abiotic processes such as hydrolysis and photolysis. Toxicity was assessed by performing acute growth inhibition tests on *Selenastrum capricornutum* (freshwater algae, ISO guideline 14442), cyanobacteria (OECD guideline 201 and acute immobilisation tests *Daphnia magna* (freshwater water flea, OECD guideline 202).

1: Kim M-S, Lim J-H, Hwang Y-H, Park B-K, Song I-B and Yun H-I (2010) Vet. Par. 169:51-56.

2: Boxall ABA, Fogg LA, Kay P, Blackwell PA, Pemberton EJ and Croxford A (2003) Tox. Let. 142:207-218

3: EMEA/CVMP/345752/2008

TU 061

The impact of carbamazepine on concentrations of acridine in river systems in Northern Germany

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Carbamazepine (CBZ) is a pharmaceutical product which belongs to the dibenzazepine group. Due to high usage of the substance, concentrations found in the effluent of waste-water treatment plants (WWTP) ranged from 100-6300 ng/L. In river waters, CBZ concentrations from 30-1100 ng/L were found. A toxicological important degradation product of CBZ is acridine (ACR). However, only scant data exist concerning occurrence of ACR in WWTP. Moreover, ACR has not been analyzed in surface waters so far. In line with our very recent measurements of heterocyclic compounds in river systems in Northern Germany, relatively high concentrations of up to 55 ng/L for ACR and for CBZ of up to 320 ng/L were detected. Surprisingly, concentration ratios of ACR to CBZ in different rivers were found to be similar. Based on these results experiments were conducted to explore if concentrations of ACR in river water were influenced by degradation reactions of CBZ.

Monthly water samples were taken between May-November 2011 in a water section of 4.6 km from the River Fuhse. Additionally, water samples from the River Lippe were taken in November in a water section of 7.6 km. Samples were filtered (glass filter 1.6 µm) and immediately enriched on SPE cartridges (Lichrolut EN, Merck). A fractionation on the SPE-cartridge was performed using different pH-values. Extracts were concentrated to 0.5 ml by using a rotary evaporator and analyzed by LC-MS/MS (Agilent 6430). Recoveries obtained at two concentration levels were 84% for ACR and 70% for CBZ. Photolysis experiments were performed with CBZ for artificial and natural water samples (River Fuhse) using a Xe-light source with a 290 nm optical filter.

Seasonal c_{ACR} values in the River Fuhse range from 20-55 ng/L and c_{CBZ} = 120-320 ng/L. Concentrations were a factor of 4-5 higher compared to the larger River Lippe. In order to render concentrations and discharges comparable, the ratio $R = c_{ACR}/c_{CBZ}$ was calculated. Surprisingly, similar values of $R = 0.13-0.15$ were found for both rivers in November, even comparable to values found in the effluent of WWTP. The ratio increases to 0.33 in summer months in the River Fuhse. Besides the presentation of concentration levels and ratios R for both compounds in rivers and WWTP, photolysis experiments and a model are presented on the poster to discuss the impact of degradation reactions of CBZ on concentration levels of ACR in river systems.

TU 064

European environmental risk assessment for Trimethoprim in surface waters

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An environmental risk assessment for the aquatic compartment was developed for the old antibiotic Trimethoprim (CAS 738-70-5). The exposure assessment is based on documented human use figures in western Europe from IMS Health and average removal in wastewater treatment; this predicted environmental concentration (PEC) is compared with measured environmental concentrations (MECs) from Europe. On the effects side, acute and chronic ecotoxicity data from the literature were complemented by additional, new chronic results for fish; based thereon, acute- and chronic-based predicted no effect concentrations (PNECs) were derived. The risk assessment compares surface water PEC and MECs with aquatic PNECs for Trimethoprim.

TU 065

Comprehensive evaluation of the impact of Triclosan in the terrestrial environment

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A comprehensive evaluation of the effects of the antimicrobial personal care product triclosan (TCS) in the terrestrial environment was performed. This evaluation included the effect of TCS on soil microflora respiration and nitrification, impact on terrestrial arthropods, acute and chronic toxicity to earthworm (*Eisenia fetida*), and effects on emergence and growth terrestrial plant species. Microbial respiration and nitrification were evaluated in soil treated with 0.1-2.0 mg/Kg TCS for 28 days. The acute and chronic toxicity of TCS to earthworms were tested in an artificial soil in accordance with OECD guidelines 207 and 222. Test concentrations for acute and chronic toxicity studies with earthworms ranged from 64-1,026 and 0.3-100 mg/Kg soil (dw) for acute and chronic studies, respectively. The effects of TCS on the predatory mite *Hypoaspis aculeifer* exposed to 0.3-320 mg/Kg (dw) TCS for 14 days were evaluated in accordance with OECD guideline 226. Phytotoxicity studies evaluating survival, emergence, shoot biomass and length, and normalcy of development in 10 species of terrestrial plants were performed in accordance with OECD guideline 208 using 0.2-1,000 mg/Kg (dw) TCS for an exposure period of the median emergence time plus 14 days. No Observed Effects Concentrations (NOEC) and Lowest Observed Effects Concentrations (LOEC) for soil respiration and soil nitrification were 2 and >2 mg/Kg (dw), respectively. NOEC and LOEC values for acute and chronic toxicity to earthworms were 1,026 and >1,026 mg/Kg (dw) [survival], and 100 and >100 mg/Kg (dw) [survival and reproduction], respectively. NOEC and Lowest Observed Effects Concentrations (LOEC) values for predatory mite survival and reproduction were 10 and 32 mg/Kg (dw) and 3.2 and 10 mg/Kg (dw), respectively. NOEC and LOEC values in terrestrial plants ranged from 75 and 100 mg/Kg (dw) [biomass in lettuce] to 1,000 and >1,000 mg/Kg (dw) [emergence and phytotoxicity in vetch and phytotoxicity in cucumber]. Overall, these studies demonstrate that TCS poses minimal risk in the terrestrial environment which is consistent with Reiss et al. (2002).

TU 066

Risk assessment of pharmaceuticals incidentally discharged to the terrestrial environment

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The Environmental Risk Assessment (ERA) of pharmaceuticals mainly considers the aquatic compartment, since the majority of active pharmaceutical ingredients (APIs) are hydrophilic. However, some APIs with more hydrophobic physical-chemical properties (e.g. Log K_{oc}>4), may partition to sewage sludge during municipal sewage treatment, and following application of sludge to land as fertilizer, there is a potential for chemical residues in soils. With the rapid growth of the human population, water re-use practices are diverse and increasing globally. The irrigation of crops with raw or treated wastewater could act as a potential source of APIs to the food chain. Exposure via this route and its significance is not currently considered within ERA guidelines. The disposal of unused medicines is also of interest, and recent monitoring studies have shown that drugs disposed to landfill can potentially leach out and contaminate local groundwater and surface water. This poster presentation describes a risk assessment for pharmaceuticals associated with emission potential routes to the terrestrial environment: application of biosolids to agricultural land; wastewater irrigation of agricultural land; unused medicines to landfill. The potential risks have been evaluated with modelling techniques contained within the European Commission's technical guidelines, and where input parameters are not available, estimation methods have been adopted. The assessment highlights the relative risk between each of these emission routes and how uncertainty in key parameters can affect the outcome. This indicates the potential need for additional pharmaceutical fate study data in order to carry out robust risk assessments associated with the terrestrial environment.

TU 067

The ScenAT exposure model: a novel spatial method to inform environmental risk assessments of personal care products in China

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China's economy has grown significantly and so has the demand for home and personal care (HPC) products. The detection of chemicals used in HPC products is increasing in profile as China develop models for use in prediction of environmental concentrations (PEC). Data required to accurately determine PEC's of HPC chemicals include product usage information, physico-chemical properties, environmental behaviour (e.g. persistence, adsorption) and use of environmental information (e.g. receiving water bodies, sewage treatment plant (STP) infrastructure). A GIS-based exposure estimation framework has been developed to predict the fate of HPC chemicals in freshwater ecosystems allowing PECs to be generated at a more spatially resolved level (e.g., 2769 counties in China). Per capita water use values were sourced at the province level and the population connected to STP's were calculated using per capita water use and the STP domestic consumption discharge. Dilution factors were calculated per country using the runoff, contributing area and waste water flow. Predicted environmental concentrations were determined using the fraction removed in STP's or in river, the domestic water use per capita, the annual tonnage of ingredient used, the population dilution factors in receiving waters. The highest PEC's can be seen in North and East China. These areas have increased water scarcity and contain larger densely populated cities like Beijing and Shanghai that will contribute to the increased concentrations. Model validation is difficult due to the rarity of long term measurements of down the drain chemical concentration. Therefore a qualitative assessment was performed to determine if spatial patterns of water quality were consistent using data published by the Ministry of Environmental Protection. The results show the PECs are qualitatively consistent with other spatial assessments of water quality. The model fits neatly between a unit world approach (e.g. EUSES, RAIDAR and USEtox), which are quick and easy to run and catchment models (e.g. GREAT-ER and PhATE) which give a better approximation of real world but are complex and data heavy.

TU 068

A concordance assessment of Predicted-No-Effect-Concentration (PNEC) aquatic toxicity data for pharmaceuticals

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Environmental safety assessments are required for market approval of new Active Pharmaceutical Ingredients (APIs) (in the EU and to a lesser extent in the US), however, such assessments may be lacking for older drugs. To determine whether available environmental toxicity data can be used to make PNEC predictions for APIs for which effect data are lacking, we analyzed publically available data for 197 APIs. The APIs included in the Stockholm County Council booklet, 'Environmentally Classified Pharmaceuticals' - January 2011 Edition were used as a representative sample of the universe of APIs for the purposes of the analysis. For each of the APIs indicated in the booklet as having a PNEC generated (195), and an additional two, the supporting data were extracted from the Swedish National Formulary website (www.FASS.se) and parsed to facilitate statistical treatment. Following an outlier analysis, the dataset was used to evaluate statistical correlations between the PNEC and trophic level (algae, fish, invertebrate), benchmark type (EC50/NOEC), assessment factor, and Anatomical Therapeutic Chemical (ATC) Classification System group. Based on the findings of the statistical analyses, we consider two potential approaches for generating PNECs for APIs for which no effect data are available, discuss their strengths and weaknesses and future development needs.

TU 069

Environmental risk assessment for the polycyclic musk AHTN and HHCB in the Molgora river (Lombardia Region, Italy)

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Polycyclic synthetic musk (PCMs) compounds are used as fragrances in a wide array of personal care products. The two most important PCM compounds are galaxolide (HHCB) and tonalide (AHTN), used respectively at amounts of 1473 and 385 t in Europe in 2000. In Europe such substances are classified as High Volume Product Chemicals. The removal of both substances during municipal sewage treatment process has been estimated as being approximately 40-60% of the initial amount. For these reasons, it is not surprising to find AHTN and HHCB as contaminants of the aquatic environments. Indeed, the concentrations detected in environmental samples range from ng/L to µg/L in surface waters and effluents. The fact that both compounds show high values of log K_{ow} and potential for bioaccumulation raised serious concerns on their potential adverse effects on the aquatic ecosystems. Furthermore, it has been suggested that both chemicals can act as chemosensitizers and could therefore have indirect effects by allowing normally excluded toxicants to permeate cells. Literature data, reporting the presence of PCMs in the Italian aquatic systems, are scarce and limited. Very recently, a monitoring survey for HHCB and AHTN started in 2010 along the Molgora river, located in a very densely populated area of Lombardy Region (Italy). Water samples were taken seasonally in several monitoring stations located up and downstream of the three sewage treatment plants (STPs) present along the river. Objective of this study is a spatial and temporal risk analysis for the Molgora aquatic ecosystem by comparing the Predicted No Effect Concentration (PNEC_{water} and PNEC_{sediment}), derived for aquatic and sediment dwelling organisms, with the relative measured concentrations of AHTN and HHCB in water and sediments.

The observed concentrations of both chemicals dissolved in water are far below the risk threshold, while HHCB adsorbed to particulate organic matter can pose a risk for aquatic sediment dwelling organism. A temporal and spatial variability in PCM concentrations has been observed and the risk to the aquatic communities varies along the river and through the year. In particular, during the reduced water flow, the risk characterization highlights that the entire river downstream the first STP is at risk for the presence of polycyclic synthetic musks.

TU 070

Does price pressure on medicines result in more pollution?

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Pharmaceutical industries outsource more and more of their manufacturing of active pharmaceutical ingredients (API) to low income countries. We have previously shown that some bulk drug manufacturers in Patancheru, India pollute the surrounding environment with very high concentrations of a variety of APIs, including broad-spectrum antibiotics. Additional examples in other parts of the world suggest that this is not an isolated problem. However, information on by whom, where and under what circumstances the API of a given pharmaceutical product is produced is not publically available. This effectively prevents prescribers and consumers to make environmentally informed decision, and thus reduces incentives for API manufacturers to invest in green production technologies. The Swedish Medical Products Agency has information on where and by whom the API is produced for products approved for the Swedish market, data which can be accessible for research purposes. A smaller, previous study of ours revealed that the factories producing the API for 31% of 242 investigated products sent their waste to a treatment plant in Patancheru with insufficient waste water treatment. This disturbingly high figure can however not be extrapolated to the entire Swedish market since we preselected 9 APIs known to be present in the effluent of the Indian plant. Also, actual sales figures were not accounted for. The aims of the present study are therefore to describe where the APIs in pharmaceutical products, sold in Sweden, are produced and to what extent the price of interchangeable products correlate to the country of origin of the API. We will also investigate if price correlates to corruption and environmental performance indexes in the manufacturing country. Such surrogate measures are used as conclusive information on pollution level is lacking for most

production sites. Furthermore, we will analyze if there are general differences between the country of origin of the APIs in generic and original products. To accomplish this we are analyzing sale statistics (price) and information on the production site for APIs for roughly 60% of all products (7836) that were sold on the Swedish market in 2010. To date, all data has successfully been compiled into a workable database. We believe our analyses will highlight some of the international aspects of the environmental challenges linked to pharmaceutical production.

TU 071

Do pharmaceuticals with evolutionary preserved drug-targets in non-target organisms pose a greater environmental risk?

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Human pharmaceuticals are designed to target specific molecules involved in a particular metabolic or signaling pathway in humans to obtain desirable effects at low doses. Although not all of these drug-targets are present within the diversity of biological life found in nature, some are evolutionary well-preserved. It has therefore been hypothesized that non-target organisms with highly conserved human drug-targets may be at a greater risk to be affected by pharmaceutical residues in the environment. In this study, we tested the hypothesis that pharmaceuticals with well-preserved drug-targets in the cladoceran *Daphnia magna* have greater effects on gene expression and RNA content. Three different pharmaceuticals were tested: the anti-fungal drug miconazole, the anti-histamine promethazine (both with highly conserved drug-targets in *D. magna*), and the progestogen levonorgestrel (with a low drug-target homology). For each pharmaceutical, juvenile daphniids were exposed to sublethal concentrations and raised to instar 3. The effects were assayed at the level of (1) gene expression (vitellogenin and cuticle protein mRNA using β -actin as a housekeeping gene), (2) relationship between total RNA and DNA (as a proxy for protein synthesis rate), and (3) body size. The exposure to miconazole resulted in a two- and three-fold decreased gene expression of vitellogenin and cuticle protein genes, respectively, as well as a significant increase in RNA content in relation to DNA. The down-regulated expressions of vitellogenin and cuticle protein genes are indicative of endocrine disruption, whereas the increase in RNA can be a result of enhanced protein synthesis as a response to stress. By contrast, promethazine and levonorgestrel did not alter RNA-DNA relationship. No effects on the body size were observed for either test substances. Thus, our preliminary results indicate that for miconazole, the drug with well-conserved drug-targets, the endocrine and protein synthesis disrupting effects were indeed observed at sublethal concentrations. To fully test the hypothesis, complementary analyses on the other two pharmaceuticals are being conducted.

TU 072

Androgenic activity of levonorgestrel in three-spined stickleback (*Gasterosteus aculeatus*)

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Synthetic progestins are widely used in contraception and have emerged as widespread contaminants in the aquatic environments. They have been shown to impair reproduction in fish at concentrations in the low ng L⁻¹ range. The mechanisms behind the reproductive toxicity of synthetic progestins are largely unknown. Some synthetic progestins, such as levonorgestrel (LNG), exert androgenic effects in humans and other mammals by binding to the androgen receptor (AR). Studies in fish indicate that AR activation by progestins may partly mediate their reprotoxic effects, and cause female fish to develop male secondary sex characteristics. In order to acquire a quantitative determination of the androgenic potency of progestins in fish, we exposed three-spined stickleback (*Gasterosteus aculeatus*) to LNG. Male sticklebacks synthesize a unique glue-like glycoprotein called spiggin, which is used in nest building. Spiggin is produced in the kidney under the influence of androgen binding to the AR. Spiggin production is absent in females but can be induced by exogenous AR agonists, and serves as the best known biomarker for androgens in fish. Adult stickleback females in duplicate groups of eight were submitted to semi-static aqueous exposure to LNG at 5.5, 40 and 358 ng L⁻¹ for 21 days. The effects of LNG on the mRNA expression of spiggin and vitellogenin, kidney epithelium height and organosomatic indices were studied. Spiggin and vitellogenin transcripts were measured using real-time quantitative PCR. Kidney epithelium height was determined in histological sections. The results show a clear androgenic effect of LNG at concentrations ≥ 40 ng L⁻¹. Spiggin expression, nephrosomatic index and kidney epithelium height were all significantly increased at concentrations ≥ 40 ng L⁻¹, and vitellogenin expression was significantly reduced at 358 ng L⁻¹. Our in vivo quantitative data show that LNG acts as a potent androgen in fish, giving support to the contention that androgenic effects of certain progestins may contribute to their reproductive toxicity.

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TU 074

Transcriptome analysis of the brain of the gilthead sea bream (*Sparus aurata*) after exposure to environmental concentrations of human pharmaceuticals

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Pharmaceuticals are being released into the environment in extremely large quantities on a regular basis. Ingested drugs are eventually excreted from individuals, as well as they are released from manufacturing and disposal of unused or expired drugs ultimately winding up in the effluent of wastewater treatment plants and aquatic environments. In order to assess the long term effects of exposure to environmentally relevant concentrations of representative pharmaceutical compounds we have exposed the gilthead seabream (*Sparus aurata*) to representative drugs found at ng- μ g L⁻¹ levels in surface- ground and coastal waters. Acetaminophen (APAP), Carbamazepine (CBZ) and Atenolol (AT) were used as model compounds to assess the effects of low level, long term exposure on the expression of the brain transcriptome.

The treatment with the greatest number of differentially expressed genes was CBZ with 467 features, compared with 226 for APAP and 4 for AT. The selected vehicle, DMSO, didn't show any significant expression changes in comparison with the SW control. Out of all these features, only one was common between all the three treatments. This feature was identified by homology search as *Sparus aurata* cytochrome c oxidase subunit I mRNA. No other feature was common both between APAP and AT nor CBZ and AT. However, there were 130 common features between the APAP and CBZ treatment.

Functional annotation clustering and enrichment analysis of APAP and CBZ candidate genes in DAVID revealed treatment specific activation of different pathways and processes, with energy-related features present in all the applied treatments.

TU 075

Chronic effect of atenolol on physiological indices in rainbow trout (*Oncorhynchus mykiss*)

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Atenolol is a representative of second generation of the beta-blockers, which is often detected in the surface water, due to its extensive use and relatively high stability. In this study, the negative effects of atenolol were studied on juvenile rainbow trout, *Oncorhynchus mykiss*, by chronic semi-static bioassay. Fish were exposed to sublethal concentrations of atenolol (1, 10 and 1000 μ g/L) for 42 days. Multiple biomarkers were measured, including morphological indices, haematological parameters, antioxidant responses and CYP450 activities. Parameters measured in this study displayed various dependent patterns to atenolol concentrations and exposure time. In short, the multiple responses in fish indicated that atenolol induced physiological stress and could be used as potential biomarkers for monitoring residual atenolol in aquatic environment. Molecular and genetic mechanisms of these physiological responses in fish are not clear and need to be further studied.

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TU 076

Investigating the genotoxicity effects of pharmaceutical photo-transformation products

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All types of urban wastewater discharge and reuse practices, including irrigation of landscape and agricultural areas, groundwater replenishment, discharge into inland surface waters and sea, cause the release of organic xenobiotic substances into the environment. Amongst them, pharmaceuticals, which are widespread-used and dissimilar chemical compounds, specifically designed to interact with living organisms, represent an important concern for humans and the ecosystems. It has already been proved that conventional treatment taking place in urban wastewater treatment plants is incapable of eliminating these substances and as a result pharmaceuticals, their metabolites and/or transformation products are released in the environment. Research has been concentrated mainly on the effects the parent compounds may have; whereas investigation of possible effects caused by metabolites and transformation products is still at its early stage.

This study has been focused on investigating effects of photo-transformation products of ofloxacin, a photolabile second generation fluoroquinolone, widely used and frequent found in the environment. Photo-transformation of ofloxacin was achieved by photolytic and photocatalytic treatment at various times. Nine photo-transformation products and degradation mechanisms based on piperazinyl dealkylation and decarboxylation are proposed. Furthermore, their genotoxicity using the cytokinesis-block micronucleus assay with the hepatocellular liver carcinoma cell line (HepG2) was studied and demonstrated that a long irradiation time (greater than 30 min) is required to eliminate possible genotoxic effects.

TU 077

Ecotoxicity of 14 serotonergic pharmaceuticals in the crustacean *Daphnia magna*, the microalgae *Pseudokirchneriella subcapitata* and the marine gastropod *Haliotis tuberculata*

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The serotonin, also named 5-hydroxytryptamine (5-HT), acts both as a neurotransmitter or as an hormone, depending on its localization. The presented work investigated the ecotoxicological effects of 14 pharmaceuticals having serotonergic properties, i.e. enhancing the effects mediated by serotonin in the central nervous system. The tested

molecules are mainly antidepressant (fluoxetine, sertraline, paroxetine, fluvoxamine, citalopram, clomipramine, amitriptyline, mianserin, milnacipran, duloxetine, venlafaxine) but also antipsychotic drug (clozapine), cough suppressant/halucinogen drug (dextromethorphan) or beta-blocker (propranolol). These molecules have the common property to play directly or indirectly a role on the serotonin transporter and/or serotonin receptors. The 14 molecules were tested for their ecotoxicological effects using chronic and acute bioassays on the microalgae *Pseudokirchneriella subcapitata* and the crustacean *Daphnia magna*. Additionally to these conventional bioassays, a novel bioassay was developed using primary cell culture of hemocytes from the marine gastropod abalone (*Haliotis tuberculata*). This assay was used in order to address the question of pharmaceutical effects in a marine species. The acquired data were used to build quantitative structure activity relationship (QSAR) modeling in order to (1) determine if the chemical properties of the molecules can explain their toxicity, (2) identify the mode of action of the selected pharmaceuticals.

TU 078

Environmental effects of anticholinesterase therapeutic drugs on a crustacean species, *Daphnia magna*

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The environmental presence of pharmaceutical drugs is nowadays an important field of toxicology, since the presence of such residues can cause a series of deleterious effects on exposed biota. This study assessed the ecotoxicological effects of two anticholinesterase therapeutic drugs, neostigmine and pyridostigmine in *Daphnia magna*. This evaluation involved acute (immobilization and feeding behavior) and chronic (growth and reproduction) responses to different concentrations of the two compounds. The pharmacological effects of neostigmine and pyridostigmine derive from their capacity to induce a reversible inhibition of cholinesterase activity, and have thus been used to treat the human disease myasthenia gravis. We observed alterations at a sub-individual parameter directly related to the anticholinesterase activity. Our study calculated 48 h-EC₅₀ values in the immobilization assay of 167.7 µg L⁻¹ for neostigmine, and 91.3 µg L⁻¹ for pyridostigmine. In terms of feeding behavior, we calculated a 5 h-EC₅₀ for filtration rates of 7.1 and 0.2 µg L⁻¹ for neostigmine and pyridostigmine, respectively; for the ingestion rates, the calculated 5 h-EC₅₀ values were, respectively, 7.5 and 0.2 µg L⁻¹ for neostigmine and pyridostigmine. In the reproduction assay, the most affected parameter was the somatic growth rate (LOECs of 21.0 µg L⁻¹ and 2.9 µg L⁻¹ for neostigmine and pyridostigmine, respectively), followed by the fecundity (LOECs of 41.9 µg L⁻¹ and 11.4 µg L⁻¹ for neostigmine and pyridostigmine, respectively). We also determined a 48 h-IC₅₀ for cholinesterase activity of 1.7 and 4.5 µg L⁻¹ for neostigmine and pyridostigmine, respectively. These results demonstrated that both compounds are extremely toxic for *D. magna* at concentrations in the order of µg L⁻¹. By comparing the here-obtained results with the actual concentrations of pyridostigmine previously reported in the aquatic environment (0.22 µg L⁻¹), it is possible that this compound can significantly alter the feeding behavior of *D. magna*. Hence, the ecological implications of these data are of great importance and must be considered under the risk assessment framework of both pharmaceutical drugs.

TU 079

Acute and chronic tests of Tamoxifen and its metabolites on *Daphnia pulex*

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Drugs administered in humans are eliminated through the excreta in either intact or metabolized form. As other drugs, chemotherapeutic drug residues were measured in wastewater affluent and effluents. They reach continuously the environment where they have the potential to persist. Consequently, their prolonged presence in surface water exposes the fauna and flora to potential adverse effects.

Tamoxifen is a synthetic non-steroidal anti-estrogenic analog inhibiting competitively estrogen receptors. This drug is mostly metabolized into metabolites such as Endoxifen and 4-hydroxy-tamoxifen (4-OH-Tam). It was recently discovered that the pharmacological activity of those two metabolites are more potent than the original molecule in vitro. As no literature exists on acute and chronic effects of these metabolites on aquatic organisms, *Daphnia pulex* have been exposed to Tamoxifen and its two metabolites in acute and chronic bioassays. In acute tests, the concentrations that induced an effect on 50% of the tested organisms (EC₅₀) were quite similar for Tamoxifen and 4-OH-Tam, i.e. 0.52 and 0.60 µg/ml respectively. Even if endoxifen showed higher EC₅₀ (1.09 µg/ml) signs of distress were observed on daphnids exposed to low concentrations of this molecule (0.79 µg/ml) such as lethargy compared to the control. On-going chronic tests will determine whether the side effects reported during acute tests are observed on *Daphnia* and their offspring exposed to low drug concentrations. In these tests, *Daphnids* (<24h) are placed individually in increasing concentrations (between acute NOEC/100 and acute NOEC) until juvenile instar is reached. The number of neonates produced by each mother, as well as any kind of other stress signs, is recorded for 21 days, and the viability of the neonates is also evaluated. Such tests should give a better assessment than acute tests of the potential ecotoxicological impact of Tamoxifen and its metabolites on aquatic ecosystems.

TU 080

Calmodulin inhibition as a mode-of-action of antifungal imidazole pharmaceuticals in non-target organisms: implications for mixture toxicity assessment

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Although mixture toxicity is not addressed in current regulation of pharmaceuticals chemicals, several theoretical models have been developed and applied to predict mixture toxicity in environmental research. The Concentration addition (CA) model describes the mixture toxicity of components with similar mode of action (MOA).

Imidazoles are primarily known as antifungal substances interfering with synthesis of ergosterol in fungi. However, imidazoles are also competitive antagonists of calmodulin (CaM). CaM is a calcium binding protein expressed in all eukaryotic cells, where it participates in many signaling pathways (e.g. nitric oxide signaling), thereby affecting many different cellular functions, particularly neurosecretory activity. Since arthropods are unable to synthesize sterols relying on dietary sources, the sterol synthesis inhibition is not likely a primary mode of action. Here, we hypothesized that exposure to imidazoles disrupt CaM-dependent nitric oxide synthesis in microcrustaceans. Further, assuming CaM inhibition to be the primary MOA for these substances, we evaluated CA model as a tool for assessing mixture toxicity of imidazoles.

We tested our hypothesis by assessing single and combined effects of four imidazoles (econazole, miconazole, clotrimazole and ketoconazole) in adult specimens of the crustacean *Nitocra spinipes* (Copepoda, Harpacticoida). The mixture was tested using fixed ratios of each individual substance' LC₅₀ value. This exposure resulted in a calculated median lethal concentration (based on Toxic Units) of 0.86 (95% CI: lower 0.74; upper 1.01), which shows that the mixture displays concentration additive toxicity, in agreement with the CA model. In parallel, levels of CaM, nitric oxide and nitric oxide synthase were monitored to support the hypothesized MOA of imidazoles for non-target organisms and to justify the application of CA models for assessment of imidazole mixture toxicity.

TU 081

Behavioural and physiological responses to pharmaceutical exposure in *Gammarus* spp. and *Fucus vesiculosus*

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Gammarus spp. and *Fucus vesiculosus* are two foundation species of the Baltic Sea. These species inhabit the littoral community and are thereby exposed to substances released in coastal areas. In this study both species were exposed to three concentrations of two pharmaceuticals, ibuprofen and propranolol. Both physiological and behavioural parameters were estimated to examine potential effects in the organisms. Respiration, feeding rate, activity with and without predator cues by a Multispecies Freshwater Biomonitor (MFB[TRADEMARK]) were estimated for *Gammarus* spp. and gross production to respiration ratio (GP/R) and chlorophyll fluorescence for *F. vesiculosus*. Ibuprofen did not affect any of the measured parameters of *Gammarus* spp. significantly and only slightly affected the algae. The strongest effect was related to activity of *Gammarus* spp. measured by the MFB[TRADEMARK], and results showed that propranolol decreased the activity. The addition of predator cues into the exposure water increased the activity in all treatments, but the *gammarids* could not compensate for the reduced activity caused by the pharmaceutical. The feeding rates of *Gammarus* spp. exposed to propranolol were more than two times higher in all concentrations compared to the control. Also the alga was more affected by propranolol measured as lowered GP/R-ratio. These data indicate that the effects on both behaviour and physiology of *Gammarus* spp. in combination with the stress responses in the alga, might cause unexpected indirect and cascade-effects which have implications for the littoral communities.

TU 082

Acute effects of psychiatric drugs on *D. magna* under insecticide exposure

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The rates of production, release and use of pharmaceuticals and of psychiatric drugs in particular is expected to increase in the next 10 to 50 years for several reasons, and so is the loading into the environment. In recent years, there has been a growing concern about ecotoxicological risk of these psychiatric drugs that interact with the central nervous system. They include antiepileptic drugs, which decrease overall neuronal activity and antidepressants, which inhibit the re-uptake of serotonin. Indeed, these compounds have been detected in many countries in sewage treatment plant effluents, surface waters, seawaters, groundwaters and some drinking waters. However, little is known about effects of the interactions between psychiatric pharmaceuticals and other substances like neurotoxic pesticides. Because neuroactive insecticides like are usually present in aquatic environments and can co-occur with psychiatric pharmaceuticals it is important to assess this possible interaction

In this work we assessed the acute toxicity of the antidepressant Fluoxetine and the antiepileptic carbamazepine (some of the more prescribed psychiatric drugs) on *Daphnia magna* mortality under exposure to carbaryl or fipronil. *D. magna* was exposed to binary mixtures of each pharmaceutical and an each insecticide in full factorial designs. To address mixture effects, the observed mortality was then compared to the expected effects of mixtures calculated from effects of single compound exposures, based on existing conceptual models for the evaluation of contaminant mixture exposures: concentration addition (CA) and independent action (IA).

Although the risk for acute toxic effects of psychiatric drugs is unlikely, our results contribute with ecotoxicological data for risk assessment of psychiatric pharmaceuticals in the aquatic environment and are discussed in terms of possible ecological effects of environmental relevant concentrations of these substances on wildlife. We advocate that sub-lethal toxicity of these psychiatric drugs should be assessed under relevant exposure scenarios such as co-occurrence with neurotoxic pesticides.

TU 083

Assessing the environmental hazard of mixtures of pharmaceuticals: combined acute toxicity of fluoxetine and propranolol to the crustacean *Daphnia magna*

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The aim of the present work is to evaluate the acute toxic response of the crustacean *Daphnia magna* exposed to individual pharmaceuticals and mixtures. We tested fluoxetine, a selective serotonin reuptake inhibitor widely prescribed as antidepressant, and propranolol, a non selective β -adrenergic receptor-blocking agent used to treat hypertension. Acute immobilization tests were performed according to OECD 202 and ISO 6341 guidelines. Less than 24 h old daphnids were exposed for 48 h. Six replicate vessels with five individuals per vessel were tested at each treatment level. Single chemicals were first tested separately; estimated EC50 were 7.0 mg/L for propranolol and 7.8 mg/L for fluoxetine. Toxicity of binary mixtures was then assessed using a fixed ratio experimental design. Five concentrations (from 0.5 to 2 total toxic units) and 5 percentages of each substance in the mixture (0, 25, 50, 75 and 100%) were tested. The MixTox model was applied to analyze the experimental results. This tool evaluates if and how observed data deviates from the CA (Concentration Addition) or IA (independent Action) models, and tests if significantly better descriptions of the observed data can be achieved using a set of deviation functions. These functions allow a differentiation between synergism and antagonism, along with deviations based on the dose-level and chemical ratio dependency. The conceptual model of Concentration Addition was adopted in this study, as we assumed that the mixture effect mirrors the sum of the single substances for compounds having similar mode of action. This analysis showed a significant deviation from the CA model that indicated antagonism. Concentrations tested were much higher than those detected in the environment; however these results are to be considered as a first step in an ongoing project aimed at assessing chronic ecological effects of mixtures of pharmaceuticals.

TU 084

Assessing health status of *ruditapes philippinarum* exposed to caffeine, carbamazepine, ibuprofen and novobiocin using the neutral red retention assay

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Despite the fact that pharmaceuticals have been detected in the environment at the $\mu\text{g L}^{-1}$ to ng L^{-1} range, it has been demonstrated they can cause adverse effects at environmentally relevant concentrations. To detect possible adverse effects of a contaminant in the environment, standardized short-term, sensitive and low-cost methods are usually applied to estimate chronic toxicity against organisms. Lysosomal membrane stability (LMS), has been successfully applied as a screening tool to determine the health status of a wide range of organisms. The feeding style and habitat of the Manila clam (*Ruditapes philippinarum*) make this species vulnerable to organic contaminants bound to water and it has been widely used in ecotoxicological studies. LMS was evaluated in clams' haemolymph by the neutral red retention assay (NRRA). Clams were exposed in the laboratory to concentrations of caffeine (psychoactive stimulant), carbamazepine (anticonvulsant and mood stabilizing), ibuprofen (non-steroidal anti-inflammatory) and novobiocin (antibiotic) during 35 days in a semi-static 48 h renewal assay. Filtered sea water was spiked every 2 days with caffeine (0.1, 5, 15, 50 $\mu\text{g L}^{-1}$), ibuprofen (0.1, 5, 10, 50 $\mu\text{g L}^{-1}$), carbamazepine and novobiocin (0.1, 1, 10, 50 $\mu\text{g L}^{-1}$). Stock solutions of pharmaceuticals were prepared in DMSO (0.001%) to ensure solubility. Test with this solvent was undertaken to ensure no solvent effect. Results showed that neutral red retention time (NRRT) measured at the end of the bioassay was significantly ($p < 0.05$) reduced (50 %) ($p < 0.05$) when exposed to environmental concentrations of pharmaceuticals (caffeine = 15 $\mu\text{g L}^{-1}$; ibuprofen = 5 $\mu\text{g L}^{-1}$; carbamazepine = 1 $\mu\text{g L}^{-1}$ and novobiocin = 10 $\mu\text{g L}^{-1}$). Results showed dose-dependent effect of pharmaceuticals ($p < 0.05$). Clams analyzed after exposure to 10, 15 and 50 $\mu\text{g L}^{-1}$ were considered to present a diminished health status (NRTT < 45 min) ($p < 0.05$). LMS appears to be a sensitive tool that enables evaluation of the health status of clams after exposure to concentrations of selected pharmaceuticals under laboratory conditions and could thus be used as a biomarker for pharmaceutical contamination in aquatic environments.

TU 086

Cyto-genotoxic effects induced by cocaine on the freshwater bivalve *Dreissena polymorpha*

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The increase in global consumption of illicit drugs has caused both social and medical problems, but also the onset of a potential new environmental hazard. After human consumption, in fact, drugs of abuse enter the aquatic system posing a potential risk for aquatic biocoenosis. Even if many studies have showed the presence of both drugs and their metabolites in freshwater in the high ng/L to low $\mu\text{g/L}$ range worldwide, at present any information on their potentially harmful effects on non-target organisms is available. The aim of the present study was to investigate the cyto-genotoxic effects induced by the cocaine, one of the most used illicit drug in Western Countries, as well as, consequently, one of the most found in the aquatic environment. Our goal was reached through the application of a biomarker battery on a classical freshwater biological model, the zebra mussel *Dreissena polymorpha*. The raise of genotoxic effects was investigated by the Single Cell Gel Electrophoresis (SCGE) assay, which evaluated primary DNA lesions, and by the DNA diffusion assay and the micronucleus test (MN test), which investigated fixed genetic damage. The Neutral Red Retention Assay (NRRA), by evaluating the lysosome membrane stability, was used to assess cocaine cytotoxicity. 96 h exposures to three increasing nominal concentrations of cocaine (40 ng/L , 200 ng/L and 10 $\mu\text{g/L}$), comparable to those currently measured in environment, were performed under semi-static conditions. Our results highlighted that cocaine exposure induced significant ($p < 0.05$) increases of both primary and fixed DNA damage, above all at the highest administered concentrations. In addition, since cocaine significantly ($p < 0.05$) decreased the stability of lysosome membranes, our data also highlighted its cytotoxicity and the possible implications of oxidative stress for the observed genotoxic effects.

TU 087

A comparison of cyto-genotoxic effects induced by some pharmaceutical and personal care products (PPCPs) on the freshwater bivalve *Dreissena polymorpha*

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Pharmaceutical and personal care products (PPCPs) are considered new environmental pollutants, since hundreds of these molecules are commonly revealed in the aquatic environment in the high ng/L to low $\mu\text{g/L}$ range worldwide. Among them, the occurrence of the antibacterial triclosan (TCS), the antibiotic trimethoprim (TMP) and the non-steroidal anti-inflammatory drugs (NSAIDs) ibuprofen (IBU), diclofenac (DCF) and paracetamol (PCM) is well documented both in surface and sewage waters. Notwithstanding, at present, few studies have evaluated their potential toxicity towards non-target organisms. In order to enlarge this topic, sub-lethal effects induced by these therapeutics were investigated by using a multi-biomarker battery applied to the freshwater bivalve *Dreissena polymorpha*. According to a semi-static *in vivo* approach, zebra mussels were exposed for 96 h to an environmentally relevant concentration (1 nM), similar for each drug, corresponding to the level measured in surface waters. This choice allowed an easier comparison among their toxicity and gave useful information on their current hazard towards bivalves. The induction of genotoxic effects was investigated on mussel hemocytes by three well-known biomarkers: the Single Cell Gel Electrophoresis (SCGE) assay evaluated primary DNA lesions, while the DNA diffusion assay and the micronucleus test (MN test) investigated the onset of fixed genetic damage. Besides, the Neutral Red Retention Assay (NRRA), by evaluating the lysosome membrane stability of hemocytes, was used to assess the cytotoxic potential of drugs. The biomarker results pointed out that a low environmental concentration of TMP, IBU, DCF and PCM has a slight cyto-genotoxic potential on zebra mussel hemocytes. On opposite, 96 h exposure to 1 nM of TCS was able to significantly increase both primary and fixed genetic damage, as well as to induce significant ($p < 0.01$) destabilization in lysosome membranes, suggesting an intense stress in treated bivalves. Even if our data highlighted that, at present, only TCS could represent a serious hazard to bivalves, considering the growing trend of PPCPs use and the time/concentration-dependencies found for each biomarker after the exposure to selected PPCPs, in-depth studies are an absolute necessity to clarify their true ecological hazard for the aquatic biocoenosis.

TU 088

Toxicity of the antibiotic clarithromycin to green algae (Chlorophyta) and blue-green algae (Cyanobacteria), a comparison of species sensitivity

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Antibiotics are suspicious environmental contaminants as they are biologically active substances. Drugs, which have specific modes of action, may probably cause adverse effects on terrestrial and aquatic ecosystems. Up to now, only a few eco-toxicological studies for the assessment of the environmental risk of antibiotics and their metabolites are available. In Germany, the human macrolid antibiotic clarithromycin is widespread present in surface waters and has been found in concentrations up to 0.3 $\mu\text{g/L}$. In some studies eco-toxicological effects of clarithromycin on aquatic organisms are published. Unfortunately, most of these findings are not valid according to the "Technical guidance document on risk assessment (TGD EU-Commission)", because of missing standardized test methods, missing analytical measurements of the exposed concentrations or else disregarding problems in water solubility and sorption effects. In the present study, adverse effects of clarithromycin on the green algae *Desmodesmus subspicatus* (Chlorophyta) and the blue-green algae *Anabaena flos-aquae* (Cyanobacteria) were investigated in compliance with the TGD. As *Desmodesmus subspicatus* is an established species in toxicity testing on primary producers, Cyanobacteria, a phylum of bacteria, are more and more brought into focus of risk assessors because there is some evidence that they are more susceptible organisms to antibiotics than green algae.

TU 089

Assessment of ecotoxicological effects of environmentally relevant pharmaceuticals and personal care products (PPCPs) using battery of bioassays

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In recent years a group of diverse bioactive chemicals covering pharmaceuticals and active ingredients in personal care products (PPCPs) has raised concern of public and regulatory agencies. PPCPs are used in large amounts throughout the world and are continually introduced to surface waters, mostly by untreated and treated waste waters. PPCPs, some of which have been linked to ecological impacts at trace concentrations, have been detected in various surface and ground waters. Pharmaceuticals are produced as chemicals with specific biological activity for target organisms already in low concentrations. Although they are present in the environment in concentrations of ng/L , pharmaceuticals can elicit biological effects and pose a risk for nontarget organisms. Based on frequent consumption, frequency of detection in the environment and biological activity, a set of priority chemicals has been selected from a broad spectrum of PPCPs (e. g. diclofenac, paracetamol, ciprofloxacin, ibuprofen, methyl paraben, triclosan, tonalide, caffeine, 4 methylbenzylidene camphor). Battery of ecotoxicological bioassays with producers, consumers and destructants (alga growth inhibition test, *Daphnia magna* immobilization test, Microtox) has been used to assess acute toxicity of representatives of several groups of pharmaceuticals and personal care products. In addition to conventional acute ecotoxicological tests, selected chemicals have been tested for specific effects mediated by estrogen and/or androgen receptors. Cytotoxicity and specific activities have been determined by *in vitro* reporter-gene bioassays with mammalian cell lines and recombinant yeast strain. The results show differences among ecotoxicological effects of selected pharmaceuticals and personal care products on the representatives of different trophic levels also depending on their structure, mode of

action and physical-chemical properties. Next to this, endocrine-disruptive activities have been observed for a few of the tested PPCPs. The obtained data indicate which of these environmentally relevant polluting PPCPs pose the greatest risk for aquatic environment.

TU 090

Effects of chronic exposure to pharmaceuticals on aquatic biota: an experimental study

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Purifying techniques at sewage treatment plants are constantly being improved to remove more and more compounds from effluent water but despite this a lot of pharmaceuticals still manage to pass the plants in large quantities. This constant flux of chemically still active compounds into the environment has led to concerns previously because although they are designed to work for humans these compounds can also provoke unexpected effects on aquatic organisms. Although acute effect studies indeed prove that effects on aquatic non-target organisms can occur this usually happens at much higher concentrations than actually monitored in the environment. However, less information on chronic effects is available. From a previous monitoring it became apparent that STP effluent contained relatively high concentrations of metformine, metoprolol, solatol, valsartan, irbestartan, hydrochlorothiazide, diclofenac, carbamazepine, atenolol, and guanilurea. Consequently, it was decided to test these compounds in realistic levels (MIX) next to a control and raw effluent. This poster will present the results of an indoor microcosm study testing the potential impact of pharmaceuticals on aquatic biota in a chronic exposure set-up. Indoor microcosms comprising a simple water-sediment system were seeded with the worm *Lumbriculus variegatus*, the isopod *Asellus aquaticus*, the snail *Physella* sp., and the cladoceran *Daphnia magna*. Test medium was refreshed every two weeks and test organism abundances were monitored for approximately 8 weeks in all treatments.

TU 091

Toxicity of Ciprofloxacin and Sulfamethoxazole on marine biofilm communities

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Pharmaceuticals are widely used compounds and many of them will eventually end up in the aquatic environment where they might affect various organisms. Two commonly used antibiotics that have been shown to occur in the aquatic environment are Sulfamethoxazole and Ciprofloxacin. As they are used to efficiently treat bacterial infections there is a great risk that they affect environmental microorganism similar to their target organisms if they end up in the environment. Therefore, the concentration-response relationship of these two substances was investigated on natural marine biofilm communities (periphyton) mainly composed by microalgae and bacteria.

Glass discs were submerged in the sea outside Fiskebäckskil on the Swedish west coast and the biofilms colonized the substrate during a week. Thereafter the biofilms were exposed to the two antibiotics (5nmol/L - 9µmol/L) in the lab using the semi static SWIFT periphyton test during 72 hours.

Toxicant-induced effects on the communities were analyzed for both bacteria and algae. Pigments were extracted from the algal part of the communities and the composition was analyzed using HPLC. The pigment composition was used as a way to estimate structural changes in the community. Effects on the periphytic bacteria were measured using the so called Biolog Ecoplates® methodology. The communities ability to utilize different carbon sources is used as a measure on community function.

TU 092

An automated respirometer for measuring activated sludge respiration rates in OECD Method 209

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The activated sludge respiration inhibition test (OECD Procedure 209) is used to assess the inhibitory effects of a substance on the respiration of the heterotrophic population of sewage microorganisms found in the activated sludge sewage treatment process. The original OECD guideline for the test was first issued in 1984 and remained unchanged until July 2010 when it was reissued following an extensive update to improve the statistical basis of the test design. Changes introduced in the new guideline included an increase in the level of replication of test and control mixtures to allow the determination of the No Observed Effect Concentration (NOEC) of a substance, a requirement of Guideline EMEA/CHMP/SWP/4447/00 for the risk assessment of human pharmaceuticals, together with methods for assessing inhibition of nitrification.

Whereas the original OECD 309 could be conducted by one operator with relatively simple equipment, the new requirements for replication call for more complex equipment and higher levels of effort to set up the study. To satisfy these requirements we have introduced and validated a computer-controlled respirometer system comprising 30 incubation chambers each containing an oxygen probe, aeration and headspace nitrogen purge systems and a stirrer to provide mixing and flow of media across the probe membrane. Each probe is connected to an automated, computer controlled data capture system, which records the oxygen consumption by samples of activated sludge in each incubation chamber after aeration has been terminated and the headspace purged with nitrogen.

We describe in detail our experience with the operation of this sensitive equipment and the results that we have obtained with the reference substance 3-5 dichlorophenol and other chemicals tested. We have identified a step-wise approach based on dose-response for the determination of the effects of a substance on activated sludge and recommend that a ring test is conducted to compare the performance of the method and refine the test guideline.

TU 093

Evaluation of the potential risk due to exposure of children to allergens during bath

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The use of personal care products is a common practice in developed countries. This brings as consequence a frequent exposition to chemicals likely to be damaging for human health, particularly in the case of babies and children. Fragrances are a group of chemicals incorporated in most cosmetic and other personal care products including baby care ones. The Scientific Committee on Consumer Products (SCCP), has identified 26 of these ingredients as likely to cause contact allergies. Among products for baby care, those intended for the bath such as shampoos, bubble baths, shower gels, and soaps, contain detergents that can break down the natural barrier of the skin, allowing other irritants and allergens to penetrate.

Based on analytical data previously published providing concentrations of 26 fragrance suspected allergens in bathwater samples, the aim of this work was to estimate the potential risk due to the exposure of children under 3 to these chemicals during bath. With this purpose, the Environmental Risk Assessment (ERA) methodology of the US EPA was applied and three routes of exposition were considered, namely dermal, oral and inhalation. For the compounds under analysis, reference doses were not available in scientific databases to characterize the risk. Hence, RfDs were estimated on the basis of toxicological information such as the NOAEL (Non-Observed-Adverse-Effect Level). The required data could only be completed for 8 of the 26 allergens: pinene, benzyl benzoate, cinnamaldehyde, citral, limonene, hexylcinnamaldehyde, hydroxycitronellal and linalool.

Hazard quotients for each compound and route were calculate and then aggregated to derive hazard indexes by route differentiating 3 age groups: 0-1 years, 1-2 years and 2-3 years. In all cases, HIR for the inhalation route largely exceeded the limit (1) under which adverse effects for human health are not expected, representing the most significant contribution to the total hazard index. The HQ for pinene and limonene contributed in around 99% to the total HIR for inhalation. The HIR for dermal and oral routes were always under 1, being almost negligible for ingestion and not much significant for skin contact (the larger value was 0.1 achieved for the age group 0-1 years).

As a conclusion, an exhaustive risk assessment is necessary to determine the maximum allowable concentrations in water of personal care products to preserve children's health.

TU 094

Comparative biomarkers responses of zebrafish life stages exposed to oxytetracycline and amoxicillin

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Oxytetracycline (OTC) and amoxicillin (AMX) are antibiotics that are extensively used worldwide. Both compounds are applied in fish and shrimp farming practices, and in other animal intensive farming systems, as a growth promoter or bacterial disease treatment. Their excessive application has become a threat for both human and environmental health. Considering this scenario, our study aims at assessing sub lethal effects of AMX and OTC in embryos and adults of zebrafish. To achieve these objectives a battery of biomarkers were analysed in adults (liver, head, muscle, gill tissues) and embryos (whole body homogenates) exposed to these chemicals. Representative biomarkers from different metabolic pathways were chosen (catalase (CAT), lactate dehydrogenase (LDH), and glutathione-S-transferase (GST)). No differences in the mortality rate were observed between embryos and adults for AMX and OTC tested concentrations. Adults exposed to OTC showed inhibition of the oxidative stress enzyme CAT in head tissues (96 h-LOECAThead = 25 mg/L) and an increase in the detoxification phase II enzyme GST (96 h-LOECGSTmuscle = 10 mg/L). OTC exposure also induced the GST activity in embryos at the highest concentrations (96 h-LOECGSTembryos = 100 mg/L). LDH in adults showed a range of responses according to the different organs analysed: being inhibited in the head tissues (96 h-LOECLDHhead = 50 mg/L) and induced in the muscle (96 h-LOECLDHmuscle = 10 mg/L) and liver (96 h-LOECLDHLiver = 10 mg/L) as well as in embryos (96 h-LOECGSTembryos = 150 mg/L). Adults exposed to AMX showed an inhibition of CAT in head and gills (96 h-LOECAThead = 1 mg/L and 96 h-LOECATgill = 25 mg/L). An inhibition pattern was also found in GST head tissues (96 h-LOECGSThead = 1 mg/L). However, in the gills, muscle and embryos (96 h-LOECGSTmuscle = 1 mg/L; 96 h-LOECGSTgills = 10 mg/L and 96 h-LOECGSTembryos = 380 mg/L) an induction pattern was observed in the measured GST activity. No changes in LDH activities were notice for embryos and adults exposed to AMX. Antibiotic seem not provoke alterations in liver xenobiotic metabolism at the dosage and time of exposure tested, as expected. Embryos seem to be much less responsive than adults to AMX and OTC short term exposure. Linkages between the enzymatic effects found and parameters at higher levels of organization are essential for the better understanding the effects that of AMX and OTC may have on organisms and the environment.

TU 095

Gemfibrozil and naproxen biodegradation in a river water ecosystem

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Incomplete removal during wastewater biological treatments is the main source of surface water contamination by pharmaceuticals. Degradation of a chemical in the aquatic

ecosystem depends on a variety of factors, including compound properties and environmental factors and above all the presence of a natural microbial community able to degrade it via metabolic and/or co-metabolic pathways. Although pharmaceutical and therapeutic products are widely found in the natural environment, the ecological effects on receiving ecosystems remain largely unknown.

Naproxen, a nonsteroidal anti-inflammatory drug and gemfibrozil, a fibrate drug used as lipid regulator, have been found in several natural waters. They were also found in influent-effluent from a municipal waste water treatment plant inside the city of Rome and in the receiving River Tiber. For this reason we studied the degradation of these pharmaceuticals in microcosm studies using natural river water. The biotic and abiotic degradation (in terms of the disappearance of the 50% of the initial concentration) of naproxen and gemfibrozil were evaluated in microbiologically active river water versus sterilized one. Moreover, the degradations of naproxen and gemfibrozil were also evaluated in microcosms simultaneously treated with both pharmaceuticals in order to evaluate if their co-presence could affect their environmental fate and the degradation activity of the microbial community. The overall results show that both pharmaceuticals were biodegraded. Gemfibrozil (DT50 > 70d) was more persistent than naproxen (DT50 = 27 d). In the presence of Naproxen the degradation of Gemfibrozil slowed down and its persistence increased to more than 70%.

TU 096

Determination of emerging substance as human waste indicator in the Danube River samples

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Caffeine, methylxanthine derivative, is a pharmacologically active substance which stimulates the central nervous system, increases heartbeat rate, dilates blood vessels and works as a weak diuretic. It is present in pharmaceuticals, foods and beverages and is considered to be one of the most commonly consumed drugs in the world. Caffeine can be used as an indicator of human sewage contamination in surface waters because it has no natural non-human sources. The objectives of this study were to determine caffeine presence in the Danube River samples and to evaluate its stability during two months storage of samples on 4°C. Analysis was performed by solid-phase extraction (SPE) followed by new developed reversed phase high performance liquid chromatography (RP HPLC) method. The chromatography was using a Zorbax Eclipse XDB-C8 column (4.6 mm x 150 mm, i.d., 5 µm particle size) at 25°C, with a mobile phase of 0.1% THF in water (pH 8) - acetonitrile (85:15, v/v). The flow rate was 1 mL/min, and detection by DAD at 273 nm. Samples were collected in July 2011 on seven different locations of the Danube River on a territory of Novi Sad, Serbia. Caffeine amount ranged 0-84 ng/L on the 1st day and 0-52 ng/L on the 30th day after sample collection. On the 60th day after sampling caffeine was under the limit of detection in all water samples. Presence of caffeine confirmed the existence of human waste in the Danube River. Highest values were obtained in sample collected from location near the water supply source "Ratno ostrvo", Novi Sad. Obtained results showed that caffeine amounts significantly decreased during two months storage of samples on 4°C most likely due to its degradation.

The work was supported by Ministry of Education and Science, Republic of Serbia (III46009) and NATO Science for Peace Project 'Drinking Water Quality Risk Assessment and Prevention in Novi Sad municipality, Serbia' (ESPEAP.SFP 984087).

TU 097

The effects of ibuprofen exposure on fathead minnows (*Pimephales promelas*)

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Occurrence of the non-steroidal anti-inflammatory drug, ibuprofen, has been reported in aquatic waters in the UK at concentrations ranging between 3-27 µg/L⁻¹. Ibuprofen is a widely used prescription and over-the-counter medicine, treating pain, inflammation and fever by reducing the level of prostaglandins through non-selective inhibition of the enzyme cyclo-oxygenase (COX). COX exists in two isoforms; the constitutively expressed COX-1 and the inducible COX-2. In order to evaluate whether pharmaceuticals pose a risk to aquatic organisms, we are testing the hypothesis that any potential effects will be related to the Mode-of-Action of the drug and will be seen at plasma levels in non-target organisms similar to human therapeutic levels.

We have identified the COX-1, COX-2a and COX-2b genes in the fathead minnow (*Pimephales promelas*). To assess the uptake and effect of ibuprofen exposure, fathead minnows were exposed for 96 hours using a flow-through system to 100 and 500 µg/L⁻¹ ibuprofen. Four fish were sampled at 3, 24, 48 and 96 hours after exposure. Gills, gonads, gut, heart, liver, muscle and brain were collected to determine the effects (if any) on COX gene expression. RNA isolated from tissues was reverse transcribed to cDNA and amplified by qPCR using specific primers to the COX genes and reference genes (18S and β-actin). Initial results indicate changes in COX gene expression following ibuprofen exposure.

Plasma and water samples were also collected to determine ibuprofen concentrations in the fish and the exposure water, using mass spectrometry. This confirmed that fish were exposed to ibuprofen concentrations close to nominal (105 ± 2.6 and 502 ± 49 µg/L⁻¹, respectively). Analysis of the plasma revealed uptake of ibuprofen to be up to 9-fold (100 µg/L⁻¹) and 360-fold (500 µg/L⁻¹) higher than the nominal concentrations. Variation in ibuprofen uptake was noted between individual fish in the high concentration group (range 120-700 fold), and current work is focussed on relating this to changes in COX gene expression.

EM02P2 - Fate and exposure modelling

TU 099

Large-scale spatial-temporal modeling of historical pesticides applications

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With increasingly more detailed data and faster computers, it becomes easier to model pesticide mass loadings into aquatic systems at a high-resolution with temporal variability for large geographical areas. A large-scale co-occurrence assessment was conducted to quantify spatial and temporal mass loadings of pesticides into tributaries to the Sacramento River, San Joaquin River, and Bay-Delta estuary California. The aim was to guide future risk assessments for sensitive and endangered species. As part of this project, ten years of daily mass loadings were simulated for 40 chemicals in a 164,000 km² area of California's Central Valley. Environmental fate and transport models used for the analysis included the Pesticide Root Zone Model (PRZM), modified to simulate pesticide losses in irrigation tail water, and the rice water quality model (RICEWQ). Model inputs included historical pesticide use data from the California Department of Pesticide Regulation's Pesticide Use and Registration (PUR) database, daily weather data from 19 stations in the California Irrigation Management Information System, detailed soils information (SSURGO) from the NRCS, and high resolution land use data from the Farmland Mapping & Monitoring Program (FMMP). Pesticide application sites represented in the simulations included fruit, vegetable, grain, nuts, rice, landscape maintenance, and structural applications. Approximately 9,115,000 pesticide applications were represented in the simulations, accounting for a total applied mass of 98,279,000lbs of active ingredient for the 40 chemicals. Approximately 14.2% of the applied amount was predicted to reach surface waters via runoff, erosion, drift, and discharge. Runoff from agriculture accounted for over 86% of the mass losses loadings. Erosion and drift from agricultural applications accounted for approximately 5.0% and 4.4% respectively of mass loadings. Another 4.3% was predicted to discharge and run off from rice paddies. Urban runoff accounted for less than 1 percent.

TU 100

Modelling the seasonal cycle of POPs in soil, vegetation, and cow milk from a high altitude pasture in the Italian Alps

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Pollution in soil, vegetation, fodder, milk and feces from dairy cows that graze on a high altitude plateau in the Italian Alps has been measured during the pasture season. We followed the seasonal cycle of some obsolete pesticides (DDT and its isomers and metabolites, HCH and its isomers, HCB) of some congeners of polychlorinated biphenyls (PCB) and some congeners of brominated flame retardants (PBDE) during several pasture seasons that, at those altitude, last typically from June to September. Soil act as the main repository compartment with a high dynamic role because consistent concentration changes were observed over relatively short periods (few months). Typically POPs re-volatilization process from soils occurs just after the snow melting in May/June; the PCBs emission was quantified in terms of several tens of µg/m² depending from the topographical features. During this period, soil acts as a secondary source of contamination to the pasture, which shows a concentration peak in July. Vegetation confirms itself as an efficient scavenging medium for intercepting POPs fluxes from the air and from the soil during emission periods, and its role was quantitatively evaluated. We also found some evidences that air should be considered as a two layer compartment, dividing the part that is under the vegetation cover and the upper part that is interested by turbulent air fluxes made by winds. The lower layer, when soil is covered by herbaceous vegetation, could be very thin, but nevertheless seems to be extremely important in determining input and output fluxes between soil and vegetation. Vegetation is an efficient pathways for transferring POPs into terrestrial food chains. By monitoring intake and output of pollutants we were able to develop a multicompartmental 'cow model' describing the main fluxes of POPs between the pasture environment. We observed a transfer of pollutants between soils and herbaceous vegetation (that act as a temporary filter) and from vegetation to milk and feces, with fast transfer rates. We also observed differences in PBDE fingerprint between inputs and outputs, pointing out differential absorption and/or metabolism of these compounds in cows.

TU 101

Fate and behavior of pesticide in US soil: use of kinetic modeling to assess environmental relevance of metabolites

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In the European Regulatory context of inclusion of active substances in the positive list of Regulation (EC) N° 1107/2009, authorizing their use as pesticides, studies conducted in US are usually not recommended because of significant differences in experimental design, soil specificities and objectives when compared to OECD guidelines. However when a US soil metabolism study (conducted according to US-EPA guideline i.e. OPTTS 835.4100 using US soils) was conducted and submitted as part of the EU dossier, Regulatory authorities may ask for clarifications on soil metabolites, even formed under the specific conditions of this study type.

The objective of this work is to present a case study where apparent conflicting results were obtained between US-type soil metabolism and EU-type soil metabolism studies: In the US study, additionally to one major metabolite M, two metabolites (m1 and m2) were considered to be formed directly from the parent in the metabolic pathway proposed by the laboratory in amounts continuously increasing up to around 7% of the applied radioactivity until the end of the one year incubation. These results were contradictory to the EU type studies where only M was identified.

Kinetics optimization with ModelMaker software and statistical analysis of the data according to the recommendations of the FOCUS Kinetics Workgroup (2006) were performed.

The kinetic evaluation demonstrated that, using a new metabolic pathway, this optimization of kinetic data leads to an acceptable error. Therefore, these soil metabolites were found not to be environmentally relevant according to EU guidance. This justifies the need to perform additional kinetic investigation of the metabolism of pesticides so as to set up properly the degradation pathway.

TU 102

Determination of plant uptake factors for pesticide fate modelling

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The plant uptake factor (PUF) is defined as the concentration of a compound in the solution taken up by the roots divided by its concentration in the soil porewater.

Numerical leaching models applied for the environmental exposure assessment of pesticides use the PUF to calculate the amount of a compound taken up by a plant together with the transpiration stream. The PUF is therefore an important parameter for a realistic leaching assessment.

PUF values have been experimentally derived for four substances covering a wide range of polarity (ionic, polar, medium polar, lipophilic) in three different crops (Tomato, Wheat and Maize).

In the hydroponic experimental set-up intact plants were exposed between 8 and 11 days to a nutrient solution (pH 6) spiked with the radiolabelled test compound. Volume uptake and concentration in the nutrient solution were measured regularly over the experimental period. By sealing the test vessel it was assured that the only loss process from the system for water and chemical was plant uptake.

As a result of the study, the PUF was always well above the FOCUS default value of 0.5 (FOCUS, 2009). Generally, a small variation between plant species was observed. The experimental results could be verified with a simple plant uptake model.

The data on PUF show that the use of the FOCUS default of 0.5 is a very conservative approach in terms of leaching assessment. For the test compounds exhibiting ionic to lipophilic properties and examined in three diverse crops representative for cereals, small grains and vegetables all measured PUF values were well above 0.5. It is expected that these high uptake values can be generalized to other compounds within the same range of polarity, as the plant uptake model successfully applied to confirm these values only uses generic compound parameters as input (e.g. lipophilicity).

TU 103

Development of a French national tool for pesticide risk assessment in the context of the water framework directive

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The main objective of the Water Framework Directive (2000/60/EC) is to achieve good ecological status in all surface and groundwater bodies in the EU by 2015. However, it is already clear that this goal cannot be achieved by 2015 for a number of water bodies in France in intensively used agricultural areas. It is therefore important to have powerful exposure assessment tools which are able to i) link the observed contamination of water bodies with agricultural pesticides with the agro-pedo-climatic conditions and pesticide application practices responsible for the contamination, and ii) explore the effect of risk mitigation measures a priori in order to determine the most effective and efficient measures for later implementation in practice.

FOOTWAYS has been charged with the development of a national pesticide exposure and risk assessment tool for France to be applied in the context of the Water Framework Directive. The tool will produce results at two different scales: i) river water bodies and groundwater bodies, for the purpose of risk assessment and global testing of the effects of mitigation measures, and ii) edge-of-field water bodies, for more specific testing of the effect of mitigation measures. The tool will be part of the FOOTWAYS Pro web platform for pesticide risk assessment and management.

While the first phase of the project is focusing on an evaluation of the current situation, in the second phase also scenario simulations exploring the effects of the implementation of mitigation measures will be conducted. Results from the first two phases of the project will be presented, including exposure and risk estimates for the widely used substance glyphosate and its main metabolite AMPA.

TU 104

Pesticide exposure assessment in flowing waters - Approaches to dynamic predicted environmental concentration

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Pesticide risk evaluation for surface waters in the EU is based on the FOCUSsw standard scenarios ditch, stream, and pond, which are characterized by fixed amounts of water for dilution of pesticide input. The representativity of this water bodies for existing waters in real landscapes of the EU was never verified, and there is some doubt that the FOCUSsw standard scenarios reflect the "realistic worst case" appropriate. Given the fact, that the vast majority of water courses are flowing waters and not stagnant ditches, a more realistic pesticide exposure assessment has to respect some basic properties of rivers:

(i) In flowing waters, the hydrodynamic dispersion lowers the peak concentration but spreads the longi-tudinal extension of an initial substance pulse.

(ii) Pesticide applications on fields along a river stretch occur stochastically, thus a water package moving downstream the water course may be affected by depositions via spray drift, runoff and tile drainage, respectively, more than one time.

(iii) The locations of pesticide input and the sites where ecotoxicological effects may occur are apart from each other.

The pesticide concentration in flowing waters observed at a point of a river stretch fluctuates irregular, depending on the temporal and spatial pattern of the pesticide input upstream. For the registration procedure these concentration oscillations has to be transformed into an operational exposure indicator, comparable to a regulatory acceptable concentration (RAC). For this purpose the GeoRisk project introduced the quantity $PEC_{TWA}(1h)$, the maximum of predicted environmental concentration averaged over a 1 h time period. The concentration pattern over the entire period of applications is scanned with a moving temporal window of 1 h and then averaged to the 1 h mean.

The poster presents results of the $PEC_{TWA}(1h)$ achieved by Monte-Carlo (MC) simulations for generic water courses with tiered combinations of the factors flow velocity, discharge volume, width-depth ratio, percentage of river stretch receiving spray drift deposition, and duration of exposure time. The timing of the spray drift depositions along the water course was randomized for each MC-realisation of the combinations. Additionally the sensitivity of the variables is depicted. The presentation of Trapp et al. demonstrates the application of the PEC-dynamic approach to a real brook in Germany.

TU 105

Plant uptake of eight pesticides / metabolites as a function of log Kow and of pH in a hydroponic test system

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Plant uptake of environmentally relevant chemicals like pesticides is an important process limiting their availability for leaching, run-off and volatilisation. Chemical hydrophobicity (log KOW) and for ionisable compounds the pKa value are the most important properties determining the ratio between a compound's concentration in the plant-root system to that in the pore water adjacent to the roots. The Plant uptake factor (PUF) can be used as input parameter in simulations models (e.g. FOCUS PELMO) to determine the leaching behaviour of the substance more precisely. The PUF was measured in soil-less hydroponic test systems with intact plant root system in an artificial pore water solution (1-L of 0.01 M CaCl₂) containing a given concentration of the substances. By determining the volume uptake and concentration of the test item in the solution at different time intervalls PUFs can be calculated. If 14C-labeled compounds are used the distribution of test item in the root plant system can be simply determined by sample combustion followed by liquid scintillation counting (LSC). The test system used in this study are 1-L brown glass vessels in which the plants without soil (pre-grown in soil for 5-6 weeks) are inserted into the test solution and cultivated for 8 days under controlled greenhouse conditions. For each substance, the experiment is conducted with three different plant species (Wheat, Oil seed rape, Tomato) and under three pH conditions using biological buffers (5.5, 6.5 and 7.5). The PUF of 8 test substances, chosen on a relevant range of lipophilicity and pKa value, will be determined on several sampling dates (0, 2, 5 and 8 days after treatment), as well as in the test plants at the end of cultivation. The PUFs determined will be presented crop specific as a function of the chemical hydrophobicity of the substances, and of the pH-level of the test solution.

TU 106

Exposure and risk assessment for pesticide inputs into surface waters via surface runoff, erosion and drainage: developing a new concept for German national pesticide authorization

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The new EU regulation 1107/2009 concerning the placing of plant protection products on the market requires a harmonization of the various national pesticide exposure and risk assessment approaches with each other. As a first step towards harmonization among member states, the German Federal Environment Agency (UBA) launched a project to harmonize the German national exposure and risk assessment procedure for surface waters with the procedure used at the EU level and in various other member

states (FOCUS surface water). Since a direct adoption of FOCUS surface water for the German national authorization procedure is not feasible for a number of reasons, within the project a new national exposure assessment tool for pesticide inputs into surface waters via surface runoff, erosion and drainage will be developed. This tool will i) take into account the full range of agro-pedo-climatic conditions in Germany, ii) facilitate modelling the effect of risk mitigation measures, iii) include a number of higher-tier options and iv) allow for a coupling of predicted exposure time series with standard laboratory or higher-tier effect time series. The project coordinated by Dr. Martin Bach at University Gießen started on 1 December 2011 and will run for 23 months.

The general outline of the project and results of Work Package 1 (Evaluation of the FOCUS surface water modelling concept with respect to representativity and protectiveness of the included scenarios for German conditions in comparison to the current assessment methods in the German national authorization procedure) will be presented.

TU 107

Impact of recent EFSA guidance on soil degradation rates and environmental exposure modelling

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Registration of agrochemicals under the EU Regulation 1107/2009 requires a lot of challenges and numerous guidance documents have been recently issued, thanks to EFSA for the organization of the release of these. Among them, the methodology of the derivation of kinetic parameters linked to the dissipation of chemicals in the environment is probably the most advanced in the Regulatory arena world-wide. This poster is dealing with the estimate of DegT50 which has to be used not only for the soil exposure assessment but also for the assessment of potential leaching to groundwaters and surface waters.

The general procedures for calculating degradation rates are well known but the assumptions made during this process can appreciably affect the results.

Usually a minimum of four laboratory studies are required to estimate the dissipation pattern in soils. If the degradation half-life (DegT50) in top soil at 20°C at pF = 2-2.5 exceeds 60 days additional field dissipation studies need to be conducted.

In the field, the persistence of pesticides is affected by the varying environment, i.e. soil moisture and temperature having a significant effect on the bioactivity of the microflora. Normalisation of field degradation data may need to be performed to obtain reliable degradation parameters and to be used for modelling purposes.

FOCUS (2005 & 2006 further to FOCUS,1997) developed detailed guidance on estimating degradation rate parameters from laboratory and field studies.

In the past years, the time-step normalisation procedure as described by FOCUS (2006) has become popular in the EU registration. This procedure assumes that the decline in the field can be described well by numerical models that assume first-order degradation kinetics. The procedure implies that the decline curve after normalisation can be used directly to estimate the DegT50matrix of the top soil at 20°C and pF = 2.

However, according to EFSA panel, this decline is expected to show a rapid initial phase in the period when surface loss processes play an important role followed by a slower phase that is dominated by the degradation rate within the soil matrix.

In the proposed poster, we will show for an existing substance how the methodology is being used (according to the new EFSA (2010) guidance) to derive degT50 and we will discuss the impact of this new guidance on the results and on exposure estimation.

TU 108

How reliable is the predicted leaching of pesticides into Groundwater? - Validation of the FOCUS groundwater model PELMO 4 for use in the German national registration process

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In the registration procedure in Germany the risk assessment for the leaching potential of plant protection products into groundwater is based on modelling results using simulated leaching concentrations of the FOCUS Hamburg scenario from the latest version of the FOCUS PELMO model (tier 1, 2). Dependencies of pesticide sorption and degradation data from different soil properties as well as the parameter variability are considered in the endpoint selection for modelling, which is in line with the FOCUS recommendations. The measured leachate concentrations for active substances and/or metabolites from outdoor lysimeter studies are accepted as higher tier endpoints in the risk assessment and overwrite predicted concentrations from simulation runs (tier 3). This is generally justified by comparable soil and climate conditions in the Hamburg scenario and during lysimeter studies and by the presupposition, that experimental analysis lead to more precise predictions of the leaching potential of such compounds under realistic conditions. However, the implementation of the new FOCUS PELMO model in the German authorisation procedure in combination with the adapted selection of modelling endpoints requires a validation of the national tiered approach. In a current research project, predicted and measured leachate concentrations from modelling and lysimeter experiments are compared for several active substances and their metabolites. Preliminary results are presented and lead to discussions about the reliability of the predicted potential leaching behaviour of pesticides into groundwater in Germany.

Further investigation is planned on the critical points, how the short duration of lysimeter studies in combination with a single application finally affects the prediction of the leaching behaviour. The methodology of inverse modelling with FOCUS PELMO was chosen to derive degradation and sorption values for selected substances and allow a model based extrapolation of the lysimeter results to different conditions, e.g. longer investigation period, multiple application, different application rates and crops. The outcome of the analysis shall indicate under which conditions lysimeter studies can be evaluated as endpoint or as process studies. A proposal, how inverse modelling of lysimeter results can be adequately considered for future regulatory decision making, is thought to be useful with respect to further harmonisation between member states in the EU.

TU 109

The impact of the rainfall criterion in the new EFSA soil persistence guideline on the results of field degradation trials

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In the new EFSA guidance on soil persistence (EFSA, 2010) it is intended to exclude loss processes on the soil surface (photolysis, volatilisation) for the derivation of field half-lives of pesticides. As a pragmatic approach, it is proposed that only sampling points after 10 mm cumulative rainfall should be used to derive DT50. While this rainfall criterion is meant to ensure that processes on the surface are not taken into account for kinetic evaluation of field studies, there is no clear scientific basis for the selected 10 mm used as a rainfall criterion. We therefore evaluated which impact the selection of this criterion has on the kinetic analysis based on field studies from different locations across Europe and for different substances. Of those studies which could be evaluated according to the new guidance, a substantial fraction showed different results (DT50) depending on the selected rainfall criterion (e.g. 5 mm, 10 mm, 15 mm, etc). While in some cases only the resulting DT50 varied according to the applied rainfall criterion, in other cases studies could not be evaluated anymore. We also show that the impact of the rainfall criterion on the resulting DT50 depends on both the study location within Europe and the time of application. These results show that the selection of the rainfall criterion can have a pronounced effect on the results of field studies. Hence, this criterion needs to be chosen with care. Studies on the speed of movement of pesticides in the soil, depending on substance and soil specific properties and rainfall, may help to show if the currently proposed value of 10 mm is reasonable.

TU 110

InversePELMO a specific software to perform inverse modelling simulations with FOCUSPELMO 4

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In the assessment for authorisation of a plant protection product its leaching behaviour is an important factor to protect the groundwater. In this context the sorption to soil (Parameter: Koc) and the degradation (Parameter: DT50) are processes that must be taken into account. Recently, the new FOCUS groundwater group suggested a third methodology for the input parameter setting (FOCUS 2009). The idea is to analyse outdoor studies, especially lysimeters, using the inverse modelling method that allows the estimation of Koc and DT50 parameters within a single step. For this procedure an optimisation tool, the program PEST (Model Independent Parameter Estimation), has to be combined with a leaching model (here: FOCUSPELMO 4). The aim of inverse modelling simulations is to find those Koc and DT50 values that could describe the outdoor study best by considering all data recorded during the experiments (e.g. rainfall, temperatures, percolate, and substance fluxes). Therefore all data of a lysimeter study are used to vary the input parameters Koc and DT50 until the leaching model shows the same results as the experiment. Generally, 2 steps have to be conducted when performing inverse modelling simulations. Firstly, the hydrology in soil is optimised, followed by the optimisation of pesticide fate. The software InversePELMO is able to provide both programs (the optimisation tool and the simulation model) with the necessary input files in the correct format. InversePELMO has also a built-in module to perform standard statistical tests to check the quality of the optimisation such as the determination of the 'FOCUS error' at which the chi² error passes as described in FOCUS (2006). It was also tested that PEST works under all relevant windows systems (XP, VISTA and 7) in a 32 bit as well as 64 bit version. The results of InversePELMO can be used to make predictions about the most likely behaviour if the lysimeter study had been conducted over a longer time period, translations of the lysimeter results to a different situation with respect to the environmental conditions (e.g. different climate), translations of the lysimeter result to a different situation with respect to the application pattern of the substance (e.g. change of the rate) and the use of optimised parameter settings for a refined standard tier 1 simulation.

TU 111

Multi-dimensional modelling of pesticide placement and fate in the soil-root-plant system

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Many pesticides are soil applied, including residual herbicides, soil insecticides and seed treatments incorporating fungicides and/or insecticides. They are under intense regulatory pressure with regard to water contamination. The pressure of regulation of pesticides within the EU is such that registrants are examining the option of using application strategies such as seed treatments and slow release formulations as a means of reducing environmental impacts due to off-site movement of pesticides. Experimental approaches for demonstrating environmental benefits of formulation are impractical: each active ingredient is sold in many formulations and the cost of a

full-field environmental programme is barely affordable for a new active ingredient let alone for a single formulation. Simulation models are the primary basis for regulating pesticides against EU ground- and surface-water protection standards, but the current models cannot explicitly represent plants or their roots, or the effects of formulations and formulation placement strategies (e.g. slow-release microcapsules and seed treatments). Other models exist that represent individual components of the system in a more satisfactory way.

Current models used for pesticide registration within the EU are 1-dimensional and have a simplified representation of root development and uptake of pesticides. Conversely, plant growth and uptake models can have sophisticated representation of plant processes, but often do not consider pesticide dynamics. An ongoing project has created a conceptual framework for representing local pesticide concentrations in the whole soil-plant system, and will next produce a spatially-explicit simulation model. By relating local concentrations of pesticides to product efficacy, it will be possible to use the model to design better active ingredients, formulations and placement strategies. The new model will also enable the environmental benefit of advanced formulations and placement strategies to be quantified in a cost-effective way for the first time. This presentation will introduce the project to the scientific community, overview results from the first year of work, and discuss anticipated outcomes and the potential benefits to the scientific and regulatory communities.

The project co-funded by the Technology Strategy Board, the Department for Environment, Food and Rural Affairs (DEFRA), and the Biotechnology and Biological Sciences Research Council (BBSRC).

TU 112

Last advances and perspective for a better risk assessment of the tropical use of Protection Plant Products in France

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Risk assessment of water contamination required prior the pesticides registration for French overseas territories is currently conducted using tools and guidance documents established for EU context although the agro-pedoclimatic conditions of these tropical regions would require to use specific tools and methodologies.

The aims of this communication are to (1) clarify how deriving some pesticide's properties appropriate for tropical soils from EU endpoints with the aid of a statistical analysis performed on degradation and adsorption parameters obtained from temperate and tropical soils, (2) summarize the main specificities of agro-pedoclimatic conditions and transport of pesticide in these regions to account in modelling and (3) provide a brief overview of the principles of the model currently under development and the framework planned to adapt it for regulation.

The statistical analysis shows that EU endpoints seem to be provisional acceptable entries for the risk assessment in these tropical regions but should not supersede any experimental evidence supplied with tropical soils. The knowledge about agropedoclimatic conditions and transport of pesticide in these territories underlines that (1) a minimum of four scenarios (2 soils: andosol and nitisol and 2 crops: banana and sugar cane) would be necessary for the risk assessment of the main uses of the French overseas territories and (2) the models to be used for predicting transport of pesticide have to account for preferential flow and specific redistribution of rainfall by crops canopy for banana. For water surface contamination, the module for surface water fate would likely to be initially simplified to a simple static receiving water body of dimensions defined consistently with French overseas territories and protection goals. Finally, the main features of two models - currently under test - representing surface and subsurface flow of water and pesticides in banana crops on tropical volcanic soils are summarized and the schedule of a 2 year research project (2012-2014) to adapt them for regulatory purposes introduced.

TU 113

Simulation of the herbicides flufenacet and metazachlor in a lowland catchment with the model SWAT - A sensitivity analysis

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TU 115

Spatial differentiated impact assessment of chemicals in freshwater ecosystem at multiple scales

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Spatial differentiation is a topic of increasing interest within ecological risk assessment (ERA) and Life Cycle Impact Assessment (LCIA). A key issue to be addressed in the impact assessment methods, models and corresponding impact factors is the level of spatial detail required and uncertainties related to the use of generic impact factors when the exact location of the activities is unknown. For freshwater ecotoxicity evaluations were performed in order to assess the relative influence of scale in calculation of LCIA characterisation factors, compare them with the result of site specific risk assessment. In this work, we applied the spatial differentiated multimedia model MAPPE in order to highlight the differences between a site specific calculation and the result of box nonspatial model, such as USEtox. ERA results are calculated following a site-specific ecological risk assessment procedure that integrates in the assessment the spatial distribution of aquatic ecosystem. ERA results at different scale present higher variability: key drivers of variability and relevant differences in addressing spatial differentiation will be presented and discussed. Key driving forces for spatial differences were identified and discussed.

TU 116

Combination of SFA and multi-media fate modeling to assess the consequences of global trade and informal recycling of WEEE in China

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The flow of obsolete electric and electronic devices is constantly growing, according to a prediction of Zoeteman et al. (2010) by 3 to 5 % each year. It is suspected to add up to a volume of 20 to 50 million tons every year (Brigden et al. 2005). A large amount of waste electric and electronic equipment (WEEE) containing a variety of harmful substances such as PBDE and Pb is shipped overseas to China, India and Western Africa for recycling. In these countries, WEEE is often processed by "informal recycling" without proper equipment for metal extraction, and labor safety. Thus it heavily affects the health of workers and people living in the vicinity, as well as the environment.

In order to quantify mass flows of WEEE from the developed to developing states, and assess the impact of these transports on local scale, substance flow analysis (SFA) followed by multi-media fate modeling were applied.

In the frame of the SFA the following information was gained:

- Global transportation routes and mass flows of WEEE
- Data on the composition of typical e-wastes exports with regard to the European WEEE-Directive categories
- Chemical composition of the single categories with a special focus on lead and brominated flame retardants (PBDEs and TBBPA)
- Estimated emissions of Pb and BFR to the environment as a consequence of different informal recycling techniques.

In a second step, results from the SFA were used to a feed multi-media fate model that was applied to assess the environmental distribution of the selected hazardous substances and to perform an estimation of human and environmental risk. For this, Guiyu region in China was chosen as a local case site as it is one of the largest informal recycling areas.

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TU 117

Modelling the environmental fate and effect of organic bulk emissions from industrial effluents in LCA

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Several fate and effect models have been developed to assess the ecotoxic impact of individual chemical contaminants on aquatic freshwater organisms in Life Cycle Assessment (LCA). These methods suffer when it comes to the impact assessment of complex chemical mixtures, such as industrial effluents, as the list of constituent compounds is rarely known in its entirety, leading to under- or overestimation of ecotoxicological effects. A more holistic and meaningful method of evaluating the environmental impact of industrial effluents in LCA would be to model the fate and effect of comprehensive and commonly measured sum-parameters, such as total organic carbon (TOC). We present our methodology for establishing aquatic (freshwater) ecotoxicity characterisation factors (CF) for waterborne organic bulk emissions from different industries, based on whole effluent assessment (WEA) and the principles of mixture toxicity. We discuss the advantages of the methodology, its complexities, as well as the need for spatial differentiation, and we present some preliminary results.

TU 118

Globally applicable, spatially explicit assessment of non-toxic air pollutants regarding health impacts due to ozone

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1. Introduction

This poster presents intake factors (iF) and characterization factors (CF) regarding human health effects for the pollutants NMVOC, NO_x and SO₂ accounting for health impacts due to ozone. Research on life cycle impact assessment (LCIA) for transboundary pollutants have been reviewed and it is suggested that spatially differentiated fate modelling is crucial. The present work aims to fill the gap of consistent global modelling by developing an approach to derive globally applicable and spatially explicit values, and by providing a comparison between models with a different degree of spatial resolution.

2. Materials and Methods

Within the ongoing EU-funded project LC-IMPACT (2010-2012) the "Development and application of environmental Life Cycle Impact assessment Methods for improved sustainability characterisation of technologies" is pursued. Within the area of "Non-toxic pollutant impacts" the objective is to develop globally applicable, but spatially explicit, LCIA methods and characterization factors.

Within the TM5-FASST modelling framework, the world is divided into 56 regions. Each region serves as a source region and each grid cell (resolution 1°x1°) of the whole world serves as receptor region. Population data and concentration response functions are applied in order to calculate the iF and relevant diseases. Finally, the health impacts are weighted in order to get DALYs per unit of emission, and different external cost estimates, by including and excluding equity weighting.

3. Results

A detailed comparison will be made between iFs derived by the TM5-FASST model and by the EcoSenseWeb integrated assessment tool in order to assess the inherent uncertainties and implicit variability in the estimates. iFs and CFs are calculated for several countries and larger regions. Finally, iFs and CFs suggested in different LCIA methods have been compared with the findings of this work

Acknowledgements

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TU 119

Environmental impacts of thermal emission to freshwater: Spatially explicit fate and effect modelling

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Power production is a crucial sector causing heavy impact on freshwater bodies by releasing enormous amounts of heat in once-through cooling systems. So far only few approaches have addressed impact on aquatic environment related to this issue and there is no study providing fate and effect model for assessing the impacts on a spatial explicit level and broad coverage. We started our research based on a detailed study assessing impacts of thermal release of a specific nuclear power plant in Switzerland and extracted the most sensitive parameters to derive a generic model of combined fate and effect model for the US. We created a 0.5 arc degree grid accounting for water temperatures, river flow, river width, flow velocity and distance to sea in order to model fate and effect of thermal emissions from different sizes of power plants. Various data sources are tapped and different interpolation methods are applied to cover points without measured data, allowing accounting for uncertainties. We further investigated uncertainties of the characterization factors by varying input parameters and the model assumptions, including the assessment of the affected species. The thermal impacts show significant spatial variability depending on the water body where the cooling water is discharged and the amount of heat released, because the impacts are non-linear. Furthermore this project illustrates how fate and effect model can be integrated to improve the assessment of spatially differentiated impact assessment considerably.

TU 120

Including the influence of worldwide crop cultivation on ecosystem services in life cycle assessment

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Land use is increasing worldwide leading to changes in the ecosystem. Measuring these changes allows for quantification of the impact that each land use activity causes to the environment. This can be achieved through the quantification of changes in ecosystem services. Ecosystem services include carbon sequestration, nutrient cycling, erosion regulation and biotic production potential. The goal of this research is to develop indicators that estimate changes in ecosystem services due to changes in selected land use activities namely, cultivation of rapeseed, soybean and sugarcane, on a global scale. In Life Cycle Impact Assessment (LCIA) these indicators are known as characterization factors (CFs). For this research we focus on erosion regulation and carbon sequestration. The cultivation of the crops is simulated using the Environmental Policy Integrated Climate model (EPIC). EPIC is a spatial and temporal agri-environmental model which simulates the biophysical impacts on homogenous response units characterized by altitude, slope and soil class. Inputs for EPIC simulations include weather and soil profile data, and information on land use, land cover, and crop management. By simulating processes of crop growth, water and nutrient cycles, EPIC produces, among its outputs, estimates of crop yield, sediment transportation and soil carbon sequestration. The characterization factors will be aggregated to country or biome level. Comparisons will be made between results from different spatial aggregation levels, and different management systems regarding input of fertilizer or irrigation.

RA02P - Approaches for comparative hazard and risk assessment of chemicals

TU 121

Health risk assessment of chlorpyrifos with rice farmers in Vietnam

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Vietnam is an agricultural developing country with over 80 per cent of workers employed in agriculture, mainly rice farming cultivation. Farmers use back-pack reservoirs with hand pumps to apply pesticides, but their knowledge of safety in pesticide application is low. Chlorpyrifos is the most common organophosphate insecticide registered for agricultural use in Vietnam, but health risk assessment of chlorpyrifos use has not been carried out and limited investigation in Vietnam and other developing countries. The objective of this study is to evaluate the health risk of chlorpyrifos exposure to a typical group of rice farmers after application in Vietnam, using a probabilistic approach. Biological monitoring of rice farmers was used to estimate chlorpyrifos exposure from all pathways during application. Urine samples (24 h) were collected from farmers (18), from 1 day prior to application and over 5 days post-application, and then analysed for TCP, the main metabolite of chlorpyrifos, using HPLC-MS/MS. Urinary TCP levels were converted into an absorbed daily dose (ADD) of chlorpyrifos for each farmer. The health risk of chlorpyrifos exposure for the rice farmers was characterised by comparing exposure doses (ADD) with acute guideline doses for chlorpyrifos used by USA and Australian agencies.

Post-application chlorpyrifos ADD of farmers varied from 0.4-94.2 µg/kg/d, with a 50th percentile exposure level of 8 µg/kg/d which was about 80-fold higher than the 50th baseline exposure level (0.11 µg/kg/d) (see Figure 1). In comparison with acute guideline doses, the 50th value of post-application exposure among the rice farmers in Vietnam was over 2 times higher than the acute MRL of chlorpyrifos recommended by ATSDR (3 µg/kg/d) and 1.5 times higher than that recommended by US EPA (5 µg/kg/d), but slightly lower than the acute guideline recommended by Australian NRA (10 µg/kg/d). The 95th percentile for the ADD values of the farmers exceeded the acute dose guidelines by a factor of 10 or more.

Conclusion

This case study in Vietnam has shown that rice farmers are at a high risk of chlorpyrifos exposure and resultant adverse health effects, mostly neurotoxicity.

TU 122

A soil ingestion pilot study of a population following a traditional lifestyle typical of rural or wilderness areas

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The relatively few soil ingestion studies underpinning the recommended soil ingestion rates for contaminated site human health risk assessments (HHRAs) that have been conducted to date assessed soil ingestion in children living in urban or suburban areas of the United States, and to a lesser extent, Europe. However, the lifestyle of populations living in North American urban and suburban environments is expected to involve limited direct contact with soil. Conversely, many populations, such as indigenous and Aboriginal peoples residing in rural and wilderness areas of North America and worldwide, practice traditional land use, and participate in activities that increase the frequency of direct contact with soil. Qualitative soil exposure assessments to estimate ingestion rates for Aboriginal populations inhabiting wilderness areas suggest that high levels of soil ingestion (i.e., 400 mg d⁻¹ quantities) may occur, and these levels are many times greater than those recommended by regulatory agencies for use in HHRAs. Accordingly, a study of subjects selected from a wilderness community in Canada was conducted using mass balance tracer methods to estimate soil ingestion and the results compared with previous soil ingestion studies and regulatory guidelines for the soil ingestion rates used in HHRA of contaminated sites. A pilot study of 7 subjects belonging to the Xeni Gwet'in First Nation community living in the Nemah Valley of British Columbia was conducted over a 3-week period. The study provided the first quantitative soil ingestion estimates of an Aboriginal population following a traditional or subsistence lifestyle. The mean soil ingestion rates estimated in this study were higher than those currently recommended for HHRAs of adults, and higher than those obtained in most previous studies of adults. However, the soil ingestion rates measured were much lower than earlier qualitative assessments of subsistence lifestyles.

TU 123

Health risk assessment and economic damage due to environmental pollution in a large industrial centre

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A health assessment in Perm, an industrial city in European Russia, has been performed using the WHO methodology. The findings indicate spatially differentiated chronic inhalation exposure of 95% of the Perm citizens to various chemicals (formaldehyde, ethyl benzene, benzene, nitrogen dioxide, etc) at concentrations exceeding maximum permissible concentrations (MPC) (acute exposure) and maximum permissible average daily concentrations, by 16.7 and 13.5 fold, respectively. More than 65% of the exposed population use drinking water which does not meet the standards, i.e. trichloromethane, residual chlorine, iron, manganese and other impurities levels exceed MPC by 2.3 fold. We have observed an increase in acute and chronic hazard indices up to 30 fold for 14 out of the 17 analysed critical body systems and organs. Almost all of the city population is at an unacceptable environmental risk for respiratory, eyesight, hematological and immune disorders as well as central nervous system impairments. More than 173,000 Perm children (99.4%) and all of the 809,000 Perm adults are at an unacceptable carcinogenic risk. The main risk factor is inhalation exposure. The presence of the above mentioned risks has been proven by the identification of contaminants in the blood of the exposed subjects and the deterioration of laboratory, clinical and functional health parameters. We have revealed a true relationship between elevated blood levels of acetaldehyde, formaldehyde, manganese, nickel, chromium, etc. and blood, immune, hepatobiliary disorders and other health effects in the risk group children. We have determined reference levels of the chemical compounds in blood, i.e. acetaldehyde - 0.049 mg/dm³, formaldehyde - 0.059, benzene - 0.0013, manganese - 0.039, chromium - 0.021, nickel - 0.075 and chloroform - 0.0021 mg/dm³. We have identified NOAEL values in the air: Ni - 0.00009 mg/m³, Cr - 0.0001, Mn - 0.00005, formaldehyde - 0.003, acetaldehyde - 0.002, benzene - 0.03 mg/m³; chloroform reference dose in drinking water is 0.009 mg/(kg x day). We suggest that priority environmental factors (Ni, Cr, Mn, formaldehyde and acetaldehyde) cause 3,246 additional new cases of respiratory diseases. The total economic damage for respiratory diseases is € 1.3 million per year. Acceptable risk levels can be reached by reducing industrial emissions of 19 industrial companies, using traffic optimisation and clean fuel and improving the water supply system quality and water treatment.

TU 124

Snail watch: a tool to assess the risk of metal transfer taking into account their bioavailability

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Current evaluation of soil quality, generally based on physico-chemical characteristics, does not integrate the biological aspect and so the risk of metal transfer to organisms. The use of bioindicators for soil quality assessment allows evaluating the bioavailability of metals and then their risk of transfer.

The aims of this study are firstly to determine the internal concentrations of reference (CIRef) of metallic contaminants (Cd, Pb, As, Cr, Cu and Zn) in *Cantareus aspersus* snails. Then, CIRef are used to identify abnormal metal transfer to snails. Secondly, to estimate the soil characteristics influence on metal accumulation using multivariate equation. On these basis, our last objective is to establish procedure to Evaluate the Risk of Transfer of Metals (ERITIME) to snails to determine management priorities that take into account physico-chemical and biological parameters of metal bioavailability.

CIRef have been established on the basis of concentrations in the snails exposed on unpolluted sites for 28 days i.e. snail watch. In snails exposed on other sites, when the value of the median of metal concentrations is above the CIRef, the site will be considered as presenting abnormal risk of metal transfer. Most of the time, the use of these CIRef has allowed to identify the contaminated sites. They also reveal unexpected metal transfers on reference sites and a lack of transfer on contaminated sites. The use of multivariate equations has enabled to determine pH, organic carbon content and iron oxides as parameters influencing metal bioavailability to snails.

The ERITIME tool, based on CIRef, allows to highlight the risk of transfer of each metal to snails not based on total metal soil concentration but on a biological risk assessment. A hierarchy between sites can be established using the total accumulation quotient (TAQ: mean of AQ, with $AQ = [C_{snail}]/[CIRef]$). TAQ represents the global risk of metal transfer of a site. The ranking of the TAQ emphasize the transfer risk of industrial sites. On these sites, As, Cd and Pb transfers have been identified, putting these sites on top of the management priorities. A risk of Pb and As transfer on forest modalities that are slightly or not polluted by these metals was also evidenced, highlighting the need to use biological tools that take into account metal bioavailability influenced by soil characteristics for risk assessment procedures.

TU 125

Prioritising chemicals for EQS development

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Environmental Quality Standards (EQSs) are a key tool under the Water Framework Directive (WFD) to help assess the chemical status of water bodies. However it is important that EQSs are developed for substances that pose the greatest risk to the aquatic environment, and that emerging contaminants are adequately considered.

Various methods for prioritising chemicals have been described, incorporating both exposure and effects. Approaches that prioritise on the basis of risk often require significant inputs, even a draft EQS, or detailed exposure modelling. We describe a simple approach to prioritisation that requires only modest inputs and assigns chemicals to different risk categories.

Unlike more sophisticated schemes, this method does not require a draft EQS in order to prioritise chemicals. Rather, it categorises chemicals in terms of their likely (or actual) environmental occurrence based on use and monitoring data, and the hazard they pose to aquatic life, based on persistence, bioaccumulation and toxicity criteria. Classifications based on exposure and hazard are then combined and chemicals assigned to risk categories. Those assigned to the highest risk category are taken forward as potential candidates for EQS development.

Almost a third of the chemicals considered could not be assigned a risk ranking due to a lack of data. This is of particular concern for emerging contaminants where both hazard and exposure data are often limited. Lack of exposure or hazard data contributed equally to the assignment of 'Insufficient information'. Predictive tools such as QSARs or 'read across' techniques may have a useful role in filling some gaps in hazard data. Gaps in exposure data may be filled by gathering monitoring data; in this respect the tool has helped identify monitoring needs that may lead to identifying future EQS candidates.

The prioritisation methodology is not suitable for metals and other inorganic chemicals because of biases in the way that fate and behaviour are dealt with e.g. measures of degradation and bioaccumulation. A separate approach for such chemicals may need to be developed.

Finally, it is important to peer review the outputs of the scheme because this can help introduce relevant new data or information about significant trends in likely exposure (e.g. planned restrictions on use) that will affect the chemical's priority.

TU 126

Klimisch 2.0 - raising the bar to increase the scientific quality of environmental risk assessments

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Ecotoxicological data with high reliability and relevance are needed in different European legislations and regulations, e.g. REACH, EMEA or the Water Framework Directive, to guarantee the scientific quality of environmental risk assessments for chemical substances.

Criteria developed by Klimisch et al. (1997) are widely used to assess data quality. This assessment relies heavily on the completeness and quality of the data set and conclusions often require expert judgement, which is sensitive to scientific and cognitive variations. Assessments based on data with low reliability could lead to incorrect conclusions and may underestimate risks in the environment or lead to unnecessary risk mitigation measures being introduced.

Several recent studies (e.g. Duchemin et al. 2010, Durou et al. 2011, Junghans et al. 2011a,b, Ågerstrand et al. 2011a) have shown that the Klimisch system is not accurate and prescriptive enough to guarantee consistency between different risk assessors. This presents a general problem in the data quality evaluation of all chemicals, and is not limited to specific substance groups.

We therefore recommend to amend the Klimisch system by adding a scoring system for the criteria for reliability and relevance of the data, to ensure a more consistent evaluation between assessors. The detailed methods developed by Kuester et al. (2009, 2010) and Ågerstrand et al. (2011b) provide a useful basis for refining the quality assessment criteria and could be adapted for all substance groups. Additionally, the assessment should also consider if a critical study is plausible in the context to the whole dataset. To test plausibility, we propose that critical information distributed across different studies should be evaluated with a weight of evidence assessment, e.g. Gross et al. (2011).

In conclusion, we propose a more structured reliability and relevance evaluation of critical ecotoxicological studies together with a plausibility assessment based on a weight of evidence approach for the whole dataset. This should increase the scientific quality of environmental risk assessments of substances, e.g. in Environmental Quality Standard (EQS) derivation according to the Technical Guidance Document for EQS (European Commission 2011).

The intention is to provide guidance for different interests from science, hazard assessment and regulation. However, we recognize that the weighting assigned to criteria might vary between different chemical assessment regulations.

TU 127

EQS derivation for metals in EU and USA: a comparison using copper

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Standards and targets are derived to help us take action to protect and improve water quality. However, environmental agencies throughout the world have or will develop frameworks that may differ substantially therefore resulting in different standards for the same pollutant. This presentation aims at providing an overview of the frameworks developed in the EU and USA for the derivation of EQS for metals. Central focus will be on differences in approach used for the development, validation and implementation of the Biotic Ligand Models (BLM). Indeed, in the USA acute BLMs are proposed for 2 different trophic levels (invertebrates and fish), while in the EU chronic BLMs are used for 3 different trophic levels (algae, invertebrates and fish). Once normalized for bioavailability safe threshold concentrations are calculated as the lower 5th percentile of the whole species sensitivity distribution SSD in the EU while only the 4 most sensitive genus mean values are used to derive the 5th percentile using least square regression analysis. The influence of such differences in both frameworks on the EQS setting will be illustrated using Cu as an example. EQS for different surface waters (with different bioavailability) will be demonstrated. In most cases, it seems that the differences in EQS values between both frameworks are within a factor of 2-3. Therefore, although there is a general tendency to incorporate such concepts in regulatory frameworks for metals there is a clear need for harmonization of frameworks as highlighted by the OECD.

TU 128

Efficient toxicity testing when test information is uncertain: a stochastic cost-effectiveness analysis

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Changing policy settings for chemicals' risk management, for example in Europe and in the U.S., have led to increasing awareness of the urgent need for more efficient testing of chemicals. The economic concept of efficiency calls for allocating scarce resources (e.g. money, time, human skills) such that output is maximised. Hence, if for a toxicological endpoint different testing options exist, a decision-maker must identify the testing strategy that provides the highest information gain per unit of cost. This, clearly, requires to weigh the costs associated with a testing strategy, usually measured in monetary terms, against the information gains from testing. The latter are commonly expressed in terms of the a test's predictive performance. Cost-effectiveness analysis (CEA) provides a powerful tool for solving this problem. CEA allows for a comparative evaluation and for identifying trade-offs between costs and information gains for different testing methods and strategies. While CEA has become widely used in many domains, for example in medical decision-making, there are only few attempts to apply CEA to testing in toxicology. These studies share the assumption that information gains from testing, measured in terms of a test's predictive performance, are known. The predictive performance of testing methods is, however, uncertain, irrespective of what type of testing method (e.g. a "gold standard" in vivo test, an in vitro test, or a "non-testing" method) is used. Our study, therefore, moves beyond existing deterministic CEA modelused in toxicology by developing a stochastic approach to CEA that accounts for the uncertainty about the quality of test information. The aim is to allow for a more robust and transparent evaluation of testing methods and strategies both within and across endpoints. This guides decision-makers in coherently selecting the testing method or strategy that provides the highest information gains per unit of cost. Another contribution of the study is to offer a critical synopsis of data and research needs in order to apply our approach to various endpoints and to make it operational in the regulatory context of REACH.

TU 129

IFRA Environmental Standards: risk and hazard assessment update for 2012

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The International Fragrance Association expanded the fragrance industry's self-regulatory safety program with the development of IFRA Environmental Standards for both risk and hazard in 2008. Fragrance material risk assessments for these Standards are incorporated in the Research Institute for Fragrance Materials' (RIFM) testing program in coordination with its Expert Panel. To identify materials for risk assessment refinement, fragrance materials were screened using the RIFM Environmental framework and 2008 IFRA volume of use survey as reported for both Europe and North America. The Framework for this evaluation was published in Environmental Toxicology and Chemistry (Salvito et al., 2002, 1301-1308). In addition, hazard assessment on these materials was also performed and reviewed. As a result nearly 3,000 materials were screened with preliminary risk quotients estimated to rank priority materials for risk assessment refinement. In an effort to provide greater transparency to the developing IFRA Environmental Standards, reported here are the results of these additional tests (for both risk and hazard assessments). These studies include persistence testing (ready biodegradation tests and die-away studies), bioaccumulation, and acute and chronic aquatic toxicity. Incorporating these new data in a second tier risk and hazard assessment for these materials will also be presented. IFRA and RIFM have decided to present an annual update of this test program and the IFRA Environmental Standards. This work represents the latest effort for 2011-2012.

TU 130

The HEROIC project: coordinated efforts towards the harmonization and cross-fertilization of human and environmental risk assessment of chemical substances

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Today, human risk assessment (HRA) and environmental risk assessment (ERA) are typically separated. There is a lack of mutual understanding between experts and data from toxicological and ecotoxicological studies are not readily accessible by risk assessors of the two disciplines. The need for RA will continue to increase (e.g. REACH or toxicity of mixtures) along with budget restrictions and political and public pressure to reduce the number of animal tests. Therefore more cost effective, predictive and rapid tests for high quality sustainable RA are needed, including a better exploitation of existing data.

The HEROIC project - a coordination action of the 7th FP - will provide a platform for networking among all the relevant stakeholders in the RA value chain and will provide them with the most relevant background information to contribute to the development of harmonised approaches which meet the challenges of RA. The project will enable the improvement and harmonisation of tools and methods in RA, by exploring how data generated in ecotoxicology and human toxicology can be applied across disciplines for integrated RA, and develop a framework for integrated methodologies and approaches for RA. This will increase transparency in RA and allow better risk communication to maintain public trust and to give unambiguous guidance for improved risk management.

HEROIC starts with a comprehensive landscaping exercise to identify common methodological and data needs in current human and environmental risk assessment practices. We will then evaluate existing in-vivo, in-vitro and in-silico methods for hazard and exposure assessment. The selection process ranks and weights data based on their reliability and relevance and uses a Weight-of-Evidence approach to integrate such information to develop an Integrated Testing Strategy (ITS) for decision making. A dedicated web portal called 'Tox-Hub' that presents information from diverse sources and that functions as a central point of access to the most relevant toxicological and ecotoxicological information will be created. A diverse range of dedicated activities is planned for information, dissemination, capacity building and communication.

These coordinating activities will result in enhanced sharing of knowledge, building consensus and development of clear, easily understood, transparent and unambiguous integrated RA procedures.

RA03P - Are Environmental Specimen Banks ready to face future challenges of environmental chemistry and regulatory toxicology?

TU 133

Fish from the German environmental specimen bank - patterns, relations and time trends of PCDD/F and dl-PCB in breams (*abramis brama*) from German rivers

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Environmental specimen banks (ESBs) are collections of samples which contribute widely to the understanding of connections within regional as well as global ecosystem behaviours. Furthermore they deliver valuable understandings not only of retrospective time lines but also of similarities and differences within the collected information as well as giving the opportunity for discussing trends.

The German ESB is one of the largest specimen collection programmes worldwide, monitoring the persistent and highly toxic polychlorinated dibenzo-p-dioxins and -furans (PCDD/F) and dioxin-like PCB (dl-PCB) in aquatic ecosystems in order to evaluate the overall state of environmental protection within the tributary system of the main rivers in Germany. Serving this purpose, the common bream, *abramis brama*, has been selected as a specimen for this monitoring. It is common, widespread, trophically reasonably high and easy to sample and also forming a link towards human consumption.

We present actual time trends and levels for PCDD/F and dl-PCB in breams from seven major rivers in Germany between 2003 and 2008. Levels are notably going down for PCDD/F concentrations but for dl-PCB there is a non-uniform overall picture showing rather a baseline than a clear decrease at fairly high levels. Generally, for both parameter groups concentrations are at a level which is of long-term concern for environmental safety as well as human health perspective, e.g. compared against actual maximum levels from EU feed/food legislation. This is especially true for the lower courses of the big streams Rhine and Elbe.

Furthermore, the single compound data for the analyte groups give additional understanding of pattern of PCDD/F and dl-PCB as well as their correlation. The presented data show for example the differences of ratios between those two groups as being fairly unique for the sampled river system. Discussion could point as well towards different or similar industrial influences of the different tributary system as towards unification of pattern, e.g. by metabolic influences.

TU 134

A 10 yr retrospective study of mussel condition in North Iberian Peninsula based on the 'mussel shell section' of the Biscay Bay Environmental Biospecimen Bank

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An environmental specimen bank (ESB) is an archive for biological samples that can be used to perform (traditionally chemical) analyses to obtain information about long-term variability and temporal trends in the chemistry of the biota and the ecosystem quality. Aware that chemical endpoints alone are not enough for ecosystem health assessment, the Biscay Bay Environmental Biospecimen Bank (BBEBB) was designed including specimens for biological endpoints, say biopsies for histopathology/ immunochemistry, cryo-specimens for molecular/cellular analysis, and dried mineralized tissues (shells/otoliths) for biometric determinations. Banking methods and specimen sampling and processing are being adapted for these needs. In a first practical application, a retrospective study was performed using the 'Mussel Shell Section' of the BBEBB (2001-2010; 22 localities covering the area of geographical distribution of *Mytilus galloprovincialis* in the North Iberian coast). Changes in shell size (length, L; width, W; height, H), allometry and shell weight (SW) were analyzed, and the shell thickness was estimated after regression of the log SW against SSF (Shell Size Factor, $L \times W \times H$ in mm). Overall, shell growth exhibited a decreasing trend during the study period with a seemingly more marked arrest growth in 2003-2006. These results are discussed in terms of interactions between the global environment trends and the consequences of the Prestige oil spill. However, since only specimens of $L=3.5-4.5$ cm were analyzed in most cases and mussels of the same shell length can belong to different age classes depending on the geographical variability and the influence of environmental conditions for a given locality, the incidence of the age in the interpretation of the obtained results is also discussed. In summary, the development of Mussel Shell Sections in environmental biospecimen banks is highly recommended as they may offer an excellent opportunity for retrospective monitoring after low cost processing and storage. In addition, our results also suggest that mussel watch monitoring programs and ESB archives should be designed on the basis of samples of same-age rather same-length mussels. This study was supported by the Government of the Basque Country through K-EGOKITZEN project.

TU 135

Using banked seabird eggs for determining geographic patterns of trace elements in marine regions

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Selenium and arsenic occur naturally in the environment but they are also known to be toxic in excess of trace levels. During the 1990s, elevated concentrations of arsenic were reported in liver tissue of ringed seals taken by Alaska Native hunters in Norton Sound, Alaska. To determine whether eggs of colonial seabirds might be good monitoring matrices for both arsenic and selenium in marine regions of Alaska, total arsenic (As) and selenium (Se) mass fractions were measured in 78 seabird egg samples collected in 2008 and banked at the US Marine Environmental Specimen Bank (Marine ESB). These eggs were collected from several colonies located within Norton Sound as well as long-term monitoring sites in two other major Alaska marine regions, St. Lazaria Island in the Gulf of Alaska and St. George Island in the Bering Sea and were from three species: common murre (*Uria aalge*), thick-billed murre (*U. lomvia*) and glaucous gulls (*Larus hyperboreus*). Egg samples were analyzed using collision cell kinetic energy discrimination inductively coupled plasma mass spectrometry (ICP-MS). Total arsenic mass fractions ranged from 0.015 µg/kg to 0.320 µg/kg while the levels of Se ranged from 0.411 µg/kg to 1.017 µg/kg. As and Se levels were higher in most of the samples collected from the Norton Sound colonies compared to other colonies. Norton Sound is located in a highly mineralized region of Alaska and is an area of historical gold-mining that continues today and could be a contributing factor to the bioaccumulation of arsenic and selenium in this region. In addition, As mass fractions were also measured in 43 common murre and glaucous gull eggs collected in 1999, 2000, and 2005 from colonies located throughout the Bering Sea, including 4 of the same colonies as those collected in 2008. The As mass fractions ranged from 0.060 µg/kg to 0.349 µg/kg and were also more elevated in the Norton Sound colonies than other colonies in the Bering Sea. The data reported here illustrates that seabird eggs can be a useful monitoring tool to help determine geographic patterns of trace elements in marine regions, specifically the sub-Arctic regions of Alaska.

TU 136

Specimen security in long term specimen banking - zebra mussel example in the German ESB

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In the late eighteenth century, the zebra mussel (*Dreissena polymorpha*) established itself as an invasive species - sometimes in huge numbers - in large parts of Europe and North America. These new settlers provided an excellent opportunity for environmental observers to use an effective plankton filter to indicate bioavailable and bio-accumulative anthropogenic contamination in many areas. The successful invasion of the zebra mussel can be seen as the trigger for a multitude of "mussel watch" programmes in limnic waters of Eurasia and North America, and has also led to the zebra mussel's permanent presence in the German Environmental Specimen Bank (ESB) for the last 20 years.

In the past few years many areas have seen a dramatic drop in zebra mussel numbers. Now, out of nowhere, what was once a successful model for monitoring waters is endangered of not producing a sufficient number of specimens for large samples. A possible cause is the receding eutrophication level of the waters and the resulting lack of food. Another invasive species, the quagga mussel (*Dreissena bugensis*), which has a stronger presence in many areas, is increasing the pressure on the zebra mussel populations too. Recent research has also shown that fertile hybrids between *D. polymorpha* and *D. bugensis* are possible in the wild - making biomonitoring even more difficult. Due to different filtration rates, it must be assumed that accumulation in both species is also different, which is why *D. bugensis* and hybrids of both species have to be excluded from the results of monitoring studies using *D. polymorpha* for them to be comparable.

Investigations into the current significance of the zebra mussel in long-term monitoring projects and environmental specimen banking programmes in the German ESB show the specific information the zebra mussel provides. This makes clear that, alongside the common bream (*Abramis brama*) and suspended particulate matter, it is an important component of the limnic sample set. Loss of this sample type would result in a huge loss of information.

Therefore, the most important task concerning *D. polymorpha* as a biomonitoring tool is to:

- find out the differences in how substances accumulate in *D. polymorpha* and *D. bugensis*;
- discover whether there are wild hybrids; and
- develop a method to differentiate unequivocally between both species for large samples.

TU 137

Organic compounds in suspended particulate matter - results from the German environmental specimen bank

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Since 2005 suspended particulate matter (SPM) in the river systems of Rhine and Elbe has been sampled, processed and archived in the frame of the German Environmental Specimen Bank (ESB). Sampling is carried out with sedimentation boxes which are emptied monthly. At the end of the year monthly samples are lyophilized and pooled to a homogenate. From every homogenate about 200 subsamples are produced and stored in the ESB. The whole processing is conducted under deep frozen conditions. Some subsamples are chemical characterized for inorganic substances, CHCs and PAHs.

The results of the five years period 2005 -2009 show considerable differences between the two river systems. The concentrations of PAH16 as well as of PCB6 and DDX increase downstream the river Rhine whereas in the Elbe system only few differences of concentration are observed between the single sampling sites. One exception is the sampling site Elbe/Blankenese near Hamburg harbor which represents the passage of the Elbe into the North Sea and is tidal influenced. Here, lowest concentrations for all substances are observed. For the most substances the concentrations at Blankenese are less than 20 % of the concentrations at the other samplings sites. This is probably caused by dilution of the Elbe water with salt water of the North Sea.

With exception of Elbe/Blankenese concentrations for most substances are significantly higher in the river Elbe compared to the river Rhine. Especially the concentrations of DDX and HCHs are much higher in SPM from the Elbe than in SPM from the Rhine.

Within the observation period significant reductions of B[a]A, B[e]P and B[a]P concentrations in the river Rhine could be observed.

TU 138

Standardized freshwater mussel watch for monitoring of aquatic environments: implications and possibilities

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Mussel Watch is one of the most effective approaches to study the dynamics of aquatic pollution. Unfortunately, it utilization is usually limited to the depending on collection of wild mussels. Based on our pilot studies, we recommend to establish an innovative "standardized Freshwater Mussel Watch" system. Namely, a population of "standardized" biological indicator mussel (e.g., *Anodonta woodiana*) is first established by artificial propagation techniques, which possesses the similar biometric characterization, genetic quality, and low background of environmental pollution. Subsequently, the standardized mussels with the same size will be transplanted to some of typical fishery waters (e.g., Taihu Lake) as the 'experimental groups', and be continually reared in the artificial propagation pond as the 'control groups', respectively. Consequently, a comparative assessment on temporal and spatial dynamics of pollution and bioaccumulation of persistent toxic substances (e.g., heavy metals, organic pollutants, natural toxicants) can be investigated with system error at a much smaller level and without destroying the wild mussel population. At the same time, a living individual pool of glochidia, juvenile, and adults can be established for exposure and toxicological studies, in order to assure the reliability of the field works and understand the corresponding impact degree, tolerance mechanism, and stress threshold. The present study will make it possible to setup the world's first 'Freshwater Mussel Watch' system, the standardized living individual pool, and environmental specimen bank of *Anodonta woodiana* for special use on conservation and investigation of freshwater fishery environments.

RA06P - Contaminated sediments in a changing environment

TU 140

The impact of high water discharges on sediment quality in the Elbe estuary

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Contamination of the Elbe estuary with particle bound compounds mostly (with the exception of TBT which had its source in the Hamburg Harbour) derives from “areas of risk” upstream of Hamburg such as the Czech Republic for organic contaminants like HCH, HCB, PCB, DDX and TBT, the tributary Mulde for As, Dioxins and HCH, and the Saale for a number of heavy metals like Cd, Hg, Cu, Zn, and Pb. The major transport of contaminants happens during periods of high water discharge, when historic contaminated soil or sediment in the catchment is resuspended during flood events and transported downstream. For some compounds, the yearly load of particle bound contaminants is transported during a few days of a high water discharge (Heise, Krüger et al. 2008). Up to now, the impact of contaminants on the quality of sediments in the estuary as a consequence of a high discharge event, however, has not been very well investigated.

The Elbe estuary is characterized by the Hamburg Port, the second largest port in Hamburg, about 100 km upstream of the mouth of the river. Even though the river downstream of Hamburg has been maintained as a deep water navigation channel, 94 % of the area is ecologically protected by FFH and Birds directive. Some of the ecologically valuable freshwater wadden areas are close to Hamburg, and at the mouth of the Elbe the river widens to a large marine wadden area which is a feeding and nesting site of many bird and fish species.

In order to assess the impact of a high water discharge situation on the sediment quality of ecologically important wadden areas, freshly deposited sediment (FDS) was sampled during normal and high water discharges at two freshwater mudflats close to Hamburg and at two places near the mouth of the Elbe close to the North Sea. Chemical and ecotoxicological analyses were carried out on the sampled material. Elevated discharges did not lead to any significant increase of contamination of FSD in either sampling site. An extreme high water event in January 2011, however, led to an increase in contamination in freshwater as well as coastal mud flats. Especially high was the impact on the more upstream “Heuckenlock” which is of particular ecological importance. Ecotoxicologically, little effect could be measured and it needs to be clarified how bioavailable the historic contaminants still are after their transport downstream the Elbe River.

TU 141

Toxicity of suspended matter and sediments in relation to contaminant load and expected effects of climate change on European rivers

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Climate change may not only affect river water discharges in terms of water quantity, but also in terms of water quality. During flooding periods, for instance, higher water discharges may cause a dilution of normal pollution levels, while on the other hand, higher discharges may also result in an increased mobilization of polluted sediments and in surface run-off from possibly polluted flood plains and inundated urban areas. During high water episodes huge increases of contaminant fluxes may be recorded. Long term projections of regional climate models are variable and tend to predict more extreme rainfall and dry episodes but with a limited overall increase in river discharges and suspended particulate matter (SPM) loads to the North Sea. To study the possible impact of climate change on the quality of urban and coastal waters, a battery of *in vitro* bioassays was applied to determine the toxicity profiles of environmental samples collected during climate change related events. SPM and sediment samples were collected in rivers from Norway, Sweden, Denmark, Germany, and The Netherlands during periods of dryness, flooding and extreme run-off. In addition, passive sampling was performed using silicone rubber sheets to evaluate the effects on dissolved contaminants. Extracts from SPM and passive samples were tested for respiratory toxicity (Microtox), dioxin-like activities, (anti) androgenic, (anti) estrogenic, and thyroid hormone-like activities, and for mutagenicity. Variable patterns of both increased or decreased toxicity in relation to discharge conditions were noted between rivers and different years within rivers. Marked increases of toxicity were noted for episodes of melting of ‘black snow’ (Åkerselva river, Oslo) and overflow of mixed sewer systems (Harrestrup river, Copenhagen). Contaminant concentrations of SPM and sediment were classified based on a toxic-unit approach and will be compared to the *in-vitro* toxicity profiles. Results of multivariate statistical analysis (PCA, cluster analysis) and different toxicity ranking and classification approaches will be presented. The results will be included in a web-based communication tool (Open Earth) open to the general public. The work described was performed within the context of the DiPol project (Impact of Climate Change on the quality of urban and coastal waters - Diffuse Pollution), which is funded by the Interreg IVB North Sea Region Programme.

TU 142

The effects of CO₂ leakages from marine stable geological formations on the mobility and availability of metals

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Carbon dioxide capture and storage (CCS) in marine sub-seabed geological formations, is already contemplated in international conventions as a mitigation measure to reduce the concentration of CO₂ in the atmosphere. Although this technology is currently considered to be technically feasible there is lack of information on the environmental impacts of accidental leakage. CO₂ Seepage is likely to create a considerable pH decrease in the sediment, sediment pore water, sediment-water interface and finally in the water column. Increase in acidity will lead to several biogeochemical alterations that can result in detrimental effects in the ecosystem. Changes in the solubility, speciation, mobility and bioavailability of trace metals is expected as a direct result of pH decrease

Laboratory-scale experiments were performed, involving direct release of carbon dioxide into sediment, inside non-pressurized chambers, in order to provide data on the possible effects of CO₂ leakage from geological storage sites on the fate of several metals. Marine sediments from two contaminated sites located in the south of Spain were collected and submitted to acidification by means of CO₂ injection. The test lasted 10 days and samples were collected on the beginning and end of the experiment for metal analysis. The results revealed that mobility of metals from sediment to water column depended on the site, metal and time. Metals concentrations generally increased with time of exposure to CO₂ injection. Within the metals tested zinc, copper, cobalt, lead and iron were most greatly influenced by acidification.

TU 143

Changes on the biogeochemistry of synthetic surfactants from river to marine sediments

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Aquatic systems are subjected to the influence of both urban and industrial wastewater discharges, which are among the main sources of organic contaminants. Due to their extensive use in a wide variety of applications, significantly high concentrations of surfactants can be found in wastewater. Once they reach water bodies, a significant fraction of these compounds can be detected in sediments due to their moderate sorption capacity. It is often assumed that surfactants are well preserved in the sedimentary column once anoxic depths are reached, which can happen within a few millimetres to centimetres depending on the aquatic system. In this work we have studied the diagenetic fate of the most commonly used surfactants: alcohol polyethoxylates (AEOs) and nonylphenol polyethoxylates (NPEOs), both non-ionic, and linear alkylbenzene sulfonates (LAS) and alkyl ethoxysulfates (AES), both anionics. Their concentrations in sediment and pore water were determined along the sedimentary column by sampling cores in three different aquatic systems from SW Spain: the salt-marsh environment of the Bay of Cadiz, the middle stretch and the estuary of the Guadalete River, and the Bornos water reservoir. Different distributions were observed according to the respective uses, production volumes and physicochemical properties of each surfactant. Levels of non-ionics (up to 12 mg/kg) were twice as high as to those for anionics near industrial areas and ports, whereas the opposite was found near urban wastewater discharge outlets (up to 18 mg/kg). The most remarkable result was that sulfophenyl carboxylic acids (SPC), LAS degradation products, were identified in anoxic pore water. However, this was observed only in marine, and, to a minor extension, estuarine sediments. No SPC were detected in sediments from freshwater systems. The presence of sulfate-reducing bacteria in marine sediments (or absence in freshwater environments) seems to be strongly related to the degradation process. On the other hand, no changes in the average length of AEO and NPEO ethoxylated chains were observed along sediment cores from any type of aquatic system, suggesting that their biodegradation is very limited in anoxic conditions. This may be directly related to their lower bioavailability too, as their calculated sediment - pore water distribution coefficients showed much higher values when compared to those for most polar anionic surfactants (e.g., 553 ± 98 for NPEOs vs. 11 ± 7 for AES).

TU 144

Historical trend and pollution assessment for selected trace elements in sediment cores from the Orbetello lagoon (Tuscany, Italy)

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Heavy metal enrichment in sediment may be due to both natural and anthropogenic contribution, which may be recognized by studying the concentration profiles along depth in sediment cores. A study on the different contribution due to heavy metal in the sediment was carried out in the Orbetello lagoon (Tuscany, Italy). The lagoon is located within a volcanic district, characterized by hydrothermal ore deposits and natural positive geochemical anomalies for some heavy metals may occur. Consequently, the correct interpretation of natural and anthropogenic enrichment is a main issue for a correct environmental impact assessment. In this work, selected trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb, and Zn) were analysed in two dated sediment cores (OR96 and OR98), about 2 meters long, and the non-dimensional Enrichment Factor (EF) was calculated for each element using local background concentrations. The determination of Cd, Cu, and Zn concentrations demonstrated in both the cores an upper anthropogenic enrichment referable to the last half of the 20th century, while Pb contamination was found to begin earlier, during the first half of the last century. In spite of considerable EFs showed by these elements (Pb and Cd EFs up to 6-7) such contamination do not determine environmental concern, because concentrations exceed in few cases the limits adopted by international sediment quality guidelines. As regards Hg concentration, a recent contamination, referable to the last few decades is clearly distinguishable only in one core. Conversely, a marked Hg positive geochemical anomaly dated around 1850 present in both the cores was considered as a result of specific anthropogenic activity related to the extensive mining activity and metallurgical processes in the region, which started in the mid-nineteenth century. On the contrary, the significant enrichment recorded for As in lower levels of core OR96 were interpreted as the results of increased contribution from the Albegna River catchment, where high As content is present in ore deposits of Hg and Sb. Among the investigated heavy metals only Hg, may be considered element of concern, because the measured concentrations exceed, with few exception, the reference values of sediment guidelines. For this reason, indication to investigate potential toxicity through studies on mercury bioavailability and ecotoxicological response arises from the present research.

TU 145

Rapid contact kinetic assay with *Vibrio fischeri* in the assessment of small streams sediments in the Czech Republic

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Small streams form an essential part of aquatic networks in all river basins but they are negatively affected by various anthropogenic pollutants. The monitoring of water quality is commonly based on chemical analyses of selected priority contaminants but water and sediments in small streams contain complex mixtures of different chemical classes, which may cause various toxic effects to aquatic biota. Biological assays thus serve as a complementary tool in the effect-based monitoring of water or sediment quality. In the present study, we investigated 25 localities from two different regions in the Czech Republic. Sediment samples from each locality were repeatedly collected for two years with the main goal to compare outcomes of chemical and biological/ecotoxicological analyses. Chemical analyses assessed levels of toxic metals, PAHs and POPs (PCBs, OCPs), and sediment toxicity was determined using a kinetic assay (Flash Test) with the luminescence bacterium *Vibrio fischeri*. We have observed significant differences between both studied regions in the Czech Republic as well as between different sampling periods. Statistical analyses repeatedly revealed relatively poor correlations between chemical contamination data and toxicity, while total organic carbon (TOC) appeared to be related to observed toxic effects. The present study shows successful application of the kinetic *V. fischeri* test in the effect-based monitoring of sediments demonstrating thus complementarity of both chemical and ecotoxicological data.

TU 146

Influence of feeding type and behaviour on whole sediment toxicity tests with the oligochaete *Lumbriculus variegatus*

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Lumbriculus variegatus were exposed for 28 days to 0, 180, 560 and 1800 µg Zn g⁻¹ sediment and received 2 feeding regimes. The partitioning of Zn between sediment, pore water and overlying water was enhanced by a 2-year equilibration period. Feeding regimes used in the toxicity tests were (i) food (Urtica+cellulose) spiked into the sediment with no additional feeding during the exposure (SED+) and (ii) daily feeding with TetraMin (TetraMin). This 4x2 design experiment was set up to test the hypothesis that feeding characteristics can affect the performance, response to toxicants and internal toxicant distribution of *L. variegatus*.

No adverse effects were noted in the TetraMin treatment, while in the SED+ treatment the total number of worms and biomass were significantly reduced at 560 µg g⁻¹ and 180 µg g⁻¹, respectively. For both feeding regimes, Zn body burdens were significantly increased only at 1800 µg g⁻¹ (SEMZn-AVS>0). Overall, the results show that despite similarity in Zn bioaccumulation between two feeding regimes, biological responses were only noted in worms fed via the sediment. The lack of toxicity in the TetraMin treatment was attributed to selective feeding on TetraMin and to daily feeding regime, which promoted the organisms to spend less time in the sediment thus reducing Zn exposure. Significant Zn bioaccumulation not accompanied with biological effects at 1800 µg g⁻¹ suggests internal detoxification or other regulation mechanisms. In the SED+ treatment, significant biological effects not accompanied with Zn bioaccumulation were observed at 180 and 560 µg g⁻¹. Feeding inhibition resulting from an avoidance reaction to contaminated food/sediment or different mechanisms of toxicity due to dietary Zn, were assumed to be the causes. The internal Zn distributions were analysed using synchrotron radiation based confocal micro X-ray fluorescence (XRF). The results show that in worms fed via the sediment, Zn is mainly associated with epidermis and less with the gut walls. It is, however, equally present in gut wall and epidermis in worms fed TetraMin. This supports the assumption of feeding inhibition due to avoidance behaviour toward contaminated sediment/food. The XRF data also show that at 1800 µg g⁻¹, Zn was mainly distributed in the epidermis and sub-epidermis of *L. variegatus*, confirming the role of dissolved Zn as the predominant exposure route when SEMZn-AVS>0.

TU 147

Toxicity of organic micropollutants in the Yangtze Three Gorges Reservoir - MICROTOX

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The creation of the Three Gorges Reservoir caused the flooding of former urban, industrial and agricultural areas. Consequently, substantial amounts of organic and inorganic pollutants were released into the reservoir. Beyond contaminants and nutrients enter the reservoir by runoff from adjacent agricultural areas as well as from sewage of industry, aquacultures and households. Periodical changes in water level cause flooding events and thereby a relocation of contaminated water, particulate matter and sediment onto agriculturally used areas along the reservoirs shore.

In order to assess (i) possible links between molecular/biochemical responses and ecologically relevant effects, and (ii) if ecotoxicological effects might be related to adverse effects in fish from the field, sediment samples and fish were collected at selected locations and analyzed using a weight-of-evidence (WOE) approach with several lines of evidence. The objective of the poster presentation is to introduce the conceptual framework and to present first results of the ongoing study.

As previously addressed by Chapman & Hollert (2006) a variety of lines of evidence can be used in WOE studies. Briefly, (i) a comprehensive battery of acute (neutral red assay, *Arthrobacter*, fish embryo toxicity test and sediment contact assay with *Danio rerio*) and mechanism-specific bioassays (micronucleus assay and Ames test, EROD and YES assays) is applied to characterize the ecotoxicological hazard potential. (ii) Biochemical investigations and the micronucleus assay with erythrocytes will be applied, representing *in situ* parameters as well as (iii) persistent organic pollutants (PCB, PAH and PCDD/Fs), endocrine disrupting substances, limnochemical parameters and the concentration of heavy metals will be analyzed. To identify organic contaminants causing effects in sediment toxicity assays, (iv) effect directed analysis (EDA) will be applied. So far sediment and fish samples have been taken at major cities as well as feeder rivers and their estuaries along the Yangtze Three Gorges Reservoir in order to characterize the state of contamination and the potential risk for human and wildlife. The monitoring strategy should help to observe the water body's quality and to serve as a basis to initiate if necessary counteractive measures.

Reference

Chapman PM, Hollert H (2006) Should the sediment quality triad become a tetrad, a pentad, or possibly even a hexad? JSS - Journal of Soils and Sediments 6: 4-8

TU 149

Toxicity of persistent organic pollutants from Baltic Sea sediments in the fish embryo test and the EROD assay

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The Baltic Sea, one of the largest brackish water bodies in the world, is considered to be an exceptionally sensitive and endangered marine ecosystem. The average residence time of Baltic Sea water ranges between 25 and 50 years, thus facilitating the accumulation of pollutants in sediment. For a deeper insight into the ecotoxicological effects of pollutants in Baltic Sea sediments, biological test systems were applied with exhaustive as well as mild sediment extracts. Extracts from PLE and mild methanol/water extraction, were investigated for their dioxin-like activity and their embryotoxic impact on the development of zebrafish (*Danio rerio*) embryos. PLE extracts were considered to represent the entire toxic potential and the mild methanol/water extracts to contain rather bioavailable pollutants. Applied biotests were the fish embryo test in 96-well plates and the EROD assay with RTL W1 cells. Results from the fish embryo test revealed no effects caused by methanol/water extracts in contrast to the PLE extracts, which gave strong embryotoxic potential. Results on the EROD inducing potential indicated dioxin-like activity for all investigated sampling sites. PLE extracts showed clearly higher dioxin-like activity than mild methanol/water extracts. The study revealed an ecotoxicological burden for all Baltic Sea sediments. Especially the Eastern Gotland Basin, the Gulf of Finland and the Arkona Basin appear highly polluted, but according to the methanol/water extracts, this pollution is not readily available for developing fish embryos. However, the ability of the mild extracts to resemble the bioavailable fraction might be limited. For a comprehensive ecotoxicological assessment it is necessary to thoroughly investigate the bioavailability and to identify the causative pollutants by chemical analyses.

TU 150

Identification of hazard factors and ecotoxicological risks of sediments from the Tietê River Basin (Brazil)

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The Tietê River, selected in this study as an example for a highly contaminated river system, is located in the most important economical center in Brazil, São Paulo state. The reservoirs constructed along its course are widely used for providing drinking water, as water sources for agricultural irrigation and as recreation sites. Several approaches involving different test organisms, several endpoints and different sediment phases (liquid as extract and solid as freeze-dried samples) were followed under laboratory conditions. Results document that sediment samples from various sites along the Tietê River Basin are differentially polluted with contaminants which cause not only acute cytotoxicity, but also genotoxicity and AhR-mediated toxicity in fish cells, as well as embryo toxicity. Moreover, mutagenicity was recorded *in situ* in fish caught from the field. Since major toxicities were associated to samples from the metropolitan region, sediment samples from this region were submitted to an effect-directed analysis, in order to identify key pollutants causing the high toxicity of sediments. Results indicated different sets of toxicants inducing genotoxicity and metabolic activation. Overall, genotoxicity could be mostly related to alkanes, polychlorinated biphenyls (PCBs), naphthalenes (PCNs) as well as medium polar to polar polycyclic aromatic compounds (PACs), whereas AhR-mediated toxicity could mostly be attributed to polycyclic aromatic hydrocarbons (PAHs). A comprehensive evaluation of the ecotoxicological situation of sediments requires different approaches and broad knowledge for interpreting results. As exemplified for the Tietê River basin, a battery of bioassays applied in combination with chemical analyses and effect-directed analysis represent suitable tools to function as early warning systems not only for sediment pollution, but also for hazards for the entire river system.

TU 151

Acute sediment toxicity assessment in the river Sao Francisco, Brazil

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The São Francisco River is the fourth longest river from South America and the longest that runs entirely in Brazil. Although is a very important river, there are diverse anthropogenic activities, such hydroelectric plants, metal manufacturing industry or agriculture that have impact on these ecosystem. The objective of this study is to evaluate

by means of chemical and ecotoxicological approach the toxicity of the sediments of this river, and their elutriates, in a region downstream Trés Marias reservoir, in a site near a processing industrial plant of zinc. For this, a battery of three laboratory bioassays, using two cladoceran (*Daphnia similis* and *Ceriodaphnia dubia*) and one amphipod (*Hyalella azteca*) species, was used to assess the toxicity. 12 different metals (Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) and the different geochemical sediment fractions were determined as means of assessing the potential impact of the bioavailable fractions (two first steps of the sequential extraction procedure) in order to establish the potential effect of the industrial plant. The effect of sediment metal contamination was assessed applying multivariate techniques. The results show elevated concentrations of Zn, Cd and Pb that involve an important potential risk on the aquatic environment. The sediments affected by the industrial plant presented high levels of toxicity, although the hydrodynamic of the river plays an important role in the metal distribution and consequently in the toxicity of the sediments.

TU 152

Ecotoxicological assessment of sediments from rivers impacted by a petroleum refinery

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Ecotoxicological studies are important for evaluating the effects and mechanisms of action of toxic agents in different environments. Aquatic sediments have a high potential for accumulating pollutants. As pollutants may become available in certain environmental conditions, the sediments can also become a potential source of contamination to water bodies. Thus, the assessment of sediment quality is essential to understanding the processes governing the fate and availability of pollutants in water bodies. For an efficient sediment ecotoxicology assessment, different approaches are necessary. The aim of the present study is to evaluate the ecotoxicological potential of sediment samples from three rivers impacted by a petroleum refinery, by means of Neutral Red assay (cytotoxicity), EROD assay (CYP1A induction potential), the sediment contact with fish embryos (acute toxicity and teratogenicity) and chemical analyses, among others. Since water samples from these rivers have been also investigated, these results must be associated, in order to obtain a comprehensive evaluation of the ecotoxicological situation of these water bodies. First results will be presented.

TU 153

Seasonal variation of toxicity and genotoxicity of sediments samples of the Ensenada de la Paz, B.C.S. México

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In this paper was carried out a qualitative assessment of toxic and genotoxic effects of sediment collected at 8 locations in the Ensenada de la Paz, B.C.S., in 2 contrasting seasons summer and winter of 2010, using a battery of biological tests with organisms of different trophic levels and the SOS-Chromotest microbioassay. Compounds with toxic and genotoxic effects were detected in 5 locations, located in the Canal de la Paz, near the thermoelectric power station and the Pichilingue navy. LC50 and genotoxicity values observed, indicating the presence of toxic compounds at sites near Punta Prieta and Cicimar, sediment toxicity decreases with increasing distance from these points. This fact possibly indicates a contribution of harmful compounds probably from Punta Prieta and La Paz city to the Bay of La Paz. This type of analysis is essential to identify areas of risk in studies of environmental analysis.

TU 154

Toxic effects of sediments from a semi-arid coastal system, influenced by a gold ore mine

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More than 600,000 tonnes of pure arsenolite are scattered in the area of the El Triunfo gold ore mine, in the southern most Baja California peninsula, México. The drainage basin, from which sediments were collected for this study, discharges into the Pacific Ocean, however, the river is dry most of the year, only occasionally filled during tropical hurricanes in summer. This coastal system receives a continuous contribution of toxic compounds due to mineral extraction in the zone.

The aim of this study was to evaluate the toxicity of the sediments and the level of metal pollutants in order to determine the environmental risks. Heavy metals were determined in conjunction with sediment toxicity using *Daphnia magna*, *Selenastrum capricornutum* and seed-germination bioassay analysis. The Sediment Quality Triad (SQT) was used to identify areas in the system where sediment contamination may be linked to ecosystem degradation. A multivariate analysis was carried out and indicated that the degree of pollution varied from moderate to highly polluted. The analyses for the SQT indicate that the stations nearest to the alteration zone were severely polluted. These results suggest that there are health risks to human populations and activities such as vegetable and cattle farming or rural touristic recreation in the area. In order to restore the alteration zone to healthy conditions a remediation program would be necessary.

TU 155

Bioassays in sediment assessment for investigative monitoring in the context of the WFD

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An intense industrial activity has been taking place in Wallonia (Belgium) since the Middle Ages. The proximity to major waterways has enhanced the industrial development and has facilitated pollution toward aquatic environment. Sediments are known for their ability to trap pollutants and release these substances years or even decades after pollution has ended. In order to assess the current status of sediment pollution, bioassays can be used as suitable monitoring tools to answer the Water Framework Directive (WFD) requests and ascertain the causes of a water body failing to achieve the environmental objectives. They integrate all contaminants effects including additive and synergistic effects of unknown substances and breakdown products.

A sediment quality recurrent monitoring throughout the Walloon Region has been set up, taking into consideration physico-chemical parameters (including Priority List substances from the WFD) and ecotoxicological effects of collected environmental samples in a triad-type approach. Different species (*Chironomus riparius*, *Heterocypris incongruens* for whole samples, *Vibrio fischeri*, *Pseudokirchneriella subcapitata* and *Brachionus calyciflorus* for pore waters) are used in this approach because they provide different information due to the way of exposure (ingestion, tegumentary contact) and because they represent different trophic levels (decomposer, producer or consumer). Among the sediments of 10 stations studied in 2010, four showed a relatively low toxicity toward several organisms of the bioassays battery (the Wiltz, the Sure, the Gueule and the Rhosnes), 3 showed low toxicity toward one of the organisms (the Oise, the Molignée and the Eau d'Heure) and 3 showed no signs of toxicity (the Biesmes the Maslette and Salm) even if 2 non-toxic stations exceed the maximum acceptable levels as defined by decree of the Walloon government. Results of the 2011 monitoring program will also be presented.

TU 156

Ecotoxicological assessment of contaminated rivers as a proxy for the water framework directive: an acid mine drainage example

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Metal contamination of freshwater water bodies resulting from mining activities or deactivated mines is a common environmental problem in Portugal. Despite authorities recognize its broad dimension with a variety of health-related hazards and threats to the environment, the establishment of an adequate remediation plan for each mining area is still failing or being too delayed. Braçal and Palhal, located in Central-North of Portugal in a riverside position, are both examples of deactivated mining areas lacking for recovery plans since their shut-down in the early-mid 1900's. Both mines are inserted in large underground complexes. Braçal was mainly focused on the extraction of galena ore and Palhal on the extraction of pyrrhotite, chalcocopyrite, galena, sphalerite and pyrite ore. Since their shutting down, the mining run-off are running into two subsidiaries of Vouga River. The purpose of this work was evaluating the potential risks and hazards of the contribution of mining run-off into Vouga river subsidiaries for freshwater communities and nearby population villages. Therefore, ecotoxicological tests using a battery of assays were performed on sediments collected from the river in the vicinity of the mining run-off. The results shows that elutriates obtained from the sediments collected in Palhal mining area were toxic to all tested species, including the bacteria *Vibrio fischeri*, the algae *Pseudokirchneriella subcapitata*, the macrophyte *Lemna minor* and the cladocerans *Daphnia magna* and *Daphnia longispina*. In contrast, elutriates obtained from the sediments collected in Braçal mining area showed, in general, no toxicity for the several species, with only slight toxicity noticed to *P. subcapitata*. These results might be explained by the flooding of the Braçal mine that occurred following the mining shut-down, which slower the process of acid mine drainage phenomenon resulting from the oxygen in contact with ore. In opposite, Palhal mine never faced flooding events, which may explain the higher toxicity of the samples collected there. Our study highlights the usefulness of using an ecotoxicological approach to help the prioritization/scoring of the most critical areas impacted by deactivated mines.

TU 157

Assessment of effects of agro-chemicals use in ecological status of Kilombero Flood Plain, Ramsar site

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Tanzania is rich in wetland resources with an estimated 10% of the land surface covered by significant freshwater wetlands providing essential ecosystem and livelihood support functions. Millions of people depend on wetlands (MNRT, 2003) for fishing, agriculture, livestock keeping and collection of a multitude of minor wetland products, thus destroying the ecological functions of wetlands. Tanzania ratified to the Ramsar Convention on 2002, as a commitment to maintain the ecological character of its wetlands of international importance and to plan for the sustainable use of all other wetlands in the country. The Kilombero Valley, designated in the same year, is among four Ramsar Sites in Tanzania. It covers surface of 7,967 km² with a catchments area of about 40,000 km². Kilombero Valley is rich in endemic species of flora and fauna and characterised by high concentrations of large mammals especially Puku, Buffalo, Elephant, Hippopotamus and Lion.

Over the last two decades human settlement in the valley has increased, parallel with the socio-economic activities such as livestock keeping, fishing, cultivation, and bee keeping. The agro-pastoralism, large and small scale cultivation of rice and sugar cane with an intensive application of diverse arrays of pesticides, is a major threat to this valley's ecological integrity. However, little is known about sediment chemical contamination and pesticide load of Kilombero wetlands. This is the first study to investigate the potential impact of toxic chemicals, especially agrochemicals, on the sediment-water quality of this flood plain. During a first survey, screening of the region for hot spots will be done by applying selected biotests that are sensitive to pesticides contaminated sediment and water samples. In a second tier, chemical analysis of those samples identified as hot spots will be carried out in order to identify sources. Water and sediment samples will be collected in both dry and wet seasons, the first survey being conducted between February and April 2012. Based on data from this first survey, a conceptual site model will be set up and presented.

The project's further objectives comprise the assessment of human and environmental risk due to pesticide exposure, the communication of these risks to farmers and inhabitants living in the area and development of management recommendations for a more efficient and less environmentally adverse use of this Ramsar site.

TU 158

Heavy metal pollution assessment in sediments of the Nemrut Bay, Turkey

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Surface sediments in the fraction <63 µm collected from eight stations in Nemrut Bay, Aegean Sea were analyzed for trace metals (Cd, Pb, Cr, Cu, and Zn) by using inductively coupled plasma atomic emission spectrometry (ICP-AES). Heavy metal concentrations in surface sediment varied from 0,1-0,95 mg/kg for Cd, 15-190 mg/kg for Pb, 13,8-58,65 mg/kg for Cr, 20,2-71,9 mg/kg for Cu and 8,09-178,3 mg/kg for Zn. The results show that some elements such as Cd, Pb and Cu are at risk level in the sediments near the harbour, harbour activity and petrochemical industry may be the sources of these elements.

TU 159

Determination of ecotoxicological effects of persistent organic pollutants from sediment cores of the Baltic Sea: a combination of chemical measurements and aquatic toxicity testing

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In the last recent decades persistent organic pollutants (POPs) gained increasing importance in environmental sciences and toxicology due to their carcinogenic and mutagenic effects to a diversity of organisms as well as negative impacts on human health. Attributable to atmospheric deposition and municipal as well as industrial waste water discharge POPs are distributed in the marine environment, where they are finally enriched in the sediments by the affinity to bind on particulate matter and sorption processes. Adverse impacts on the benthic fauna are caused through chemical uptake and bioaccumulation of these contaminants.

Ecologically relevant processes such as bioconcentration and baseline toxicity of POPs are mostly controlled by the chemical activity of the substance rather than by its total concentration in the sediment. To calculate chemical activity and thus to predict bioconcentration and the baseline toxic potential of a contaminant mixture the freely dissolved concentrations of PAHs and PCBs were measured in sediment pore water samples of the Baltic Sea using solid phase microextraction, a passive sampling technique based on the principle of equilibrium partitioning.

To evaluate biological effects of contaminated sediments, modern strategies not only focus on analyses of contaminants, but also test the impact on biological organisms.

In addition to the chemical studies a composition of limnic and marine microbiological assays was used in order to assess bioavailability of the contaminated material to different organisms under controlled laboratory conditions. Interpretation of our biotest results followed a weight of evidence approach by which several individual positive tests in the tier add to an overall conclusion that a risk for the environment may in fact exist. The test system proved suitable for assessing the risk of the Baltic Sea sediments.

TU 160

Triad approach for risk assessment of sediment and floodplain soil of a metal-polluted stream

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Many streams and small rivers in Europe are faced with historic metal pollution due to the activity of zinc smelters. Rehabilitation activities are executed for many streams in the Netherlands, both in the sediment phase as in the terrestrial part of the floodplain. Especially the floodplains are faced with drastically changing environmental conditions due to the periodic and unpredictable flooding. During high discharge events, sediment is resuspended, transported, and settled at floodplains downstream. This spatial distribution of contaminants in the floodplain should be taken into account when streams and rivers are being reconstructed.

This is investigated in the Tengelroyse beek, a stream in the south of the Netherlands. Local water authorities have restructured large parts of the stream. We used a triad approach to assess whether all the ecotoxicological risks have been adequately removed by the restructuring and rehabilitation activities. Sediment and soil was sampled in the stream and in the floodplain on 5 locations along the stream. Total concentration (aqua regia extraction) and bioavailable concentration (CaCl₂ extraction) of the following metals were analyzed: Cd, Cu, Pb, Zn, As. A standard Chironomus survival bioassay was conducted in the laboratory. Metal and bioassay results were compared with field inventarisations of the macroinvertebrate community.

The highest bioavailable concentrations of Cd and Zn were observed in the floodplain soils. The bioassay showed a negative effect for two locations. Combining the two measurements with the in situ macroinvertebrate community showed an ecotoxicological risk for one floodplain soil when inundated. These results are compared with previous studies along the river Dommel. Advices on optimal restructuring and rehabilitation of small streams should include the spatial distribution and bioavailability of contaminants.

TU 161

Hexachlorobenzene-contamination of sediments of the Upper Rhine River - identification of sorbents to assess bioavailability

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Due to historical emissions of hexachlorobenzene (HCB), the sediments of the ponds of Upper Rhine River in southern Germany remain highly contaminated (up to 1000 µg kg⁻¹). HCB is a bioaccumulative and toxic persistent organic pollutant (POP). By reason of its strong sorptive characteristics, HCB is mainly bound to organic substances. However, geogenic clay minerals are also capable sorbents. Regarding the contaminated sediments, the spatial distribution of HCB is considerably more heterogenic, then e. g. the distribution of polychlorinated biphenyls (PCBs). Further, maximum loads of HCB occur rather in larger particle fractions (63 - 2000 µm), than in smaller fractions (< 20 µm). Knowledge of the specific HCB-sorbents in the different fractions is of importance, because their characteristics control sorption strength, which is an important factor for remobilization as well as bioavailability of organic contaminants.

The mineral composition of sediments (measured by X-ray Diffraction, XRD) and its influence on the amount of sorption is presented, as well as composition and sorption capacity of sediment fractions (classified by particle size and content of organic matter). Sorption isotherms and partition coefficients (K_d and K_{oc}) were determined in batch equilibrium studies by solid-phase microextraction (SPME) coupled to gas chromatography (GC). In addition, adsorbents were investigated by imaging mass spectrometry and scanning electron microscope coupled to energy-dispersive X-ray spectroscopy (SEM-EDS).

Results contribute to explain mechanisms of sorption and can therefore help to predict remobilization processes as well as bioavailability of HCB in aqueous ecosystems. Results yield important physicochemical properties of sorbents for better understanding the environmental behavior and pathways of chemicals and beyond that to model environmental fate under changing climate conditions.

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TU 162

River sediment sampling and environmental quality standards

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Sediment forms an important part of the aquatic environment and provide habitat to benthic organism. However, poor water quality has been linked to contaminated sediment most of which consists of complex materials transported within river or deposited on river bed. Sediment has been identified as major transporter and sequester of fluvial contaminants such as heavy metals, most especially the fine sediment. At present, there are no environmental quality standards (EQSs) for bed sediments or fluvial suspended sediments, and one of the major problems in establishing sediment EQSs is the identification of the suitable sediment fraction to measure - the actively transported or previously deposited sediment. The variability in sediment characteristics is mainly attributed to their active properties and the characteristics of the rivers such as pH, redox, and high water discharge, which is investigated during sediment sampling.

The study aims to investigate the effect on particle size on heavy metal concentration, study seasonal variation in the concentration of Cu, Ni, Pb and Zn in Ravensbourne River, and compare the concentration of selected heavy metals between different compartments such as suspended sediment, bed sediment, bank sediment and the water column of Ravensbourne River. Sediment (bed and bank) and water samples were collected from Ravensbourne River over a period of 12 months. Sediment samples were tested for heavy metal concentration using the aqua regia, sequential extraction using a 6 hour extraction technique proposed by Maiz et al. (1997).

TU 163

The first sight into contamination of Polychlorinated biphenyls and Polybrominated diphenyl ethers in sediments of Tam Giang-Cau Hai Lagoon, Central Vietnam

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Rapid economic development and urbanization growth in Vietnam have made this country become an important subject for extensive studied dealing with environmental pollution recently. So far, there has been no information on PBDEs contamination in Central Vietnam and the information about PCBs sources and levels is limited. Tam Giang-Cau Hai Lagoon lies along the coastal line of Thua Thien-Hue Province, Vietnam. Due to recent industrial and economical growth in this region, large amount of untreated municipal and industrial wastewater have been discharged to this river - lagoon system, which may cause adverse environmental consequences and increased health risk for local communities. In this study, the contamination status, distribution and congener profiles of PCBs and PBDEs in surficial sediment of Tam Giang-Cau Hai Lagoon were investigated. PCBs and PBDEs were detected in all sediment samples with mean concentrations of 0.19 and 0.56 ng/g dw, respectively. The highest concentrations of both contaminants were observed downstream at the river mouths, indicating the river flow as a main source of pollutants into the lagoon system. The similarity of PCB congener distributions in sediments suggest the presence of a unique source over the entire study area, probably connected to local use of old electrical equipments containing

low chlorinated PCB formulations. Atmospheric transport & post depositional processes might modify to some degree the fingerprint of PCB inputs to the environment favouring the predominance of 3, 4 & 5 chlorinated congeners. The PBDEs profile in sediment was relatively uniform, with decaBDE as the dominant congeners, followed by nona- and octaBDEs, indicating DecaBDE as the major technical mixture in consumer products in this region. In general, the levels of PCBs and PBDEs in lagoon sediments were low, compared with those reported in sediments of other rivers in Southern Vietnam as well as in other river systems in developed countries. This may reflect a limited usage of PCBs and PBDEs in this region. Comparing to sediment quality guidelines currently used around the world, present PCB sediment concentrations of Tam Giang-Cau Hai Lagoon does not constitute a threat to environment. Nevertheless, the first time detection of PBDEs in the lagoon-river system might be an indicator of enhanced recent human pressure that suggests the need for a monitoring plan to prevent the possible dangerous worsening in the near future.

RA07P - Environmental problems of estuaries

TU 166

Distribution character and potential risk of PCBs and phenols in surface water from 22 tributaries and mainstream in middle reaches of Yangtze River

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47 surface water samples were collected from 22 tributaries and mainstream of the Yangtze River from Chongqing to Yichang. The contents of 28 PCB congeners and 15 phenolic compounds in surface water samples were measured using Varian CP3800/300 GC-MS/MS technique. PCB8, 28, 52, 118, phenol, o-cresol, 2,6-dichlorophenol, and 2-nitrophenol are the most predominant compounds in the samples from tributaries, PCB8, 28, phenol, o-cresol, and 2-nitrophenol are the most dominant compounds in those from mainstream. The measured level of PCBs in the samples from the tributaries and the mainstream were 20.71 ng-L⁻¹ and 13.25 ng-L⁻¹, and the measured level of phenolic compounds in those samples from the tributaries and the mainstream were 87.99 ng-L⁻¹ and 52.47 ng-L⁻¹, respectively. The highest concentrations of PCBs and phenolic compounds were detected separately in the samples from Rangdu River (61.79 ng-L⁻¹) and Jialing River (1723.77 ng-L⁻¹). The average concentrations of PCBs and phenolic compounds in the samples are far lower than the Chinese drinking water guideline values. The concentrations of PCBs and phenolic compounds in the samples were in the same order of magnitude of those reported on lower levels in European and American countries, which show that potential risk are negligible due to PCBs and phenolic compounds contamination in these samples.

TU 167

Measuring trace labile metals in the Pasig River water using DGT technique: an application for an effective water resource management

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Pasig River is an important water body in Manila, Philippines. This is a busy river greatly influenced by urbanization. It is an estuary in nature as it is in between a bay (Manila Bay: west) and lake (Laguna Lake: east). The water quality is a main concern. From being in a state of biologically dead, rehabilitation is applied to raise it to Class C. The efforts are still on-going. An efficient water quality monitoring technique and assessments are necessary. This research made use of the current available technique called passive sampling. DGT had been used as the target compounds are the labile trace metals (Cr, Co, Ni, Cu, Zn, Cd and Pb). The time of sampling campaigns captured the ENSO phenomenon (El Niño: Period 1, La Niña: Period 2 and in between summer and rainy seasons: Period 3). From these periods, DGT-labile metal concentrations had been computed. Multivariate analyses composing of the Principal Component Analysis (PCA) and Factor Analysis (FA) were employed. This is to be able to identify the parameters that play a part more in each period. The cumulative percentages of the total variance are as follow: 89.3% for Period 1; 87.11% for Period 2; and 81.25% for Period 3. Correlations among the parameters had been achieved as well. In addition, percent concentrations of each element per site were determined. Scores were given and ranking was then supplied. Based from the ranking, sites that contribute more of the selected parameters were identified. For Periods 1 and 2, highest contributions are found near mid to upstream. While for Period 3, highest rank is near at the downstream. From this also, we were able to locate the sites that can be given more priority and attention. Fluxes (in kg d⁻¹) were provided as well. Period 2 having the highest flow gave the highest fluxes per site.

TU 168

Dioxin-like compounds in Portuguese estuaries: levels and patterns in superficial sediments

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Persistent organic contaminants such as polychlorinated dibenzo-p-dioxin (PCDD), polychlorinated dibenzofuran (PCDF) and dioxin-like polychlorinated biphenyl (DL-PCBs) may enter the estuarine system through atmospheric deposition, riverine runoff and direct release from human activities. In the aquatic environment these contaminants strongly adsorb to suspended particles and tend to accumulate in underlying sediments which constitute long-term reservoirs and secondary sources. Contaminated sediments can therefore represent a significant risk to aquatic life and human health and the loss of natural and economic resources. The aim of this research is to evaluate the sediment contamination of Portuguese estuaries by PCDD/Fs and DL-PCB. Seven estuarine systems were selected along the Portuguese coast: Lima, Ria de Aveiro, Mondego, Tagus, Sado, Mira and Ria Formosa. At each estuary, superficial sediments (0-10 cm) were collected at various locations in the intertidal mudflats. Samples were analysed by high-resolution gas chromatograph/high-resolution mass spectrometer (HRGC/HRMS) to determine the concentration of seventeen PCDD/Fs and twelve DL-PCBs. Results show that PCDD/Fs and DL-PCBs concentrations are variable not only between estuaries but also within each estuary. In general, the highest values were found at sites near large populations and industrial complexes (e.g. Tagus, Ria de Aveiro and Sado). The lowest PCDD/Fs and DL-PCBs values were measured in Ria Formosa, a protected area with status of Nature Park. OCDD is the most abundant PCDD/F congener in all the samples but PCDD or PCDF were the major contributors to WHO-PCDD/F-TEQ2005. For DL-PCBs, PCB 118 was the dominant congener while PCB 126 was the major contributor to WHO-PCB-TEQ2005 in all the analysed sediments.

TU 169

Environmental monitoring and assessment of Terminos Lagoon, Mexico

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Terminos lagoon is located in the estuary of the Grijalva-Usumacinta river system, with the largest fresh water runoff in Mexico (35% of the total), and the second largest in the Gulf of Mexico. It is near of one of the main oil drilling areas in Mexico and there are many industrial facilities associated with the oil industry affecting the region. Other industrial activities, such as urban waste water, and agriculture, are also important inputs of pollutants to the Gulf. All these activities introduce pollutants such as metals, hydrocarbons, pesticides, etc.

As one of the components of the Sustainable Management of the Gulf of Mexico Large Marine Ecosystem, the pilot project on monitoring and environmental assessment selected Terminos as the site to study. The project consists of five modules: water quality, sediment quality, contaminants and biomarkers in fish, benthic community, and habitat degradation. The results are categorized as good, regular or bad according to pre-established criteria, in some cases adjusted to conditions in the southern Gulf. Sampling was random stratified, using the five zones determined from hydrology as a basis for stratification. Results show that in general pollutant concentrations in sediments are low, and have been decreasing at least since 2005. However, mercury levels exceed Mexican advisory levels in the muscle of fish in about 20 % of the fish. For organic pollutants there were no significant differences between the zones, except for HCHs and PAHs. A number of stations were found to be in "bad" condition for water quality and benthic community. Loss of mangroves forests is decreasing. Results have been presented to management authorities.

TU 170

Chemical and biological assessment of metal pollution in the Urdaibai Reserve of the Biosphere (UNESCO) using oysters as sentinels

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Oysters have been widely used as sentinel organisms in order to determine the presence of pollutants and their effects in coastal and estuarine environments. In the present work, oysters from the Oka river estuary (Urdaibai Reserve of the Biosphere, UNESCO) were collected in 7 sites at different seasons along 2010. Together with oysters, water and sediment samples were also collected. The concentration of 14 elements (Al, As, Cd, Co, Cr, Cu, Fe, Mg, Mn, Ni, Pb, Sn, V and Zn) was determined. Cell and tissue level biomarkers and histopathological alterations were also recorded. High Cu concentrations were present in the water column but tissue concentration was not high in oysters. Overall, some seasonal trend was observed in metal concentration in the water column with higher values in September, which was not reflected in oyster tissues. In contrast, a moderately high Pb concentration was found in oysters but not in water. Preliminary data in sediments indicated higher values of Cu and Mg in Mundaka. Regarding cell and tissue level biomarkers, no significant differences were observed comparing the sites. Occasionally, Arteaga presented symptoms of atrophy in the digestive diverticula in March and June. Seasonal variability was observed in the Connective-to-Diverticula ratio, with lowest values in September. Likewise, seasonal changes in the cellular distribution of metals as also observed in the digestive gland. Metals revealed by autometallography as black silver deposits (BSDs) appeared in the basal lamina (histological sense) of the digestive epithelium in March and June, whereas BSDs were localized within digestive cell lysosomes in September and especially in November. The when a significant increase in the BSD volume density was observed in parallel with an increased metallothionein content. In conclusion, the results confirmed that the Urdaibai Reserve of the Biosphere presents low-to-moderate levels of metals both in oysters and waters, with only minor biological effects in oysters. It is worth noting that, however, seasonality may play a relevant role in environmental metal levels, bioaccumulation and the biological responses of oysters against environmental insult.

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TU 171

Monitoring of sediments genotoxicity of the Yucateco, lagoon Tab, México

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The Yucateco lagoon is located in southeastern of the Mexican Republic in the Tabasco state. This system receives a continuous contribution of toxic compounds due to hydrocarbons extraction and industrial activity carried out in the zone. Since the system have been reported mass mortality events of fish and shellfish, in this research, an evaluation of the genotoxic effects of sediments over a period of 5 years were carried out to establish the state of ecosystem health. Sampling were made annually during the dry and rainy seasons. porewater were obtained from sediment samples, which were tested in the SOS-Chromotest microbioassay. In general can mention that the number of sediment samples with genotoxic effects decreased from 2003 to 2007. In 2003 was detected genotoxic compounds in 5 sampling stations, while in 2007 this effect was observed only in 3 locations. Critical areas are the stations 1, 2 and 3 located in the northern and central part of the system, the genotoxic effects decreases with increasing distance from these points in the direction of outlet the river Tonala. The critical pollutants likely to cause genotoxicity were the HAP's, PCB's (polychlorinated biphenyls) and metals vanadium and nickel. The utility of this type of analysis is essential to identify areas of risk in studies of environmental analysis.

TU 172

Bang for buck: the challenge of choosing environmental indicators of anthropogenic impacts in estuaries

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Ecological assessments over large spatial scales require that anthropogenic impacts be distinguishable above natural variation, and that the monitoring tools implemented maximise impact detection for a relatively low cost. For three heavily modified and four relatively 'pristine' estuaries (disturbance category), chemical indicators (metals and PAHs) of anthropogenic stress were measured in benthic sediments, suspended sediments and deployed oysters, together with other environmental variables. The differing ability of these data sets to explain the variances observed in infaunal and hard-substrate invertebrate communities were compared. Univariate analyses were useful for comparing contaminant loads between different monitoring tools and identified the strongest correlations between benthic and suspended sediments. However, multivariate analyses were necessary to distinguish ecological response to anthropogenic stressors from environmental "noise" over a large spatial scale and to identify sites that were being impacted by contaminants. These analyses provide evidence that suspended sediments are a useful alternative monitoring tool to detect potential anthropogenic impacts on benthic organisms.

TU 173

Metal availability and toxicity in estuarine systems in relation to chemical speciation and biological diversity

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The estuarine environment is a very dynamic system characterized by strong physical and chemical gradients and an enormous diversity in flora and fauna which undergoes major transitions moving from the freshwater to the marine areas. Along these gradients metal concentrations and speciation undergo important changes which also have an impact on the environmental distribution and biological availability of the metals. Parallel to these are changes in the physiological organisation of the biological species living in this gradient as they adjust themselves to the changes in ion composition and osmotic pressure of the external environment in order to maintain homeostatic balance. The effect of changes in salinity on metal speciation, metal biouptake and metal toxicity was studied under controlled conditions in a series of structurally and functionally different organisms with either a freshwater or marine origin (e.g. unicellular algae, water flea, blue mussel, periwinkle, sea bass,...). The results were compared and combined with information available from data bases and literature to explore whether general concepts such as the free metal ion activity model and major ion competition effects used to describe metal uptake and toxicity remain valid across estuarine gradients and biodiversity. Using a dynamic modelling approach the effects of changes in physical and chemical conditions on metal speciation, biouptake and toxicological effects have been analysed and simulated across species diversity. For some metals, such as cadmium; where a strong inorganic metal speciation effect is observed a species sensitivity distribution analysis shows a decrease in metal toxicity moving from freshwater to higher salinity environments. However, this is not the case for a metal such as copper and in any case the effect is considerably less than what would be expected on the basis of the changes in free metal ion activity. The results of these observations are further discussed in relation to the ion and osmoregulatory constraints of the different species and whether or not normalization of metal availability and toxicity on a free metal ion activity scale is warranted for risk assessment purposes in estuarine gradients.

TU 174

Ecological risk management at a site adjacent to an area of special scientific interest

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Risk assessment is currently underway for a contaminated site in Northern Ireland with a wide variety of compounds of potential concern. In accordance with the UK CLR11 framework for management of contaminated sites, risk assessment is integral in leading the site from investigation to remediation, with the aim of protecting human health and the environment and obtaining a "suitable for intended use" outcome. An important feature of this site is its close proximity to an Area of Special Scientific Interest (ASSI), a Special Protection Area (SPA) and a Ramsar site. Being adjacent to such sensitive receptors, the ecological risk management approach for this site is a high priority. This poster presentation aims to share some of the recent experiences and future directions in ecological risk management at the site, including:

- Ecosystems are complex, therefore identifying and monitoring the right parameters is key to successful management. At this site, environmental monitoring has extended beyond routine water quality parameters to include terrestrial and aquatic ecological surveys and detailed studies evaluating benthic community abundance and diversity. These will form a robust baseline for evaluating changes in ecological status as short- and long-term remedial measures are implemented.
- Early engagement with regulatory authorities is important to agree benchmarks for assessing environmental monitoring data. Being able to understand and critically review how these benchmarks are established is key in effective engagement on these issues. Identification of a subset of key risk-driving compounds has helped facilitate assessment of potential impacts from multiple sources/ stressors.
- Stakeholder engagement beyond the regulatory authorities is important to communicate the management approach and seek alignment with social as well as scientific drivers. Using sustainability indicators to support remedial decision making on the site can assist with more fully integrating ecological risk management into the process.
- Ecosystems are constantly changing, therefore it is important to continually review the monitoring and assessment approach and consider the influence of potential future stressors, such as climate change.

TU 175

Integrated estuarine Mmodeling to support watershed management for sinclair and dyes inlets, Puget Sound, WA, USA

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An integrated estuarine model was developed to simulate fecal coliform (FC) fate and transport in Sinclair and Dyes Inlets, Puget Sound, WA. The integrated model consisted of a watershed model (Hydrologic Simulation Program Fortran - HSPF), an empirical fecal coliform loading model based on upstream land use and cover (LULC), and three dimensional fate and transport model, Curvilinear Hydrodynamics in Three Dimensions (CH3D), previously calibrated to match the hydrodynamics of the Inlets and modified to include FC kinetics (CH3D-FC). The integrated model was used to simulate the release, transport, and fate of FC loading from watershed pour points corresponding to stream mouths, stormwater outfalls, waste water treatment plant discharges, and shoreline drainage areas. A total of 20 simulation scenarios were run to verify model performance, assess sensitivity and uncertainty, and provide results needed to establish management goals for the watershed. Model results indicated that for marine waters to meet standards, FC targets more stringent than the freshwater quality standards were needed for three streams that discharged in nearshore areas with reduced flushing. Overall, the integrated watershed-receiving water model performed very well. The integrated model was able to recreate a wide range of dynamic loading within the inlets, from large-scale storm events with high flow conditions to dry, low-flow conditions during the summer months. The integrated watershed monitoring and modeling approach to water quality management is assisting the development of management plans worthy of stakeholder acceptance, helping to achieve reductions in FC loading, and resulting in improvements to the environmental quality of the inlets.

TU 176

Impacts of hypersaline conditions on the endocrine and reproductive effects of bifenthrin on salmonids

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Pyrethroids are a commonly used class of pesticides in California in both agricultural and urban applications. Although pyrethroids are reported as having nominal effects in mammals, they have been shown to have toxic effects in aquatic organisms, especially invertebrates and fish. Additionally, studies have found that in parts of the San Francisco Bay Estuary system, salinity has been increasing as much as 3 parts per thousand (ppt) each year. Such increases in salinity have been shown to increase enzymes that may reduce the acute toxicity of pyrethroids, but may form metabolites that have greater endocrine disrupting (i.e., estrogenic) activity. Concentrations of bifenthrin, a third generation pyrethroid, have been observed to be as high as 73 ng/L in northern California waterways. To evaluate the effects of hypersaline conditions on bifenthrin toxicity and endocrine disrupting activity, sexually immature rainbow trout (*Oncorhynchus mykiss*) were exposed for 14 days to nominal concentrations of 10 and 150 ng/L. Prior to bifenthrin exposure, fish were acclimated to freshwater, 8 and 17 ppt saline conditions. As hypothesized, 85.6 % mortality was observed in the 150 ng/L treatment

group in freshwater fish, 14.4% in the 8 ppt and no mortality in the 17 ppt salinity treatment groups indicating protection by hypersaline conditions from acute lethality. However, significant differences were not observed in plasma vitellogenin from bifenthrin-treated fish acclimated to fresh and salt water. These data suggest that higher salinity environments enhance the detoxification of bifenthrin, but without enhancing endocrine disrupting activities at these concentrations for 14 days.

TU 177

Characterization of organic and inorganic mercury transference dynamics in a coastal lagoon food web using stable isotopes

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It is well established now that, the determination of total Hg (HgTOT) concentration is not sufficient to understand its fate in the environment. Inorganic ionic Hg is the main form in waters and sediment samples, whereas organic forms (HgORG) account for the majority of Hg found in biological tissues. Despite not representing a large fraction of HgTOT in the aquatic environments, the main form of HgORG, methylmercury (MeHg⁺), raises prime concern, due to its tendency for bioaccumulation and biomagnification within the aquatic food chain. In order to examine the trophodynamics of HgTOT and HgORG in a coastal lagoon, suspended particulate matter (SPM), sediment, seston, phyto and zooplankton agglomerates, Crangon crangon, Pomatoschistus microps, Liza aurata, Atherina boyeri and Dicentrarchus labrax were sampled. Sampling took place near the mouth of the two major freshwater sources (Vouga and Antuã rivers) to the lagoon. Vouga River runs in a narrow channel, with steep margins. Its watershed receives influences from agriculture, livestock production, urban, industrial and mine effluents. Laranjo Bay (a depositional area) receives the Antuã, which has been conveying industrial discharges, particularly an effluent from a mercury cell chlor-alkali plant (from the 1950s until 1994). From the estimated 33t of mercury dispersed into the lagoon, the majority (81%) is associated with the sediments in Laranjo bay. By determining the position of each of the sampled compartments in the local food webs, using GC-C-IRMS (Gas Chromatography Combustion isotope Ratio Mass Spectrometry) to quantify stable isotopes ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), the evaluation of this element's transference in the food web was achieved. Mercury analysis was executed using CV-AAS (Cold Vapor Atomic Absorption Spectrometry). Certified reference materials (DOLT-3 and DOLT-4 for tissues, PACS-2 for sediment) were used in the QC/QA process. A significant negative correlation (Pearson Product Moment correlation, $P < 0.05$) and a very strong positive one (Pearson Product Moment correlation, $P < 0.0001$) were found for Laranjo data, between HgTOT and $\delta^{15}\text{N}$, and HgTOT and HgORG, respectively. No other significant correlations were established.

Results for HgTOT were generally in line with published values for Laranjo Bay. Organic mercury was the most abundant of the species in the superior organisms (always >57%). The diverging patterns of HgTOT and HgORG distribution are discussed.

TU 178

Is chronic exposure to pollution able to change the physiological capability of Corbicula fluminea to respond to acute chemical stress in estuaries?

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The Asian clam *Corbicula fluminea* (Müller, 1774) is an invasive species that has been colonizing aquatic ecosystems in Europe, America and several other regions. In the freshwater tidal area of the Minho River estuary (NW Iberian Peninsula) that was colonized by this species in the 80s, *C. fluminea* is now the dominant species of the benthic community, has been contributing to the decline of several native molluscs and considerably changed ecosystem functioning. Furthermore, *C. fluminea* has been showing to be able to develop resistance to pollution. Here, the hypothesis that individuals from the river but inhabiting sites with distinct levels of historical contamination, have different capabilities of answering to pollution exposure events. To test the hypothesis, animals collected at two sites in the Minho River under different anthropogenic pressure were exposed separately to the model polycyclic aromatic hydrocarbon benzo[a]pyrene (BaP) after a period of acclimation to lab conditions. At the end of the bioassays, enzymes involved in neurotransmission, biotransformation, anti-oxidant defences, aerobic energy production and lipid peroxidation levels were used as biomarkers. Results show differences in biomarkers responses between the two groups of clams suggesting that animals inhabiting the most contaminated site developed tolerance to chemical stress. This study was carried out in the scope of the project "NISTRACKS - Processes influencing the invasive behaviour of the non indigenous species *Corbicula fluminea* (Mollusca: Bivalvia) in estuaries - identification of genetic and environmental key factors" funded by the Portuguese Foundation for the Science and the Technology (FCT) (PTDC/AAC-AMB/102121/2008) and FEDER COMPETE funds (FCOMP-01-0124-FEDER-008556). P. Vilares had a grant in the scope of the project and C. Oliveira had a PhD grant from FCT (SFRH/BD/68423/2010) funded by national funds of the MCTES and FEDER European funds (POPH-QREN-Tipology 4.2.).

RA19P - Plants and chemicals in the environment: risk assessment, pest management and phytoremediation

TU 181

Sediment contact test with *Myriophyllum aquaticum* (ISO/CD 16191): first results of an international ring test

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Although they are an important part of an aquatic ecosystem, dicotyledonous macrophytes are not yet part of the risk assessment of sediments or dredged material.

A sediment contact test system with the dicotyledonous watermilfoil *Myriophyllum aquaticum* was established by the ecotoxicological laboratory of the German Federal Institute of Hydrology. This test procedure is now in the standardisation process within ISO.

In the sediment contact test, *Myriophyllum aquaticum* whorls are exposed to environmental samples for ten days. The growth of *Myriophyllum aquaticum* in a test sample is compared with its growth in the control sample. Phytotoxic effects can be quantified as growth inhibition (%) relative to the control growth.

Several laboratories from academia, industries and government participated in an interlaboratory ring test organized by the German Federal Institute of Hydrology. The ring test aimed at i) investigating the practicability and reproducibility of the sediment contact test, ii) validating the chosen endpoint, and iii) determining the response range of the reference substance functioning as positive control.

First results of the interlaboratory ring test will be presented.

Acknowledgement: U.F on behalf of the participants of the international ring test

TU 182

Getting more ecologically relevant data from laboratory tests: recovery potential of *Lemna minor*

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The goal of ecological risk assessments for chemicals typically is to protect the status of populations. Still, the assessments are usually conducted at the level of the individual, so the results of laboratory testing need to be translated into predictions of population status. Extrapolation from laboratory tests has many limitations, among which is the fact that species differ not only in toxicological sensitivity but their potential for recovery from adverse effects.

Lemna minor and *L. gibba* are representatives of rare standardized laboratory test species that can actually provide direct information on population status upon exposure to potentially harmful substances. Still, the standardized laboratory protocols are typically completely ignoring the recovery potential after exposure. Such ecologically relevant information can be gained already from suitable minor adaptations of standard laboratory tests. In the study on sensibility and recovery potential after exposure to atrazine (Teodorovic et al, ETC, in press) we suggested that standard test protocols with *Lemna* sp. could be adapted by a) adding a recovery phase after the exposure phase and b) frequent observations and measurements (in 3 days long intervals) during the whole study.

The suitability of adapted protocol has been tested on three substances with different mode of action: standard reference substance for *Lemna* (3, 5 DCP in concentration range 0.625-10 mg/l), and two herbicides - isoproturon and trifluralin (in concentration range 0.01 - 1 mg/l). The results indicate that adding the 7 days long recovery phase after the exposure phase is suitable adaptation of the standard test protocol. Frequent observations and measurements during the whole study, particularly during the recovery phase, can provide a better understanding of response and recovery patterns. Relative growth rate calculated for the last 3 day long interval of the study (Last interval RGR) proved to be a suitable and sensitive end point which might provide good indication of plant recovery potential after exposure to toxic compounds. Estimation of recovery potential directly from suitable laboratory tests would add more ecological realism into worst case scenario responds typically obtained by single species laboratory toxicity test but also provide valuable data for population - level models.

TU 183

New developments in mesocosm testing with herbicides: recent experiences and recommendations

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A number of new developments in mesocosm testing have taken place since the most recent revisions to guidance for these complex studies (e.g. HARAP, 1998 & CLASSIC, 2001). Much of the progress in this area since this guidance was produced has been in the design and conduct of freshwater mesocosm studies to evaluate the toxicity of herbicides on algae, periphyton and macrophytes. This presentation will highlight some of the developments in this area using examples drawn from recent state of the art mesocosm studies.

We will highlight our recent experiences in this area by describing methods we have developed to evaluate the effects of herbicide exposure on a variety of macrophytes with different morphology and life histories. We will also provide recommendations for the evaluation of effects on algae and periphyton drawing on our experience in this area. It is hoped that the information provided will advance mesocosm study design and offer advice to those considering conducting mesocosm studies with herbicides whilst providing a catalyst for discussion in this developing area of higher tier aquatic ecotoxicology.

TU 184

Unforeseen effects of herbicides on wetland and terrestrial plant communities: time to vegetative and reproductive recovery following exposure

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Standard plant toxicity tests rely on the evaluation of species in short-term (ST) studies; long-term (LT) effects of low doses of herbicides on wild vegetation, or the recovery potential of plants is often not assessed. The ability of wild species to recover following sub-lethal exposure to herbicides was examined using the sulfonylurea herbicide chlorimuron ethyl. Eight wetland and nine upland species were tested with eight doses of chlorimuron (+ controls) ranging from 1 to 107% of the recommended label rate

of 9 g ha⁻¹. For each species, two sets of plants were grown: total aboveground plant biomass was assessed at four weeks after exposure for the ST plants (first set) and again for the LT plants (second set) when the controls began natural senescence (7-23.5 weeks post-spray depending on species). To assess recovery over time, measurements of maximum plant height were taken weekly for all species using the LT plants. Reproductive parameters were also recorded for 13 species. Inhibition concentrations (IC₅₀) were calculated for ST and LT biomass and for LT reproductive parameters. ST biomass was found to be the most sensitive measure of IC₅₀ for nine species and LT reproduction for three; IC₅₀s could not be predicted for five species (no effect). Delays in flowering time were apparent for several species. Twelve species had early delays in growth as compared to the controls at doses of 7.4% or less, two species were affected at higher doses (14.5 or 55%) while three species (all grasses) had no reductions in height at any dose. Of the species affected at doses of 7.4% or less, three fully recovered within an avg. 6.7 weeks post-spray, six recovered up to doses of 14.5% (avg. 6.5 weeks) and three up to doses of 28.2% (avg. 12.3 weeks). Though an overall trend towards recovery was also noted for reproductive parameters (4 spp. recovered, 3 remained the same, 3 unaffected at any dose), rates of recovery did not follow those observed for height. Delayed growth and flowering was observed at doses less than known drift rates (<10%); however, plants exposed to these low rates were often able to recover over time. While recovery did occur in this greenhouse experiment, it may be suppressed in natural communities where more resistant species (e.g. grasses) may grow faster and out-compete susceptible ones for light or resources. Such events may alter the natural species composition in exposed areas. This community approach is not considered in risk assessment.

TU 185

Effects of agrochemicals on macrophyte community structure and quality in a Canadian agricultural watershed

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Macrophytes are essential to healthy rivers and lakes. Unfortunately, macrophyte diversity, particularly diversity of submerged species, appears to have declined in some North American and European ecosystems during the last century. In agricultural watersheds, streams are intimately connected with croplands and the health of macrophyte communities may be compromised by increased levels of disturbance and exposure to agrochemicals such as nutrients and herbicides in areas of high-intensity agriculture. The macrophyte community was identified at twenty-four sites located throughout the South Nation River watershed, a sparsely populated agricultural watershed comprising 3919 km² in Eastern Ontario, Canada. The sites varied in terms of their contamination with nitrate and the commonly used herbicide atrazine, with mean spring maximum in-stream concentrations of 6600 µg/L and 1.56 µg/L respectively. In-stream concentrations of nitrate and atrazine were strongly correlated. Over 200 macrophyte species were identified along stream banks and within the stream channels. Sites surrounded by high levels of agriculture tended to have a higher percentage of non-native species, suggesting that native species are losing ground across the watershed. A decline in the quality and conservation values of macrophyte communities was observed along a gradient of nitrate contamination.

TU 186

The effect of five sulfonyle-urea herbicides to aquatic macrophytes - implications of results from outdoor growth inhibition studies for the risk assessment

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The collection of multi-species data is an option to refine higher tier risk assessments. The objectives of multi-species testing are (1) to derive a species-sensitivity distribution (SSD) and (2) to identify a most sensitive species. Lemna is the standard aquatic test organism and among the most sensitive species to herbicides. Multiple species data provide arguments to lower the assessment factor due to increased realism and lower uncertainty. The example of sulfonyle-urea herbicides (SU) outlined here shows that herbicides with the same mode of action can have the same or a similar SSD for aquatic macrophytes.

Over a period of four years Smithers Viscient (sponsored by Bayer CropScience) generated macrophyte data for five different SU herbicides. In each study 9 to 10 species from a variety of taxonomic groups were exposed in artificial outdoor ponds for 6 to 8 weeks. Each year the design was slightly adjusted based on experiences gained from former tests. Despite these changes, the data obtained are applicable to (1) comparisons of species sensitivities within one test substance and (2) comparisons between the test substances.

Numbers of replicates were 4 in the controls and lowest concentrations, 3 at medium, and 2 at the highest concentrations (combined NOEC-EC_x-design). A dose-response was observed in most species. Due to variation among replicates, the EC₅₀ is regarded as more reliable than the NOEC. As plant length could not be assessed in all species, dry weight biomass was used for comparisons. EC₅₀-levels based on biomass and biomass growth rates were very similar.

The EC₅₀-levels from biomass growth rates were compared to the Lemna-EC₅₀. In 3 of the 5 herbicides tested none of the macrophytes was more sensitive than Lemna. For 2 substances sensitivity of Lemna was ranked 2 and 3. In all 5 cases Lemna was among the 5% of the most sensitive species. Myriophyllum, the forthcoming second standard species within the frame of EU regulations, was of medium sensitivity (rank 4 to 6). Overall, the pattern of sensitivity distributions was very similar in all 5 SU herbicides. The large amount of data obtained enables refined risk assessments with low assessment factors based on the lowest EC₅₀ as well as probabilistic approaches using a hazard concentration (HC₅).

TU 187

Ecological functions of plant growth regulators in the stability of agrocenosis

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Tolerance of the system "soil-plant" in agrocenosis is the key condition of high yield. These functions in soil are accomplished mainly by humus component, and in the plant - by complex of biologically active compounds. Regulatory role in the resistance and protection of agrocenosis is studied in the example of chlorocholinechloride (CCC) and phytohormones: 24-epibrassinolide (EpiBS), gibberellins (A3). Their functioning and efficiency in "soil - plant" system is connected with a series of abiotic (temperature, nutrient elements, etc.) and anthropogenic (organic pollutants, etc.) variable factors. The complexity of agro-chemical and ecological studies presupposes the widening of methodical approaches. The wide use of bio-testing methods for summary characteristics of agrocenosis functioning allowed the revealing of protective effects of CCC, EpiBS, A3 of the, which are widely implemented today into the practice of agriculture. The protective effect peculiarities of CCC were studied at a high load of agrocenosis by chemical means of plant protection. It with the reduction of total toxicity in soil, root system, in plant and reproductive organs were revealed during using CCC Correlation of biological activity by bioassay used in agricultural lands, was found, depending on the plant nutrition system. Therefore, foliar effect of CCC influences on the plant, changes the activity of root system functioning (growth, absorption and root excretion), that actively effects on the dynamics of microbial association. The important ecological role of exogenous use of EpiBS phytohormone was proved experimentally. The optimum created in agrocenosis artificially (providing plants with elements of mineral nutrition) is shifting under the influence of abiotic factors of external medium (temperature, humidity, light, etc.). EpiBS takes part in the regulation of plant adaptation by constantly changing the complexity of these factors, depending on each other. Use of EpiBS is compatible with gibberellins (A3). Efficiency from their joint use is manifested in personal contribution of each to the performance of definite functions in plant ontogenesis. Thus, the biologically active compounds directly and indirectly affect the stability of agrocenosis. Intensive implementation of plant growth regulators into the practice of agriculture is connected with their expressive, smoothing and systematic effect.

TU 188

Biochemical responses in the aquatic macrophyte Myriophyllum quitense exposed to the strobilurin fungicide azoxystrobin

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Strobilurin fungicides constitute a relatively new fungicide class developed from natural fungicidal derivatives and the great impact of them on agriculture is reflected by the widespread use of azoxystrobin (AZX), a compound approved for use on almost 100 different crops. Its mechanism of toxicity is well understood in fungi but it effects in plants is scarcely studied. Aquatic macrophytes have been used as environmental biomonitors because of its bioaccumulation capacity of xenobiotics. The main goal of this study was to establish a concentration- response curve of AZX in *Myriophyllum quitense*, by using oxidative stress enzyme activities and chlorophyll content. Particularly, the activity of Guaiacol Peroxidase (POD), Glutathione- S -Transferase (GST), Catalase (CAT), and the chlorophyll a and b contents were tested. Plants were exposed to 0 (negative control); 0.1; 1; 10; 50 and 100 µg/L of AZX for 24 hours. All the parameters were measured spectrophotometrically. Inhibition of CAT and POD at 50 µg/L was observed (p<0.05), showing the stress condition exerted by this fungicide. GST activity was not significantly different to the control at any concentrations tested (p>0.05). Although there was no change in the chlorophyll a/chlorophyll b ratio among treatments an increased content of both pigments at 100 µg/L with respect to control was observed (p<0.05). Our results shows the importance of using biomarkers of different sensitivity, in the evaluation of potential negative effects of emergent agrochemicals in the environment, being a more detailed studied highly recommended.

TU 189

Impact on surface water, stream sediments and macrophytes from the Aljustrel mining area (Portugal)

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The main environmental problem in the vicinity of Aljustrel mining area, as in other massive sulphide deposits of the Iberian Pyrite Belt, comes from the tailings deposited at the site which contain large concentrations of trace elements (namely As, Cu, Pb and Zn) and can react with oxygen and water producing acid mine drainage (AMD). The AMD produced will end up reaching the downstream areas, affecting surface water, groundwater and stream sediments. That impact is evident at Água Forte stream, which receives the AMD from the Aljustrel mine area, a tributary of the Roxo stream.

The aim of the study was: a) to evaluate the extent of pollution in stream waters and sediments at Água Forte and at Roxo, and b) to assess the potential of the macrophyte *Scirpus holoschoenus*, collected along the stream banks, for phytoremediation of AMD. Several sampling sites were selected at Água Forte stream and at Roxo stream, upstream and downstream from the confluence of the Água Forte stream. Water quality was assessed considering physico-chemical characteristics and ecotoxicological parameters. Sediment samples were analyzed considering general physico-chemical characteristics and a sequential extraction procedure was used to quantitatively assess trace element pool (As, Cu, Pb and Zn). *Scirpus holoschoenus* samples were analyzed in order to assess trace element concentrations in the aboveground plant material.

Both surface water and sediments were extremely acidic at Águia Forte stream, with pH values <2.92 for surface water and <3.32 for sediment samples. Surface water at Roxo stream presented pH values >7.26, apparently not affected by the confluence of the Águia Forte stream, but the same was not true when considering the pH and other characteristics of the sediments. Ecotoxicological bioassays (luminescence inhibition of *Vibrio fischeri* and 48-h immobilization/mortality assay with *Daphnia magna*) were consistent evidencing that water samples collected at the Águia Forte stream were extremely toxic, with very EC50 values. Although high As, Cu, Pb and Zn concentrations were found at Águia Forte stream, the same was not true in Roxo stream. Trace element content in water, sediments and *Scirpus holoschoenus* samples was analyzed and correlated in order to ascertain if this particular macrophyte, which seems adapted to the extreme conditions found at Águia Forte stream, can be potentially used for metal removal in constructed wetlands to treat AMD.

TU 191

Species-specific responses to zinc in Lemnaceae: zinc storage and impacts on photosynthesis along frond developmental gradients

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Lemnaceae are used to represent all macrophytes in standardised aquatic toxicity testing, with protocols allowing for the use of different species. Recently, differential sensitivity in Lemnaceae species to zinc, based growth and chlorophyll-a fluorescence parameters, has been reported. Chlorophyll-a fluorescence is an effective tool for sensing and assessing the impact of metals on the photosynthetic apparatus, used extensively to investigate effects of various toxins on Lemnaceae. Plant age and developmental stage strongly influence the effects of metals on the photosynthetic apparatus. However, there is little information on how this may affect toxicological assessment in plants. Zinc accumulation, morphological distribution and cellular storage, in either bound or soluble form, are also important for zinc sensitivity/tolerance and will be affected by increased zinc concentrations. Differential metal sensitivity of plant species has been linked with differences in accumulation and distribution. This study investigates how frond age and developmental stage, as well as zinc accumulation and storage, determine the impact of zinc on Lemnaceae. The maximum quantum efficiency of photosystem II, Fv/Fm, the effective quantum efficiency, Y(II), and photochemical quenching, qP, were measured in mature and young fronds as well as along a developmental gradient within fronds over seven days. Zinc accumulation and storage patterns (assessed as (i) zinc stored in fronds and roots and (ii) zinc stored in the soluble and bound forms) in the plants were also measured after seven days. *L. punctata* and *L. minor* accumulated more zinc in their tissues than *L. gibba*. Partitioning of zinc in *L. minor* was notably different from the other two species, zinc was stored mainly in roots and the bound-fraction. Fv/Fm and Y(II) in young fronds were more severely impacted by zinc than in mature fronds in all species. However, younger proximal sections of *L. punctata* fronds were more impacted than older distal frond sections compared to more homogenous impact in single Lemna fronds. Single colony, time-point or leaf-zone analyses may not show the full biological picture of the impact of a toxicant, especially not in *L. punctata*. Therefore, selection of Lemnaceae species and developmental stages of fronds should be given careful consideration when using chlorophyll-a fluorometry for assessing chemical toxicity in Lemnaceae.

TU 192

Alleviative effects of magnesium on copper rhizotoxicity to grapevine: macroscopic and microscopic point of view

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Intensive and long-term application of copper (Cu)-containing fungicides (e.g. Bordeaux mixture) in vine-growing areas has led to the accumulation of Cu on the surface of some vineyard soils around the world, thus posing the problems of Cu toxicity to the organisms therein. Recently, a number of studies have found that some cations, such as magnesium (Mg) and calcium, may alleviate the rhizotoxicity of Cu to plants. In this study, a hydroponic experiment was conducted to determine the influence of Mg concentration on the growth inhibition effects of Cu to grapevine roots. In addition, optical microscopy was used to examine the histological changes in root tissue at the cell level under Cu stress. Results indicated that the median inhibition concentration of Cu for root elongation increased significantly with increasing solution Mg concentration. On the other hand, the Cu concentration in roots was negatively correlated with the solution Mg concentration. From the light micrographs of root tip cells, the Cu concentration that caused histological changes in rhizodermal cells (i.e., increased vacuolization and plasmolysis) ranged from 10 µM, in the 0.2 mM Mg treatment, to 25 µM, in the 4 mM Mg treatment. In the 8 mM Mg treatment, no histological change was observed even at an exposure concentration of 25 µM Cu. In conclusion, this study demonstrates that Mg can alleviate Cu rhizotoxicity to grapevines effectively.

TU 193

Metal effects in *Fraxinus angustifolia* and its endophytic communities

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Soil contamination with metals is a serious problem requiring urgent measures to counteract the impacts. The conventional soil remediation techniques are mostly inefficient and expensive. Phytoremediation comes as a promising alternative with a great potential for recovering ecosystem services. Besides looking for the best suitable plant, remediation programs should also focus on the microbial communities associated with the roots of the plants, namely endophytic and rhizosphere bacteria. In this work, we studied the potential of the species *Fraxinus angustifolia* for the phytoremediation of metal contaminated soils from the Cunha Baixa uranium mine (Mangualde, Portugal). The strategy consisted of the exposure of the plants to the contaminated soil, a reference soil and a control soil, for a period of about 3 months. To access the ability of *F. angustifolia* to tolerate the contamination and its physiological performance, a set of parameters (above-ground growth, leaf area, relative water content, maximum efficiency and quantum yield of photosystem II and leaf chlorophyll a and b, carotenoid, proline, and malondialdehyde contents) was measured during the test. Furthermore, the genetic profiles of the endophytic communities from the roots of the plants were also analyzed. This analysis was made via PCR-DGGE technique, targeted for a conserved region of 16S rDNA, pre- and post-exposure. Only above-ground growth was a suitable indicator of the negative effects of the contamination on the plants throughout the experiment. The remaining parameters were able to indicate the physiologic status, including recovery to a normal state. The comparison of the bacterial profiles pre- and post-treatment showed notable changes, mainly for plants exposed to the contaminated soil. The bacterial profiles from the control and reference soils showed more similarity with each other and with the pre-exposure profiles than with those from the contaminated soil. This study showed that *F. angustifolia* plants have the ability to resist and adapt to the adverse conditions of contamination, revealing a potential which can be exploited for phytoremediation. It also revealed that changes exerted on the bacterial root communities exposed to contamination, resulted in profiles considerably different from those of the remaining communities. The performance of the plants might be related to these microbial alterations.

TU 194

Dose-dependent effects induced by uranium (U) at pH 4.5 in *Arabidopsis thaliana*

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To evaluate the environmental impact of U-contamination, it is important to unravel the mechanisms by which plants respond to U-stress. It was already shown that U-exposure at pH 5.5 can disrupt the cellular redox balance and induce oxidative stress related responses in *Arabidopsis thaliana* plants (Vanhoudt et al., 2008). However, U-speciation and as such its toxicity strongly depend on environmental parameters such as pH. In a previous experiment, it was shown that U-toxicity at pH 4.5 was higher than at pH 7.5. Therefore, we want to investigate dose-dependent effects at low pH.

Arabidopsis thaliana plants were exposed to U-concentrations ranging from 0 to 100 µM at pH 4.5 during 3 days. U-concentration, fresh weight, lipid peroxidation and photosynthetic efficiency were analysed. On protein level, the enzymes of the antioxidative defence system were analysed to evaluate the importance of the cellular redox balance in *Arabidopsis thaliana* plants exposed to U.

Results indicate that the U-content in roots and shoots increased with increasing U-concentration added to the nutrient solution. However, there was a low root-to-shoot transfer. Fresh weight of roots and leaves decreased after exposure to 50, 75 and 100 µM U. In contrast, plants exposed to 6.25 and 12.5 µM U had an increased fresh weight as compared to the control plants, which alludes to a hormesis effect as was observed before (Vanhoudt et al., 2008; Straczek et al., 2009).

One of the most important physiological processes in plants is photosynthesis. Apparently, the photosynthetic efficiency of plants exposed to 25-100 µM U was increased. This indicates that the photosynthetic system is not damaged but in contrast plants try to optimize their photosynthesis under U-stress.

In addition to the reduced biomass production, plants exposed to 25-100 µM U showed an increased lipid peroxidation. This increase indicates an affected membrane integrity and functionality. Assessing antioxidative enzyme capacity of the plants indicated an increased defence against ROS in the U-exposed plants as evidenced by increased activities of ROS scavenging enzymes. The increased activity of guaiacol peroxidase could indicate an increased cell wall lignification as a defence reaction that limits the entry of toxic metals. In conclusion, this study indicates that elevated U-concentrations at low pH can cause important morphological, physiological and biochemical effects in *Arabidopsis thaliana* seedlings.

TU 195

The effect of pharmaceutical active compounds on oxidative stress response enzymes of the macrophyte *Ceratophyllum demersum*

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Pharmaceutically active compounds (PhACs) in the environment have recently been acknowledged to potentially constitute a health risk terrestrial and aquatic ecosystems and thus ultimately for humans. The occurrence and fate of selected human PhACs belonging to different therapeutic classes (non-steroidal anti-inflammatory drugs, anti-epileptics, contraception's and β -blockers) have been detected in many countries in sewage treatment plant effluents, surface waters, seawaters, groundwater and some drinking waters. The biological effect of even low concentrations of PhACs on non-target organisms, such as aquatic macrophytes has so far received little attention. In nature aquatic organisms are constantly exposed to xenobiotic compounds that cause the overproduction of oxidants or reactive oxygen species (ROS) resulting in oxidative stress. Therefore, we have investigated the detoxification enzyme glutathione S-transferases (GST) in the plant extract to metabolize PhACs in vitro and the results shown the inhibition of GST activity under the influence of the used PhACs. The data of the peroxidase (POD) and catalase (CAT) suggests that PhACs do not directly cause oxidative stress by an increase in ROS production. Due to increased ROS accumulation PhACs may be selectively toxic to some organisms leading to ecosystem alterations.

TU 196

The toxicity of iron nanomaterials to aquatic and terrestrial plants

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Zero-valent iron (Fe⁰) as well as ferrate (FeV, FeIV) nanoparticles are well known especially for the ability to degrade a wide range of contaminants in both soils and ground waters. Although these materials are widely used in remediation processes across the world, almost no data are published about their ecotoxicological properties. This study aim was to provide new information about their effects on both aquatic (*Lemna minor*) and terrestrial (*Sinapis alba*) macrophytes. Our experiments show large differences in toxicity between tested species as well as oxidation state of nanomaterials. The higher an oxidation state the more pronounced toxicity and the higher EC50 values have been found, following the order as FeV > FeIV > Fe⁰. We used the frond numbers, root length and dry weight after 7 days of incubation as endpoints of the inhibition tests.

TU 197

Selection of suitable aquatic plants for phytofiltration of arsenic-contaminated water

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The work presented here is part of a larger on going study about the metals and metalloids accumulation in aquatic plants of uraniferous geochemical province of Central Portugal. It is oriented for the use of aquatic plants as indicators of metal contaminated waters and their potential use in phytoremediation.

In submerged and free-floating plants the concentrations of As were much higher than in emergent plants, with the exception of *Oenanthe crocata*. The highest concentrations of As were found in the submerged species *Callitriche brutia* (436.92 mg/kg DW), *Callitriche stagnalis* (354.03 mg/kg DW), *Callitriche hamulata* (160.37 mg/kg DW), *Ranunculus trichophyllus* (268.53 mg/kg DW), *Ranunculus peltatus* (103.98 mg/kg DW), in the free-floating *Lemna minor* (279.42 mg/kg DW), and in emergent plant *Oenanthe crocata* (157.94 mg/kg DW). The measured concentrations in most of the emergent plants, such as *Apium nodiflorum*, *Typha latifolia*, and *Juncus effusus* were significantly lower when compared with the previously species, even in the rhizomes/roots. Other metals, such as Cu and Zn, are also accumulated by plants from the Callitrichaceae family, namely, Cu by *Callitriche verna* and Cu and Zn by *Callitriche stagnalis* with maximum concentration values of 132 mg/kg and 1395 mg/kg in dry biomass, respectively.

The abundance of *Callitriche stagnalis* and the ability to accumulate arsenic and several heavy metals at the same time made this plant our first choice for rhizofiltration methodologies development.

TU 198

Genotypic variation in metal-tolerant *Silene vulgaris* clones

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Silene vulgaris is a facultative metallophyte with multiple tolerance and co-tolerance to heavy metals, capable of colonizing contaminated sites due to its fast and vigorous growth by producing seeds and rhizomes. In order to evaluate its potential for the purposes of phytoremediation, genetic approaches are required to uncover the allelic variation at both Mendelian and quantitative genes. Analytical tools based on the identification of useful genetic diversity are needed for plant genotype selection aimed at enhancing phytoremediation efficiency.

This study considers the genetic relationships among different *S. vulgaris* clones. Fourteen clones from ten populations collected from Madrid (Spain) were analysed using chloroplast DNA (cpDNA) markers. Ten primers pairs chloroplast SSR loci were tested and 3 out of the 10 primer pairs revealed the existence of intraspecific length polymorphisms. The intergenic spacer between the *trnH* and *psbA* genes was amplified with the primers *trnH* (GUG) and *psbA*. All PCRs produced a major robust band per primer pair in all samples studied. Two size variants were detected for ccmp1 (120 and 121pb), two for ccmp4 (113 and 114pb), five for ccmp2 (190, 191, 192, 193 and 198pb) and six for *trnH/psbA* (328, 338, 349, 354, 355 and 357pb).

Regarding haplotypic diversity, ten unique cpDNA haplotypes were identified and seven of them were private, being found in only one population. Despite de limited number of clones examined, a considerable intraspecific polymorphism was detected and can be explained by *S. vulgaris* out-crossing breeding system. Conservation of genetic resources in this allogamous species requires maintenance of the genetic diversity within each clone to avoid inbreeding depression and loss of rare alleles.

TU 199

Biosorption of Pb by *Chlorella vulgaris* (Chlorophyceae) at laboratory scale

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In the lower Salado River (Santa Fe Province, Argentina) were reported levels of Cu, Cr and Pb in water, higher than permitted standards, thus highlighting the urgency of implementing remedial measures. Among the conventional methods to capture metal ions from aqueous solutions are ineffective when the volume of wastewater is high and the concentration of metal ions to be removed is low (1-100 mg/L). An alternative is biosorption technology, using materials of biological origin. In the present study we analyze the efficiency of *Chlorella vulgaris* (Chlorophyceae) to remove Pb at laboratory scale.

The microalgae were harvested in exponential growth phase, centrifuged and resuspended twice in ultrapure sterile distilled water. After measure of algae concentration, we used three real concentrations of Pb+2 (Pb (NO₃)₂: 1.951 [C1], 2.826 [C2] and 4.830 [C3] mg/L with their respective controls, all in triplicate (50 mL-1). The vessels were maintained in an incubation chamber under controlled temperature (23±1°C), continuous illumination (3000 lux) and daily shaking. At 10 and 30 min, 1, 12 and 24 h of exposure, they were centrifuged for 10 min at 3000 rpm, separating the supernatant from the pellet of algae. Both matrices were analyzed by atomic absorption spectrophotometry. For each time and concentration, we calculated the % of removal, the concentration factor (CF= [metal algae] / [metal water]). Pearson correlations were performed to relate the concentration of Pb accumulated by *C. vulgaris* and the remaining in the supernatant.

C. vulgaris removed 50% and 47% of Pb at 10 min of exposure; 100% and 87% at 1 h exposure in C1 and C2 respectively. The maximum percentage (72 %) was obtained at 12 h and 24 h in C3. After 12 h, we observed desorption of Pb, with concentrations ranging from 2.62 mg/L to 2.58 between 12 h and 24 h in C2. In C3, 3.27 mg/L (12 h) and 3.01 mg/L (24 h). The CF in C1 was >1 at 1 h of exposition, in C2 at 12 h was 0.93, falling slightly at 24 h. In C3 the CF was <1 in all the treatments, with higher values at 12 h (0.66-0.68) and decreasing at 24 h (0.61-0.63). There were significant negative correlations between the final concentration of Pb in algae and in the supernatant: $r = -0.849$, $p < 0.0001$; $r = -0.880$, $p < 0.0001$; $r = -0.880$, $p < 0.0001$ in C1, C2 and C3 respectively. *C. vulgaris* is efficient to reduce Pb concentrations from the order of ppm to ppb from aqueous solutions, so it is recommended for wastewater treatment at a larger scale.

TU 200

Bioaccumulation and distribution of selected metals in the macrophyte *Ceratophyllum demersum*, in a polluted South African river

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Selected metals were measured in the submerged macrophyte *Ceratophyllum demersum*, introduced to the Diep River, Cape Town, South Africa. The Diep River passes various possible sources of metal pollution before reaching the Atlantic Ocean. Previous studies on this river have shown high levels of metal contamination, however, studies on metal bioaccumulation in aquatic biota such as plants are limited. Plants were obtained from a reference site and placed in baskets at two sites along the river (one upstream and one downstream from urban pollution sources). Plants, sediment and water were collected every fortnight for twelve weeks. Samples were analyzed for aluminium, iron, zinc and copper using nitric acid digestion and analyzed by means of ICP-MS. The results generally indicated that plants collected upstream had significantly higher concentrations of all four metals, compared to those collected downstream, possibly due to intensive agricultural activities upstream and the filtering effect of aquatic macrophytes between the two sites. Also, *C. demersum* generally rapidly bioaccumulated higher concentrations of metals in its leaves, compared to stems, possibly as a result of having a greater surface area for uptake. Additionally, metal-laden leaves may be shed as a detoxification method. In conclusion, it is clear that the metals in the Diep River are bioavailable for uptake in aquatic biota. Also, *C. demersum* may possibly be used in biomonitoring programmes. However, further research is necessary to investigate the toxic effects of the metals and how it relates to metal exposure.

TU 201

Selenium pathway in water moss *Fontinalis antipyretica*

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Water mosses are good indicator of polluted watercourses. We investigated the uptake of Se in widely distributed truly water moss *Fontinalis antipyretica* in its natural environment. Nine locations in Notranjska region, Slovenia, with different land use in the catchment, were sampled. Samples of water and moss were taken in all four seasons

during years 2009, 2010 and 2011. The concentration of Se in water from all locations did not exceed 0.2 µg Se L⁻¹. *Fontinalis antipyretica* took up Se in the range between 343 - 3039 ng Se g⁻¹ (on dry matter basis; DM). The Se content varied, depending on the location and season. The highest content of Se was measured in the stream of Zerovnišćica that flows through agricultural area with pastoral farming, the value being 3039 ± 170 ng Se g⁻¹ (DM). The amount of insoluble Se compounds after enzymatic hydrolysis using Protease (XIV) was around 75%. In soluble Se compounds only traces of Se (IV) and Se (VI) were found. No other Se compounds (SeMeSeCys, SeMet) were detected.

TU 202

The organic matter contribution of salt marsh vegetation to coastal wetland ecosystems: a case study in Jiangsu, China

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Stable isotope techniques and different calculation models were used to analyze the potential sources of sediments organic matter in the core area of primary wetland and to calculate the contribution of salt marsh vegetation. By using multiple resources linear mixing model and the Euclidean distance model, we can conclude that micro-algae and *Spartina alterniflora* are the main contributors, under the assumption that the δ¹³C value of micro-algae is -23‰, the average contribution rate of micro-algae is 40%, which is the main contributor to the salt marsh wetland ecosystems in northern Jiangsu. The contribution rate of *Spartina alterniflora* to its marsh is 56% and to the intertidal is 57%. By comparing the two quantitative models, we can find that the Euclidean distance model would overestimate the contribution of incidental resources and average the resource of high contribution rate.

TU 203

Genotoxicity study on *Vicia faba* L. grown on natural and spiked contaminated soils

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Contaminant bioavailability and toxicity varies among soil types and may depend on soil properties and on contaminants characteristics. Metals contamination could have different potential risks depending not only on the total content but on their bioavailability. Operationally, metal bioavailability is often evaluated by chemical extractions characterizing different chemical forms. The use of plant bioassay may be an amenable tool to screen the phytotoxicity of contaminated soils by metals.

In this work different soils contaminated by Boron (B) were studied in relation to plant bioavailability and toxicity. B is an essential micronutrient for plants and generally it is absorbed from soil in the form of boric acid. Previous studies demonstrated that both excess or deficiency of B can affect normal plant development.

Natural B polluted soils and artificially B spiked soils, with B total concentrations ranged between 20 and 100 mg/Kg, were tested on *Vicia faba* L., plant commonly used for detecting the genotoxic effects of environmental pollutants. Artificially contaminated soils showed the highest B bioavailability (about twofold) when compared with the natural polluted soil at similar B total content. Cytological analysis was carried out on root tip meristems of *Vicia faba*, after 3 days of seed germination in the different B polluted soils; mitotic index and micronucleus assay (MNC) were determined for genotoxicity evaluation. Moreover, developmental and physiological parameters were analyzed during plant growth in the B contaminated soils. The B content in distinct organs of the plants was determined and compared with B content in soil (distinguishing the total from the bioavailable) and with the genotoxicity effects.

The results showed the close relationship between B bioavailability, genotoxicity and B content in the plant. The natural polluted soil and spiked soil with similar B content in the bioavailable fractions showed the same pattern of phytotoxicity and genotoxicity in *Vicia faba*.

It is interesting to stress that bioavailability of B, and its content in the different plant organs, resulted higher in artificially spiked soils when compared with the same B concentrations in natural soils.

A close correlation between high B concentration in roots and major level of cytogenetic defects was found. These results confirmed plant genotoxicity of B polluted soils and showed clear evidences between soil properties, B bioavailability and phytotoxicity.

TU 204

Acute and chronic in vitro bioassays vs. autochthonous plants used as sentinel: comparing genetic biomarkers as tools in ecological risk assessment in Del Plata basin

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Del Plata basin is the fifth largest river basin in the world, its area covers four million km² approximately and contains a great variety of natural environments and resources; these resources are shared by Bolivia, Paraguay, Brasil, Uruguay and Argentina.

The lack of wastewater treatments from 100 million inhabitants is the main source of chemical and biological pollution that impact on all tributaries and affects negatively to the biota.

Samples of surface river water from along 1200 km of river coast were analyzed according APHA, AWWA, WEF (1992); water quality indexes (WQI) were calculated. Value were adjusted to Argentine guidelines and sampling sites were qualified, being Excellent (95-100) 11%, good (80-94) 21%, Fair (65-79) 26%, Marginal (45-64) 26% and Poor (0-44) 16% of sampled places. Bioassays using water from Uruguay and Río de la Plata rivers were performed to evaluate toxicity. Allium cepa were exposed chronically and semi-statically while *Latuca sativa* assay was acute and static according IRAM standards. Samples of *Echinodorus uruguayensis* Arechav. (Alismataceae), *Eichornia crassipes* (Mart.) Solms (Pontederiaceae), *Pistia stratiotes* L. (Araceae), *Scirpus giganteus* Kunth (Cyperaceae) were fixed in field to evaluate biomarkers. The biomarkers included in this study were chromosome count, chromosome characterization, occurrence of cytogenetic abnormalities and Mitotic Index calculated as (MI= # total of dividing cells x 100/nro total of observed cells) were studied. An average of 1800 + 500 cells were analyzed per individual. Uni- and Multivariate analysis were applied.

Pearson was a pristine site localized downstream of the Río de la Plata and it was considered as reference point. While chronic bioassays showed higher MI in samples from Uruguay River, samples of water from the Río de la Plata were characterized by abnormalities in mitosis. Classical end points might be masked by abundance of nutrients in water, in fact, differences were registered among, overpopulated and poor populated areas, industrial and agricultural developing areas. On one hand we found higher responses to multiple stressors in laboratory bioassays than in field specimens indicating an overestimation of water toxicity. Our results revealed that different sources of pollution depending on the characteristics of field could show similar results in some biomarkers in situ but integrating data with field results and WQI it could be possible to assess the ecological status.

RA20P - Risk assessment of chemical mixtures: where do we stand? what are the next steps?

TU 205

Can we possibly derive environmental quality benchmarks for chemical mixtures?

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In reality, many chemical pollutants are indeed coexisting in the aquatic environment. For example, many antifouling biocide residues are often detected as a cocktail in water and sediment samples collected from coastal environments. Based on literature review of documented studies on the combined ecotoxicity of antifouling biocides, we found that both additive and synergistic effects together account for 80% of all cases in which about 35% cases are synergistic. To allow more accurate risk assessment of concurrently occurring chemicals, there is a need to develop environmental quality benchmarks (EQBs) for their mixtures. In this presentation, I will introduce and discuss several possibilities of deriving EQBs for chemical mixtures. First, if all components in a chemical mixture are known to share a similar toxic mode of action, we can assume that the combined toxicity of the mixture would follow a simple concentration addition model, and the concept of toxic equivalency quotient (TEQ) could be applied to derive the EQBs based on lethal and/or effect concentrations expressed in terms of TEQ and/or TEQ concentration. This method has been applied to polychlorinated biphenyls, dioxins and dioxin-like compounds, and chlorinated polycyclic aromatic hydrocarbons. Second, if the mixtures contain chemicals with different toxic modes of action, it is possible to explore the use of the multidimensional species sensitivity distribution (m-SSD) approach. Here, I use binary mixtures of copper (Cu) and zinc pyrrithione (ZnPT) as an example to illustrate the m-SSD method. We first conducted standard acute toxicity tests with an array of marine organisms for each chemical alone, and for their mixtures. The Cu-ZnPT mixtures showed a strong synergistic toxic effect to all test organisms. By utilizing all the toxicity data, we are able to construct a two-dimensional SSD in form of a response surface, from which we can derive any specific hazardous concentration (i.e., EQB) for the two compounds. This novel method can be potentially applicable to a more complex mixture by employing non-parametric response surface models. Third, I will highlight the field-based SSD approach, which is integrated with the quantile regression method, can be used to derive sediment quality guidelines for any target chemical with consideration of the presence of chemical mixtures and biological interaction. Finally I will discuss the pros and cons of each described method.

TU 206

Evaluation of available frameworks for mixture risk assessment in biocide and plant protection products in the EU

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Pesticides are regulated in the EU by the Biocide Product Directive (98/8/EC) (BPD) and the Plant Protection Product Regulation (Regulation (EC) No 1107/2009) (PPP Reg).

A new Biocide Regulation (BP Reg) is proposed and will likely come into force during 2013. Environmental and human health risks are evaluated before a product may be authorised and sold on the European market. Simultaneous exposure to several substances based on the use of biocidal products and plant protection products are to be evaluated, according the PPP Reg and the BP Reg, respectively. This is more explicitly stated in the BP Reg compared to the BPD.

Guidance on how to perform mixture risk assessment on biocide or plant protection products is very limited. However, several frameworks for tiered risk assessment of the combined effect of substances within products have been suggested or are being developed by e.g. WHO-IPCS and Competent Authorities from several EU Member States, such as DE, DK, ES and SE.

In the present work several such frameworks have been evaluated for risk assessment of both environmental and human health. We have used model products which are based on actual biocide or plant protection products, and the outcomes of the evaluations are compared from a regulatory decision making perspective. The suggested frameworks are all based on tiered approaches that include various levels of precaution and conservatism in order to be sufficiently protective of human health and of the environment

even in data poor situations. The evaluation of the frameworks provides estimates of how protective and conservative they are in realistic regulatory assessments for both human health and environmental risk assessment. The various frameworks and decision trees are easier to apply for risk assessment on higher biological organization, i.e. easier to apply to environmental risk assessment than to human health risk assessment. Suggestions on how to prioritise refinement efforts in the product dossiers should be included in forthcoming guidance.

TU 207

DPD+ - an appropriate method to assess the risk for mixtures under REACH?

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According to REACH chemical substances need to be registered at the European Chemical Agency in Helsinki. For hazardous substance produced in amounts above 10 t / y the manufacturer or importer of the substance has to conduct a risk assessment and document the assessment in a chemical safety report (CSR). Operational conditions (OC) and risk management measures (RMM) for the safe use of a substance are documented in exposure scenarios (ES). ES are attached to the CSR. In addition, manufacturer or importer have to create a safety data sheet (SDS) for substances to communicate conditions of safe use within the supply chain. ES are attached to the SDS. The formulators must assess the risk for the mixture they produce and create a SDS for the mixture on the basis of the information they get with the SDS and ES for the single substances. Although emissions to the environment results mainly from mixtures there is no guidance to assess the risk resulting from the use of mixtures. Industry developed the DPD+ method (based on the dangerous preparation directive) to assess the risk from mixtures. Aim of the method is to consolidate the information in the SDS and ES of the single substances to derive conditions of safe use for the mixture. The method is based on the identification of a lead substance based on the classification of the substances in the mixture. Substances without classification are not considered in the derivation of OC and RMM for the mixture. The Federal Environment Agency, Germany (UBA) conducted a study to analyse the DPD+ method. The analysis tried to identify which potential risks of a substance could be overlooked by DPD+ and would hence not be adequately addressed in the selection of lead substances and the derivation of safe conditions of use. The above considerations question if DPD+ is a reasonable instrument to facilitate the consolidation of environmental information on mixtures. Proposals for possible improvements of DPD+ are mentioned above. This proposals need to be further elaborated and included in a method to assess the risk of mixtures. The interpretation of DPD+ (only substances with classified for environmental hazards need to be addressed in the risk assessment for the environment) is regarded as not in line with the interpretation of the legal text by Commission, ECHA and MS. Beyond that the possibility of an integration of scientific methods for the assessment of mixtures in the regulatory framework should be investigated.

TU 208

Application of the TTC Concept to focus cumulative exposure assessment in environmental media: a special case for pesticides?

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The concept of Threshold of toxicological concern (TTC) is based on establishing an exposure level for chemicals below which no significant risk is to be expected. This level is so low, that a risk assessment could be based only on structural or de-minimis considerations, even in the absence of effect data. Originally it was applied to toxicological evaluations of substances such as flavourings, food contact materials, and for the risk assessment of chemicals (EFSA, US FDA, WHO IPCS). Later it was adapted for ecotoxicology, and an "environmental threshold of no concern" (ETNCaq) of 0.1 µg/L was proposed for polar narcotics, non-polar narcotics and also reactive chemicals (de Wolf et al. 2005). However, because of their specific MoA pesticides were considered as different and some showed lower NOECs and thus were not included.

The TTC concept could be helpful to focus the assessment of chemicals that occur in environmental media. The recent SCHER opinion on Toxicity and Assessment of Chemical Mixtures recommended that no further risk assessment is needed for substances that do not exceed the TTC. Thus the TTC could serve as a filter to avoid making mixture toxicity assessment unnecessarily complex and laborious by including a multitude of substances which are not relevant for the assessment.

An analysis of the Bayer CropScience portfolio suggests that regulatory acceptable concentrations (RACs) for most pesticides are well above the ETNCaq of 0.1 µg/L suggested for chemicals other than pesticides. However, the original application of the ETNCaq was to derive safe thresholds in the absence of effect data, whereas for pesticidal active substances robust acute and chronic ecotoxicity data are always available. Thus the limited number of substances that have lower threshold of concern can easily be identified and should then be included separately in a mixture toxicity assessment, even if present at concentrations below the ETNCaq. The application of the ETNCaq as a generic TTC in the field of mixture toxicity assessment in environmental media would primarily serve to filter out irrelevant contributors, not to substitute measured effect data.

That TTC for surface water could also guide where to set the generic LOD in monitoring studies rather than to try to detect substances at levels "as low as possible". Further analysis is needed to assess whether a generic TTC of 0.1 µg/L is suitable and this should be tested on relevant real-world cases.

TU 209

Relevant potency threshold: reducing uncertainty by calibration of cumulative risk assessments

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Relative approaches, i.e., toxic equivalent (TEQ)-like approaches, for mixtures risk assessment have been established for some time. TEQ-like approaches assume that if certain premises are met, e.g., chemicals are true congeners, are metabolized and detoxified by the same biological processes, produce the same spectrum of biological effects by the same mode of action, and exhibit parallel dose response curves for the biological effect being modeled (Safe 1990), then those chemicals may be assumed to be dose additive (DA) for specific toxic effects. In 2008, the National Research Council published a report entitled "Phthalates and Cumulative Risk Assessment: Tasks Ahead," containing recommendations for cumulative risk assessment practices at the U.S. EPA. The Panel rejected the underlying premises of TEQ-like approaches, asserting that cumulative risk assessment should be conducted by applying DA to chemicals that produce "common adverse outcomes" (CAOS) rather than to chemicals with similar modes or mechanisms of action. Using the data available, published mixtures data upon which NRC based its recommendations and a published human health risk assessment that used the DA-CAOS concept were evaluated to determine how firmly the concept is supported by the data and with what level of certainty the results may be extrapolated to lower doses and to humans. The underlying assumptions of the model and the risk assessment were probed for consistency with available human data, using data for two human pharmaceuticals as test cases. Predictions based on the DA-CAOS concept were compared with human clinical experience to determine whether those predictions are verifiable and consistent. Based upon this analysis, an alternative approach was developed - the Human-Relevant Potency-Threshold (HRPT) - that appears to fit the data better and avoids the contradictions inherent in the DA-CAOS concept. The proposed approach recommends application of independent action for phthalates and other chemicals with potential anti-androgenic properties at current human exposure levels. This example should be instructive for the inevitable discussion of the suitability of DA-CAOS for addressing environmental cumulative risk assessment for hormonally active agents, and environmental mixtures in general.

TU 210

Application of delayed fluorescence to estimate influence of chemical mixtures on alga

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Algae are important primary producers in aquatic ecosystems. The OECD Test Guidelines 201 (TG201), alga growth inhibition test, is a conventional method that evaluates chemical toxicity on alga. The conventional method evaluates influence of chemical substance on algal growth from a 72 h exposure. In the study of chemical mixtures, a method that can estimate chemical toxicity in shorter time or provide information on the mode of action of chemical substance would be advantageous. Applications for a new method include improving the evaluation efficiency for toxicity screening of a large number samples for regulatory filings, toxicity identity evaluation of chemical mixtures, and assessment of effluent toxicity. We are investigating a new rapid estimation method of the growth inhibition using delayed fluorescence (DF) from algae. The DF is a special type of luminescence; it detects the growth of only those cells that have photosynthetic capability. Since the DF originates from re-excitation of chlorophyll by a reverse reaction of photosynthetic electron transfer, the DF inhibition reflects inhibition of photosynthetic activity that is necessary for growth. Therefore the DF is a potential endpoint for estimation of the influence of chemical substance of algal growth in a shorter time than the conventional 72 hours growth inhibition test (e.g. TG201). In addition, the intensity of DF shows a time decay curve. Chemical exposure changes the decay curve to patterns that provide insight into the mechanisms of action, and also has potential for application to the identification of contaminants in mixtures. We discuss characteristics of the evaluation of the influence of chemicals (herbicide, other chemical compounds and its mixtures) on alga by both the DF inhibition and analysis of the DF decay curve. We also compare the detection characteristics of DF and other rapid estimation methods, including chlorophyll fluorescence-based photosynthetic activity measurement (PAM), and Microtox®, a common rapid bioassay that uses luminescent bacteria to evaluate complementary sensitivity between photosynthetic and non-photosynthetic microbial bioassay

TU 211

Species sensitivity distribution for the prediction of herbicides mixtures toxicity on benthic diatoms

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Coastal zones of lakes could be contaminated by a cocktail of substances coming from urban discharges or diffuse watershed run off. This type of contamination especially concerns micro pollutants such as pesticides. Benthic diatoms, which are a major component of benthic biomass, are then exposed to several pesticides that could interact together and present a modified toxicity (synergy, antagonism). Considering risk assessment, Species Sensitivity Distribution (SSD) models are partly used to extrapolate protective concentrations for a community exposed to a single or a cocktail of contaminants. To predict the toxicity of a mixture, two concepts are used depending on the mode of action of the mixture components (Concentration Addition _CA_, or Independent Action _IA_). The main objective of the study was to assess 1/ if SSD (using CA or IA models) were reliable in terms of toxicity prediction of herbicide mixture on benthic diatoms and 2/ if this SSD could be used as a management tool for aquatic ecosystems. As a first step, eleven species of benthic diatoms were exposed to 4 herbicides, separately (diuron, isoproturon, terbutryn, atrazin), in 96h monospecific growth inhibition tests.

EC₅₀ values derived from dose-response curves allowed to build a SSD curve (SSD-EC₅₀) for each herbicide. The different individual SSD-EC₅₀ curves were combined, using CA or IA hypothesis, in order to predict the toxicity of several mixtures. This first step allowed us to predict hazardous concentrations (HC). Then each species was exposed to three different mixtures (96h monospecific growth tests). Two binary mixes (atrazin/terbutryn; diuron/isoproturon) and one quaternary mix (diuron/isoproturon/terbutryn/atrazin) were tested. EC₅₀ obtained for each species-mixture combination allowed to build a SSD curve (SSD-EC₅₀) for each mixture. Measured HC were obtained from these curves. Finally, we compared the toxicity thresholds predicted by CA and IA models to the measured toxicity thresholds. We also assessed the species ranking between the mono herbicide SSD curves and the mixture SSD curves.

TU 212

Effects of Atrazine and 2,4-D mixtures on *lemna minor*

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Thailand is country where agriculture is very important in terms of the economy. Pesticides are therefore widely used in Thailand and this usage is increasing. As a wide range of pesticides is used in Thailand, aquatic will be exposed to a mixture of compounds. In order to understand pesticide impacts, it is therefore important to understand the potential toxic interactions of pesticides in use in Thailand. The aim of the present study was therefore to test the interactive effects of two herbicides, atrazine and 2,4-Dichloroacetic acid which are some of the most widely used and imported pesticides in Thailand. The test was carried out on *Lemna minor*. The effects of atrazine and 2,4-D on their own and in mixtures were assessed using OECD method 221. For the single compound studies, the effects of a range of concentrations of pesticide on the growth of *L. minor* were studied. For atrazine the test concentrations were 0.05, 0.1, 0.2, 0.4 and 0.80 mg/L and 2,4-D were 5, 10, 20, 40, 80 and 100 mg/L. *L. minor* one colony, 3 healthy fronds was exposed to the test solutions for a period of 7 d. The design of the mixture studies was used seven mixture ratios and seven chemical dilutions. Dose response curves were obtained and fitted in logistic regression by using sigma plot. Isobole analysis was used to interpret the mixture toxicity results.

Results from the single substance studies showed that atrazine was more toxic to *L. minor* than 2,4-D. Due to 2, 4-D being an auxin hormone, it is absorbed through the leaves and translocated to the meristems of the plant in dicots and it does not affect monocots which may explain the low toxicity to *L. minor*. The studies into the combination effects of atrazine and 2,4-D on *L. minor* clearly showed that the two compounds interact in an antagonistic manner. The antagonism has been found to occur more frequently in mixture where the herbicide mixture belongs to different chemical groups and monocot species.

In terms of this mixture study, the results showed that the interaction between atrazine and 2,4-D was antagonistic. These results are re-assuring and indicate in terms of impacts on aquatic macrophytes in Thailand, mixture effects are less than additive. If additivity is assumed for risk assessment purposes for macrophytes in Thailand, then this should be protective. Work is ongoing using a wider range of pesticides and test organisms.

TU 213

Predictive mixture toxicity assessment of pesticides in Swedish surface waters

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It has been demonstrated that combined effects of chemical mixtures give rise to stronger toxic effects than any of the compounds applied individually. The Scientific Committee on Health and Environmental Risks (SCHER) has recently put forth concentration addition as a conceptual basis for the determination of environmental quality standards for mixtures. In this study we have predicted the risks from pesticide mixtures within Swedish surface waters using the concentration addition concept. Data from the national pesticide monitoring program in Sweden has been combined with experimental data from the US EPA's database for single chemical toxicity and predicted toxicity from QSAR's to determine the risks from pesticide mixtures in Swedish surface waters close to agricultural areas. The Swedish monitoring program has been taking samples in six different locations since 2002 and currently analyses the samples for more than 80 compounds, out of which 78 has been detected. All and all 751 weekly samples have been analysed bringing the total number of analyses performed close to 60 000. The abundance of information enabled the study of not only the individual site risks but also additional questions. Among these the effect on risk from season, taxa most at risk, average error factor when ignoring mixture effects as well as the sensitivity of the risk assessment to different ecotoxicological data has been determined. The results show that the average risk quotient of the mixtures from the six different sites range between 2.3 and 18.9 thus calling for additional assessments. The study also show no effect on risk from season implying that potential for recovery from pesticide stress is very limited. Finally the high ratio between predicted risk of the mixture and the average risk of the individual substances clearly demonstrates the need for going beyond the standard compound-by-compound assessment.

TU 214

A proposal for considering mixture toxicity with EQS compliance checking

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Environmental quality standards (EQS) are used under the EU Water Framework Directive to determine the chemical status of a water body. At present, compliance with EQS is only checked on the single substance level. However, a large and growing body of evidence from mixture toxicity studies indicates that this procedure might underestimate the toxicity of the chemicals present in a water body, as the effect of mixtures has been shown to be higher than the highest single substance effect in the majority of cases. This propensity to underestimate potential effects of mixtures is underlined by a recent review published by Kortenkamp and co-workers. Two approaches have been proposed to date to account for mixture toxicity in assessing the chemical status: (i) application of a default assessment factor of 100 as used for EQS derivation by RIVM (NL) and (ii) application of mixture toxicity models based on species sensitivity distributions (SSD). The latter method predicts the 'multi-substance potentially affected fraction' (msPAF) of species proposed by Posthuma and co-workers. In this paper we propose a third approach for cases where the EQS was not derived from SSDs for all mixture components, which allows more accurate assessment of environmental mixtures even where there is incomplete SSD information. The approach is based on the identification of the most susceptible taxonomic group and can be combined with the mixture cumulative ratio [MCR] approach recently proposed by Price and Han. It will also take into account the fact that specifically acting substances will only be specifically toxic to some taxonomic groups while exerting baseline toxicity to other taxonomic groups. This novel approach will be illustrated using case studies based on real monitoring data and published EQS values. The everyday applicability of the approach will be discussed along with the decrease in uncertainty that it achieves when assessing the chemical status of water bodies containing more than one substance of concern.

TU 215

Increasing the number of data points does not necessarily reduce the probability of erroneously conclusions about interactive effects in mixture toxicity experiments

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The importance of mixture ecotoxicity studies is increasing exponentially. Developing requirements in regulatory risk assessment are further fuelling the need to gain more knowledge about mixtures, mixture experiments and the subsequent data analysis. In particular, little attention has been given to the choice of an appropriate experimental design for a given reference model (e.g. independent action and concentration addition). Indeed, it has been shown that some experimental designs might not be able to detect (false negative) or erroneously conclude (false positive) deviations from a reference model. In this study, we simulated experimental data from a full factorial and equitox design and subsequently tested if these data deviated significantly from the concentration addition reference model. Experimental data were simulated for synergistic, antagonistic and additive mixtures. We then determined false positive and false negative rates for each experimental design. The number of data points, the lowest concentration of each chemical and the step size between two subsequent concentrations were varied. Results demonstrated a marginal increase in false positive rates with increasing number of design points. This contrasts with the general belief that adding design points will reduce the false positive rate. Varying the two other parameters resulted in a significant decrease in false positive rates. An optimal combination of these two parameters leads to the lowest false positive rates for a given design. In conclusion, we have shown that an optimal experimental design for mixture toxicity focuses on optimizing the lowest tested concentration and the concentration step size rather than increasing the number of design points. Furthermore, datasets based on suboptimal or imperfect designs lead to erroneous conclusions and care should be taken in analyzing and evaluating such datasets.

TU 216

Proposed PAH relative potency factors will greatly increase risks at all sites with PAH mixtures

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The United States Protection Agency (USEPA) is proposing to modify the approach that it has been using since 1993 to evaluate polycyclic aromatic hydrocarbon (PAH) mixtures. While USEPA previously considered seven PAHs to have carcinogenic potential, the proposed approach increases the number to 26. In addition, many PAHs are being assigned a Relative Potency Factor (RPF) that is greater than 1 relative to benzo[a]pyrene. Several proposed RPFs are greater than 10, including benzo[c]fluorene (20), dibenzo[a,l]pyrene (30), and benz[j]aceanthrylene (60). If this approach is adopted, it will be necessary to quantify all 26 compounds in media at contaminated sites, despite the lack of standard analytical methods. In addition, it may be necessary to re-open and reassess sites that have already received regulatory closure. However, there are many scientific criticisms of the proposal. Most importantly, no Weight of Evidence Evaluation was performed. Second, USEPA assumed that all PAHs act by a similar mode of action with no supporting evidence. USEPA also failed to validate the derived RPFs using cancer response data from real world complex mixtures, such as coal tar. This paper summarizes several validation exercises that demonstrate that USEPA's RPFs overestimate the true carcinogenic risk observed when the interactions between components are inherently taken into account in mixture studies. Lastly, there were many technical problems with proposed RPFs: many RPFs were based on a single study or "low confidence" studies; some RPFs were based on in vitro assays; some RPFs were derived from studies with no confirmation of the chemical identity of the test substance; some RPFs were based on studies with unusual modes of administration, such as lung implantation; and many studies exceeded the Maximum Tolerated Dose. This paper presents the proposed approach to evaluating PAH mixtures and discusses key scientific criticisms.

TU 217

Derivation of environmental risk limits for polycyclic aromatic hydrocarbons (PAHs) based on internal residues

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Polycyclic aromatic hydrocarbons (PAHs) are substances that have both natural and anthropogenic origins. They can be formed as a result of combustion, and are constituents of many petroleum products as well. Environmental risk limits (ERLs) were derived for 16 PAHs that were also considered in the European Risk Assessment Report (RAR) on coal tar pitch, high temperature, but completed with a substantial amount of additional data. It can be assumed that toxicity of all PAHs is similar and possibly caused by narcosis. The toxicity of different PAHs differs only as a consequence of different environmental distribution and accumulation potential. The sum of the internal concentrations of different compounds gives rise to the same effect as that of a similar concentration of an individual compound, which is referred to as concentration additivity. This concept has been successfully applied before for total petroleum hydrocarbons (TPH) and will be investigated here for PAHs.

All ecotoxicity data were collected and carefully evaluated for their usefulness and reliability. To calculate the total internal residues, pore water concentrations were calculated first for soil and sediment, by considering partitioning between organic carbon and water. From water concentrations, the internal residues were calculated using a partition coefficient between the membrane and water.

From all chronic toxicity data for individual PAHs expressed as internal residues, a set of no observed effect residues (NOERs) for 54 species was obtained that showed no significant differences between the compartments (e.g. between aquatic, benthic and terrestrial species) and between the individual PAHs, which confirms the assumption that indeed accumulation from (pore) water is the determining factor for toxicity. On basis of these data, a species sensitivity distribution (SSD) was constructed, which appeared to be very similar to the SSD for TPH, suggesting a similar mode of toxic action. To derive a value for a generic ERL, an assessment factor of 5 has been applied to the HC5 to account for the potential of certain PAHs to exert a high acute toxicity through phototoxicity.

For the PAHs it is confirmed that the equilibrium partitioning method is a useful method in setting quality standards. Because toxicity is driven by equilibrium partitioning, monitoring of these PAHs could be focused on measuring free water concentrations, e.g. in pore water, with solid phase extraction techniques.

TU 218

Prediction of the mixture toxicity of PAHs and their derivatives in the Ah receptor based H4IIE-luc assay

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Polycyclic aromatic hydrocarbons (PAHs) form a large group of widespread pollutants of high environmental concern. Many of the PAHs are toxic and exposure can result in mutagenesis and carcinogenesis in humans and animals. PAHs are transformed to oxygenated PAHs by chemical reactions in the air and metabolic reactions in organisms. Oxygenated PAHs (oxy-PAHs) are mutagenic and in addition more mobile than their parent PAHs. Today, the generic guideline of PAH contaminated areas are based on the 16 U.S. Environmental Protection Agency (EPA) priority PAHs, even though often hundreds of PAHs and PAH derivatives exist in the soils. Aryl hydrocarbon receptor (AhR) mediated toxicity of PAH contaminated samples has been shown in several studies, but knowledge of the mixture interactions is insufficient. The aim of this study is to investigate the predictability of the AhR mediated activity of mixtures containing both oxy-PAHs and PAHs, using the concentration addition model (CA). It will be done by testing 26 individual PAHs and 12 oxy-PAHs and different mixture combinations of these compounds in the Ah receptor based H4IIE-luc assay. The observed mixture toxicity will then be compared with the mixture toxicity predicted by the CA model, assuming for additivity. The results presented here will aid future research using mass balance analysis of environmental samples and bring additional knowledge regarding mixture effects of PAHs and PAH derivatives via the Ah receptor.

TU 219

Hazard evaluation of shale oil to the environment

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Estonia is the country with the largest commercially used oil shale basin. Oil shale is used for the firing of local power stations but also for the production of shale oil. The latter is used in boiler houses, industrial furnaces but also for heating of houses, etc. The current annual production volume of shale oil is about 0.5 million tons and that will be increased up to one million tons per year in the near future. Increased production, transportation and use of shale oil in Estonia entail risks of environmental contamination. Different shale oil fractions contain various hydrocarbons, phenols, sulfur- and nitrogen-containing compounds, etc. The knowledge of shale oil toxic properties and behaviour in the soil and water ecosystems is limited. This current study aims to contribute to filling of these data gaps. In order to study the mobility and fate of the shale oil, the spiked soil samples were incubated in laboratory (i.e., controlled test conditions) and outside (natural climatic conditions) for several months. The dynamics of chemical composition of spiked soils was followed using different analytical methods. Potential hazard of shale oil to terrestrial (soil microbial community, higher plants) and aquatic organisms (bacteria *Vibrio fischeri* and crustaceans *Daphnia magna*) was investigated using various test formats. The assessment of risks related with contamination of soils and surface waters by different fractions of shale oil based on the obtained results will be presented. This research is supported by Central Baltic INTERREG IV A Finnish-Estonian project: Risk Management and Remediation of Chemical Accidents.

TU 220

Application of predictive mixture models to differentiate P-gp and MRP type efflux transporter activities in zebra mussel (*Dreissena polymorpha*) and its environmental implications

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Aquatic organisms have developed strategies to cope with a variety of natural and anthropogenic toxicants. A central defense mechanism is the *Multi Xenobiotic Resistance* (MXR) system. MXR is mediated by transmembrane transport proteins belonging to the ABCB, ABCC and ABCG sub-families of the *ATP binding cassette* (ABC) superfamily of proteins. ABC transporters translocate their substrates in an ATP-driven process out of the cell. MXR transporters are particularly abundant in tissues with a barrier function, such as gills in mussels.

This work aimed at identifying P-glycoprotein (P-gp; ABCB subfamily) and multidrug resistance related protein (MRP; ABCC subfamily) efflux activity in gill tissue of zebra mussel (*Dreissena polymorpha*) and determining inhibitory effects of chemicals on P-gp and MRP efflux activities. Calcein-AM, a substrate of both pumps, was used as proxy dye for P-gp and MRP activity along with the specific inhibitors Reversin 205 for P-gp and MK571 for MRPs. Thus, a calcein-AM uptake assay was applied to study single and combined effects of the inhibitors on the efflux transporters. Enhanced accumulation of calcein in the gill tissue as reflected by increased calcein fluorescence indicated decreased efflux activity. Next, joint effects were modeled using the independent action and concentration addition concepts. Reversin 205 and MK 571 showed a distinctive calcein accumulation kinetics and their joint effects were greater than those predicted by the independent action concept. This indicates that the studied inhibitors targeted different P-gp and MRP efflux pumps but act in a cooperative manner in *Dreissena* gills. Furthermore the ability of twelve pollutants to inhibit P-gp and MRP pumps was tested. In addition to calcein-AM, also assays with rhodamine B, supposedly a substrate of P-gp but not MRP, were performed. Most tested pollutants had an equal or greater inhibitory effect on rhodamine B efflux than on calcein-AM, which indicates that they are specific P-gp inhibitors. However, e.g. perfluorooctane sulfonate (PFOS) had a greater inhibitory potential when calcein-AM was used, pointing to specific inhibition of MRP pumps by this chemical. This is the first study attempting to distinguish between inhibitory effects of environmental chemicals on either P-gp or MRP type efflux activities in a freshwater mussel.

TU 221

Characterization of the multixenobiotic resistance (MXR) mechanism in *Daphnia magna* and studies on its role in tolerance to single and mixtures of toxicants

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The study of the cellular mechanisms of tolerance of organisms to pollution is a key issue in aquatic environmental risk assessment. Recent evidences indicate that multixenobiotic resistance (MXR) mechanisms represent a general biological defense of many marine and freshwater organisms against environmental toxicants. In this work, the detection and functional characterization of toxicologically relevant efflux transporters were studied for the first time in *Daphnia magna*. P-gp and MRP transporter gene expression was studied by RT-PCR, and its associated efflux activity was monitored using the fluorescent dye rhodamine 123 combined with specific MXR transporter inhibitors (chemosensitizers).

Toxicity bioassays with model substrates of P-gp (mitoxantrone) and MRP (chlorambucil, mercury) applied singly and in combination with different chemosensitizers were performed to elucidate the tolerance role of P-gp and MRP efflux transporters.

Results provide evidence for the existence of the genes and the associated efflux activities of ABC transporters in juvenile stages of *D. magna*. Specific inhibitors of P-gp and/or MRP transport activities including cyclosporine A and verapamil but not MK571 and reversin 205 resulted in a dose dependent inhibition of rhodamine 123 transport in *D. magna* juveniles. Binary mixtures, of the tested transporter inhibitors, with the pharmaceutical drugs and P-gp substrates chlorambucil and mitoxantrone and the MRP substrate, mercury, increased the toxicity of the studied pollutants more than additively as predicted by the independent action concept. These results indicate that MXR transporter activity is present in *D. magna* and that this may play an important role in its tolerance to environmental contaminants.

TU 222

Validation of the simulated earthworm gut (SEG) for widespread use in soil invertebrate toxicity testing

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The Simulated Earthworm Gut (SEG) is an in-vitro test that can be used to estimate the bioaccessibility of contaminants found in soil. The main objective of this research is to validate the SEG as an indicator of soil invertebrate toxicity for soil contaminated with petroleum hydrocarbons (PHCs). Current practices for determining site-specific toxicity for soil organisms can be expensive and time consuming. By validating an in-vitro toxicity test such as the SEG, site specific remedial objectives can be determined faster and at a lower cost. To validate the SEG, we compare the toxicity of six PHC contaminated soils for three invertebrate species (mite, collembolan and earthworm) against the bioaccessible estimate obtained from the SEG. Furthermore, due to the lipophilic nature of hydrocarbons we compare SEG bioaccessibility with and without the addition of a lipophilic sink to see which model better characterizes in-vivo exposure.

TU 223

Prediction of mixture toxicity for metals in soil: a reality-check

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Today, EU Chemicals legislations are based predominantly on assessments carried out on individual substances. However, mixture toxicity is an upcoming issue in the regulatory area because of growing concern about the potential adverse effects of interactions between substances when present simultaneously. Only a few models are available so far to predict mixture toxicity (dose/concentration addition and independent action), and dose/concentration addition seems to be commonly selected as a default for regulatory purposes because of its higher protection level and the limited information about the mode of action of most substances. The applicability of these models in a regulatory framework for assessing the ecological effects of naturally occurring elements, such as metals, in soil may not be straightforward, however. For several metals, the predicted no-effect concentrations are close to their natural background concentration in soil, and adding the potential effect of several metals may lead to over-protective results and e.g. identification of risks in uncontaminated natural soils. Consequently, a reality-check is carried out on the use of common models for assessing the mixture toxicity of metals in soils and the selection of the number of metals to be included, based on exposure data for several metals in arable land and grassland soils across Europe (data from the GEMAS project). The distribution of the predicted total risk ratio across arable land or grassland in Europe is evaluated with respect to the mixture model applied, the number of metals included, the incorporation of bioavailability corrections, etc.

TU 224

Toxicological evaluation of chemical mixtures posing hazard to human and environment

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Exposure of human and other organisms to chemical mixtures found in an environment can occur in different forms, with regard to reason of the contact and composition of the chemical mixture. Beyond all doubts is the fact that the chemical risk assessment, due to higher and higher contamination of environment with multi-component chemical mixtures, plays crucial role in toxicology and ecotoxicology.

This work illustrates the most important issues concerning toxicology of chemical mixtures, with reference to an experimental design and special attention paid to the types of study methods, mainly acute and chronic studies, as well as legal aspects relating to European regulations connected with REACH and CLP.

TU 225

Assessing human health risk from farmed milkfish consumption: considering toxic interaction among arsenic, copper, and zinc mixture

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Various studies indicated that a strong association between cultured fish and waterborne metals of arsenic (As), copper (Cu), and Zinc (Zn) in coastal areas of Taiwan regions. Generally, the impact of mixture toxicity of metals on the aquatic ecosystems is higher than that of single metal. The purpose of this study was to assess the human health risk for toxic interactions among As, Cu, and Zn mixture from farmed milkfish (*Chanos chanos*) consumption in blackfoot disease (BFD)-endemic area in Taiwan. The interaction risk assessment model proposed by the U.S. Environmental Protection Agency (EPA) and Agency for Toxic Substances and Diseases Registry (ATSDR) was used to estimate the interaction-based hazard index (HIINT) of non-carcinogenic mixture toxicity. The mixture toxic interactions of As'Cu and As'Zn were both antagonistic, whereas Cu'Zn was no significant influence. The 90th percentiles of HIINT for As'Cu, As'Zn, and As'Cu'Zn ranged from 0.74 to 1.78, 0.37 to 1.37, and 0.73 to 2.53, respectively. Our study indicates that As, Cu, and Zn mixture exposure may pose potential non-carcinogenic risk to human consuming farmed milkfish. The present interaction risk assessment model provides an illustration for assessing the potential threat to human health posed by the mixture toxicity of metals from seafood consumption.

TU 226

Whether BDE209 interacts with Cd hepatotoxicity?

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During the last ten years, mixture toxicology has undergone a remarkable and productive development. In fact, combined toxicity, which more accurately represents real environment conditions, may assist in the determination of toxicologically relevant effects. The aim of this study was to examine whether decabromodiphenyl ether (BDE 209) influences cadmium (Cd) hepatotoxicity. Wistar rats (200-240 g) were exposed orally to BDE 209 (1000 mg/kg/bw), Cd (2.5, 7.5 or 15 mg/kg/bw) or their three combinations, by gavage, for 28 days. Control groups were exposed to saline or DMSO as vehicle. The following end points were examined: liver weight, morphology, histology as well as liver enzymes activities (aspartate aminotransferase-AST, alanine aminotransferase-ALT and γ -glutamyltransferase- γ -GT). Study was approved by Ethical Committee of Military Medical Academy (No. 9687/11). Liver weight increased in all groups comparing to controls. Interestingly, the highest increase was seen in Cd groups (20-42%), than in BDE209 group (28%), while in mixture groups it ranges between 96 and 115%. Morphological changes in liver were not observed while histological analysis indicated degenerative changes in hepatocytes, hyperemia, vascular bleedings and disruption of vascular membranes. Average histological scores on scale from 0 to 5 were: 0.13, 1.26, 2.26, 3, 3.4, 2, 2.03 and 2.26, for control, BDE2091000, BDE2091000+Cd2.5, BDE2091000+Cd7.5, BDE2091000+Cd15, Cd2.5, Cd7.5, and Cd15, respectively. AST values in general did not change, ALT decreased, but more in single compound groups than in mixtures. Furthermore, ALP was significantly lower in Cd groups, while in mixture and BDE209 groups decrease in ALP activity was observed, but not so intensive like in all three Cd groups. Activity of γ -GT was higher in the mixtures than in groups where Cd or BDE209 were administered alone. Watching scores of histological changes and γ -GT activity, we may assume that BDE209 potentiate Cd effects on liver. (Partly supported by Ministry of Science project III46009)

TU 227

Waste recovery - a special treatment under REACH

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According to REACH, a manufacturing process is considered any production or extraction of substances in the natural state. This includes chemical modification as well as mechanical processes. Although the entry products at recovery companies are mainly waste (covered by the Waste Regulation), the status of the end products might "cease to be waste", and thus fall under the REACH regulation, and thereby having a major impact on the legal obligations of the recovery operator. However, under certain conditions recovery operators can benefit from registration exemption by applying Article 2.7(d) of the REACH regulation.

As the end products at recovery plants are often complex mixtures containing substances whose presence is not always intended, the process of substance identification is challenging. Each compound in the mixture has to be identified as either substance (mono-constituent, multi-constituent or UVCB), or impurity.

Once it is established that Art.2.7(d) can be applied, the recovery operator needs to fulfill certain obligations, such as the derivation of the classification of substances and mixtures, the notification of substances, the preparation of Art.2.7(d) dossiers (substantiating the application of the exemption article) and the development of SDSs.

Obviously, little information is available on the mixture itself due to the often changing composition, nor on the impurities due to limited access to the raw data as recovery operators are not participating in SIEFs or consortia. This lack of data influences the derivation of C&L of the mixture since this will often be derived based on the impurities' classification alone (e.g. summation method). Furthermore, with the exemption from registration, the recovery operator is not required to perform an exposure and risk assessment.

One should stimulate the re-introduction of former waste products on the market, because this contributes to the recycling of natural resources and the products may have economic benefits in the time of raising commodity prices. Nevertheless, sufficient efforts should be made to assure safe use of these, potentially heavily classified, mixtures. In addition, the hazard and risk assessment should be performed in such a way that overestimation due to the application of conservative assumptions and worst-case scenarios is avoided. Although these concerns mainly address issues related to metal-based waste products, some may also apply to other types of recycling industries.

TU 228

Toxicity evaluation of disperse red 1 textile dye using freshwater organisms from different trophic levels - a PNEC proposal

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Azo dyes have been found in surface water especially because of textile production; however data about ecotoxicity of dyes are scarce in the literature. Commercial azo dyes are mixtures of a main dye, surfactants and other synthesis impurities. The commercial dye Disperse Red 1 containing 60% of the main dye N-Ethyl-N-(2-hydroxyethyl)-4-(4-nitrophenylazo) aniline, CAS number 2872-52-8, was obtained by PCIL industries Ltda, Brazil. This product is used for dyeing synthetic fibers. Previous data showed the main dye was mainly responsible for the commercial dye toxicity at least for *Daphnia*. This same dye was found in an effluent and a receiving water sample collected in Americana region, São Paulo Brazil. The aim of this study was to compare the results of ecotoxicity of the commercial dye Disperse Red 1 obtained for organisms from different trophic levels and propose a PNEC (predicted no effect concentration) for aquatic life. For chronic tests, the NOEC (72h) obtained for *Pseudokirchneriella subcapitata* was 0.1 ppm and the NOEC obtained for *Ceriodaphnia dubia* in a reproduction inhibition test (8 days) was also 0.1 ppm. For the secondary consumer *Hydra attenuata*, the NOEC obtained was 1 ppm in a reproduction inhibition test (7 days). For planarian and fish, only acute tests were performed. For *Girardia tigrina* newborns the EC50 (96h) was 80 ppm and for fish (*Danio rerio*, larvae, 96h), it was >50 ppm. This dye also showed mutagenic activity in the *Salmonella/microsome* assay but did not present estrogenicity in a bioluminescent yeast estrogen assay. Based on these data we propose a PNEC for the main dye (N-Ethyl-N-(2-hydroxyethyl)-4-(4-nitrophenylazo) aniline of 0.6 ppb, considering that this compound would be responsible for the toxicity of the commercial product. This value was based on the lowest NOEC 0.1 ppm (chronic assay *P. subcapitata* and *C. dubia*) divided by an assessment factor of 10 because no chronic assay for fish is available and additional 10 because of its mutagenic potential. More studies are being conducted to verify the mutagenic effect of this product in microcrustacea as well as monitoring studies in river waters that receive textile discharges to determine exposure concentrations.

TU 229

Efficiency of chemical mixtures in the industrial control of biofouling by the Asian clam *Corbicula fluminea*

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The freshwater bivalve *Corbicula fluminea* (Müller, 1774), commonly known as the Asian clam, is one of the most invasive species of aquatic systems around the world. In addition to the adverse effects on ecosystems, this pest is well-known for its biofouling activity on freshwater-dependent industries. Developing environmentally-friendly methods to control the Asian clam in the industrial context and thus prevent significant economic losses is a major challenge.

The biocide activity of the cationic polymer polydiallyldimethylammonium chloride (polyDADMAC) against the Asian clam has been previously demonstrated. In this study, binary mixtures containing polyDADMAC and either potassium chloride, the molluscicide niclosamide or the insecticide dimethoate were formulated in an attempt to further increase the susceptibility of the clams to the polymer. The three combinations were tested in standard laboratory bioassays and the mortality results interpreted using an isobologram-based methodology. Through this preliminary study, different types of interactions between the chemicals were explored and the extent to which the performance of a potential combined treatment depends on the chemicals' mode of action was assessed. In general, the combined effect of the compounds was found to be antagonist and, therefore, these mixtures are not promising control tools. In order to understand the antagonist nature of the chemicals, preliminary experiments were conducted to elucidate the mechanism of toxicity of polyDADMAC and its interaction with dimethoate in *C. fluminea*. This study indicated that polyDADMAC neither affects the ability of osmotic regulation of the cells as suggested by the literature for other surface-active molecules nor interacts with the hydrolytic activity of the enzyme cholinesterase, which is the specific target of dimethoate.

TU 230

Industrial enzymes - an example of the environmental risk assessment of an UVCB (unknown or variable composition, complex reaction products or biological materials)

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Industrial enzymes for technical applications like for example detergent enzymes have to be safety assessed and registered under REACH. Enzymes are categorized as UVCB's because they are produced by fermentation and are thus substances from biological origin with varying content of constituents. The enzyme protein together with the constituents derived from the fermentation process is considered to be the substance. Before enzymes with the same catalytic activity, but produced by different production strains, can be considered to be the same substance from a safety point of view, it is necessary to establish the safety of the production strains including the safety of the other constituents. To assure the safety of the other constituents the enzyme industry uses safe strain lineages (i.e. the production organism and methods of modification are demonstrated to be safe) which have been well established over the years as also indicated by scientific publications. This is a prerequisite for establishment of sameness so further ecotoxicological evaluation can be focused on the enzyme protein itself.

Industrial enzymes are general of low ecotoxicity except for proteolytic enzymes, where some exhibit acute aquatic toxicity. Based on the available ecotoxicity data industrial enzymes can with regard to their ecotoxicological profile be grouped in proteolytic and non-proteolytic enzymes. Within these two groups read-across can be applied. Enzymes are readily biodegradable to peptides and amino acids, then further to environmentally harmless carbon dioxide and water as degradation products. Additionally, for the majority of ecotoxicity tests required for REACH registration data waiving can be applied including long-term aquatic toxicity testing and toxicity to terrestrial organisms. These tests are considered not relevant due to the fast biodegradation of enzymes, the low absorption and bioaccumulation potential and because enzymes are primarily active in aquatic environments. Thus only short-term aquatic toxicity tests are considered relevant for industrial enzymes.

Based on the current knowledge on industrial enzymes and their intrinsic properties future ecotoxicological testing of enzymes may be avoided by applying read-across and the data waiving approach.

In conclusion, environmental risk assessment of UVCB's like enzymes requires an alternative approach compared to the risk assessment of 'classical' chemicals or well defined mixtures due to the biological origin.

TU 231

A partial least squares based integrated addition model for estimating mixture toxicity

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Studies on mixture toxicity among chemicals find that mixture components at levels below no-observed-effect concentrations (NOECs) may elicit toxicity resulting from the joint effects among the substances. However, chemical risk assessment frequently focuses on individual chemical substances, although most living organisms are substantially exposed to chemical mixtures rather than single substances.

The concepts of additive toxicity, concentration addition (CA) and independent action (IA) models, are often applied to predict the mixture toxicity of similarly- and dissimilarly acting chemicals, respectively. However, living organisms and the environments are exposed to both types of chemicals at the same time and space. Therefore, from the scientific perspective, it still needs to develop an integrated model to predict mixture toxicity from different chemicals practically, regardless of whether mixture components produce similar, dissimilar, or both similar and dissimilar modes of toxic actions.

The objectives of this study are to develop and evaluate a partial least squares-based integrated addition model (PLS-IAM) for not only to overcome the multicollinearity problem which can be occurred between the two independent variables, CA and IA, but also to combine them into the integrated addition model by using the latent variable. In this study, the PLS-IAM was validated by different datasets on mixture toxicity. The results showed that the prediction capability of the PLS-IAM outperformed reference models, the CA, IA, and IAM based on ordinary least squares.

LC04P - Latest developments in uncertainty management – adding value to LCA studies

TU 232

Using sensitivity analysis in developing a characterization model for noise impacts

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Pilkey and Pilkey-Jarvis [1] claim the "virtual impossibility of accurate quantitative modelling to predict the outcome of natural processes on the Earth's surface", due to the inability of environmental model makers to consider all possible sources of uncertainties in their models or due to their unrealistic or even false assumptions.

In Life cycle assessment (LCA) we do use models whose outcome is always affected by a certain level of variability and uncertainty. The numerical solution of these models is, in fact, itself a source of uncertainty [2]. LCA studies often fail to communicate to what extent models, parameters, inventory data and results are influenced by uncertainty [3]. Practitioners and developers often communicate single values for model parameters and characterization factors, negatively impacting on the transparency, usability and trustworthiness of the throughput of the models.

The use of LCA for the support of public decisions has contributed to the increased attention to the quality of data reported by LCA studies. In many cases the result of an assessment used by a policy maker can make a difference to the quality of life and health of (animal or human) populations living at a certain location. In the context of the study of noise, an underestimation error could, for instance, prompt a local authority to cease investing in systems which could prevent the propagation and impact of noise. We proposed a new approach [4] that allows for the characterization of any noise-emitting source and its impact on humans, creating the necessary theoretical structure to accommodate noise in LCA and possibly also make it available for policy advice. Our follow up research aims at testing, analysing and further developing the noise characterization model applying sensitivity analysis (SA) techniques to demonstrate how uncertainty from the input of the model propagates to the output and how each single variable affects the overall variance of the output.

TU 233

Quantifying and propagating uncertainty in regionalized impact assessment: the relevance of spatial aggregation

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Most LCA methods do not provide uncertainty information to be coupled with uncertainty of LCI. Furthermore, regionalized differences are often not properly addressed, leading to additional uncertainty due to aggregation of spatial explicit characterization factors (CF) on regional or global levels. This lack is a main challenge for practitioners applying proper LCA including uncertainty and sensitivity analyses. On the other hand, concepts for reporting spatial uncertainty by method developers are still under development. In this study, different types of uncertainty for water consumption impacts are analyzed and combined on the endpoint level, showing how uncertainty information can be added to spatially explicit CFs.

In current Life Cycle Impact Assessment, human health impacts due to water consumption have only been addressed by two published methods so far. Both methods assess impacts as DALY (Disability Adjusted Life Years lost) due to lack of water for human use, but the modeling approach and considered impact pathways differ and cannot be used for model scope uncertainty quantification. The method of Pfister et al. (2009) was used as a starting point. Parameter uncertainties were estimated based on analysis of original data sources and, where applicable, on quantitative assessment of the model uncertainty of the data provided by third parties. Spatial variability was also considered when using aggregation on country level. The uncertainties were propagated within the cause-effect impact model by applying the stochastic procedure of Latin Hypercube with the software @Risk.

The average k-values (dispersion factor denoting the 95% confidence interval if the median is divided (lower bound) and multiplied (upper bound) by k) was equal to k=2.76 on the midpoint level and 18.1 on the endpoint level. The aggregation from watershed to country level resulted in an average uncertainty of k=19.2 for the endpoint characterization factors and represents the variability of watershed factors within the country. The results show high spatial diversity of k-values and make it difficult to derive generic estimates of uncertainty, especially regarding the endpoint CFs. However, they can be used to produce maps of uncertainties and also to indicate in which regions

country level data might not considerably add to uncertainty. Such information helps to improve efficiency when dealing with regionalized LCIA. Overall spatial aggregation added considerable uncertainties.

TU 234

Sensitivity analysis for archetypes development in impact assessment of chemicals

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Emission of chemicals is increasing over years and the related impacts are greatly influenced by spatial differentiation. Chemicals are usually emitted locally but, due to persistence and physical chemicals properties, may exert global impact. Variability of environmental parameters may affect the fate and the exposure up to orders of magnitude of difference. Accounting for spatial differentiation of chemical impacts requires the use of multimedia models, at various levels of complexity (from simple box model to computational intense and high spatial resolution model). Trade-off between complexity of high detailed models and global applicability requires the development of suitable archetypes.

In the present study, sensitivity analysis techniques have been applied to MAPPE (Multimedia assessment of pollutant pathways in the environment) to build archetypes of emissions and fate. MAPPE is an advanced, multimedia, spatially resolved (1x1 degree) model, that allows the evaluation of chemicals removal rates from air, water and soil. We evaluate the relative influence of substance properties and of environmental characteristics on the variability in the environmental fate. In particular, using a Monte Carlo framework, we applied variance-based sensitivity analysis techniques to find out those environmental parameters explaining the highest share of the variability (namely the variance) in the model outputs. This information is crucial in order to define the environmental dimensions to be used for the definition of the archetypes of emissions and fate, accounting for major differences in the potential impact of the different pollutants.

We present the result of the analysis for the pesticide Lindane. The overall variability of the removal rate from air is up to 5 orders of magnitude, mainly driven by four out of the eleven environmental parameters proposed in the model. These four parameters were then used as basis for building archetypes.

TU 235

Dealing with uncertainties in UNEP SETAC toxicity model

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In the field of LCA, the issue of uncertainty generates a growing interest. Although this issue has not yet been systematically addressed in existing databases and models, it neither can be neglected, nor addressed too simply. Although uncertainty has partly been quantified in USEtox model, several questions remain open to date.

A significant share of this uncertainty arises in model itself, based on the relationship between the data and the intended reality being modeled.

As regular users of the USEtox model, we identify the following sources of uncertainties:

- substance for which no data is available, or missing input parameter value
- parameters for which only an inappropriate or inaccurate value is available
- data for which more than one value is available (multiple sources and conflicting information)

Given the relative complexity of the mathematical model, uncertainty propagation has been performed using a numerical method.

Well known monte-carlo simulation has been used to take into account the potential variability of the input parameters (substance data and landscape model).

This approach allows us to describe statistical properties of a characterization factor value, based on input data uncertainties. It can also be used to study parameters sensibility.

Taking into account the fact that uncertainty in USEtox database cannot be fully captured within the input parameters variability, other approaches have been studied, which aims at reducing uncertainty.

In approaches to reduce the uncertainty, database documentation, substance coverage, as well as user interaction are key issues.

The determination of the true value for a parameter requires research, experiments and therefore time. Involving stakeholders, discussing and finally deciding on a consensus, so has the current model been built, so the database should be.

Possibilities must be given to each user to view, add, or revise the documentation of a specific parameter. Reporting an error in a parameter value also have to be easy, as well as the possibility to discuss validity of each parameter value.

Our practical experience and ideas, related to management of these uncertainties in USEtox model will be presented.

Those approaches have been implemented in a web application prototype that allows uncertainty calculation and documentation completion.

TU 236

Standard information modules as basis for LCA reliability in the manufacturing context

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In such work the standard-information-modules approach is presented as methodology to reduce uncertainty in the Life Cycle Assessment. In particular such approach aims to overcome common barriers in the modelling of complex systems within the manufacturing context. An application is illustrated with reference to the uncertainty characterization of environmental profiles referred to energy flows. The use of standard modules for environmental profile characterization seems to improve the comparison of different studies by polarizing the improvement analysis on key life-cycle stages.

TU 237

Taking into account variance of primary data in decision making: a method for the building sector

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Life Cycle Assessment (LCA) can be a powerful tool to drive society toward sustainability if used for help to decision. Nevertheless decision step comes often early in the design process where very few data is available. To overcome this issue analysts are forced to use poor quality data or generic data even for foreground system description which is hurtful to results liability and could mislead the decision-maker. In this paper we focus particularly on construction sector which represents about 40% of the total energy consumed in Europe [1]. In the building field, help to decision comes at the planning phase. At this step, only rough data are available but still the planning manager has to choose the best environmental solution regarding its expectations among several building team proposals. The method presented in this paper allows decision-makers choosing between solutions taking into account variance of primary data. It is tailor made to building sector.

In the first step of the method, selection of key parameters is made taking into account both sensitivity of parameter on the results and variance of these parameters. Then standard deviation is propagated using Taylor series. This method has been implemented in a real case where decision needed to be made between three building projects. The case study has shown that the method presented in this paper is easy to implement and suitable for complex products as buildings which are tough to describe completely. In some case, it is possible to identify the best building solution even with up to 50% standard deviation on some primary data.

TU 238

Uncertainty evaluation of LCA models input data using Monte Carlo Method

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This is a contribution to the development of methodologies in considering uncertainties in LCA studies. The main results are a solid methodology for dealing with uncertainties in LCA and two stochastic LCA models that enable practitioners to validate their results. The case study consists of an acoustic panel made up of cotton fibers, polyethylene and scraps of jeans. Its LCA model is developed with SimaPro® software. The Monte Carlo tool available in this software is used in order to develop the Monte Carlo Process model. Another model, called Monte Carlo Inventory model, is developed in Matlab® codes, with the model inventory table and in the CML 2 baseline 2000 impact assessment tables, all exported from SimaPro®. The same steps are followed for both models. First, a sensitivity analysis is made in the deterministic LCA model. Next the random parameters are chosen and modeled as random variables associated to a probability density function. Next, the Monte Carlo Method is applied and the convergence is observed. Finally, the results are analyzed. Both approaches are compared in order to observe if the method based on the inventory has the same performance of the method based on the input data. The objective is to obtain a basis of comparison for the stochastic LCA model and the focus is on the methodology development.

TU 239

How certain can we be about LCA after 20 years of harmonisation and standardisation? A critical comparison of methodology and results from ten LCAs comparing disposable cups

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Harmonisation and standardisation of LCA-methodology started in the 1990ties. It happened more than once in this period that contradictory LCA-results were released for a same product (particularly packaging materials). This resulted in intensive scientific debate, and also heated societal debate, especially where product claims were involved. Differences in LCA-results at that time could often be traced back to different decisions and assumption related to product system boundaries, functional unit definitions, technology choices, data sets etc.

In the last two decades LCA-methodology development has made immense progress. We meanwhile have a solid LCA frameworks. ISO 14044 describes the procedures to be followed, and specifies the assumptions and decisions to be made in LCA. Comprehensive and detailed guidelines are provided by national handbooks that still show few differences, but far greater overlap in methodology. With this in mind it is interesting to evaluate whether the progress in harmonisation LCA-methodology has also resulted in harmonisation of LCA-results. For that purpose we evaluated 10 LCA studies, each comparing a range of different types of disposable cups for hot and cold drinks. We

EC05P - Occurrence, fate and impact of atmospheric pollutants on environmental and human health

TU 241

Urban background levels of novel brominated flame retardants in ambient air in Southern Bavaria, Germany

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After the technical penta- and octabrominated diphenyl ether mixtures (PBDE) were banned in the European Union in 2004 and decabrominated diphenyl ether is not any longer permitted in electrical and electronic equipment since 2008, there is an increasing demand of 'novel' brominated flame retardants. At present, little is known about the concentrations of these substances in ambient air. Therefore, ambient air was collected between March and September 2011 at the urban background air monitoring station on the premises of the Bavarian Environment Agency, Augsburg, Southern Germany. A sample volume of 960 - 3900 m³ was collected over a period of 35 days using cartridges with a glass fibre filter and two polyurethane foam plugs. Two low volume samplers were run in parallel. 1,2-bis[2,4,6-tribromophenoxy]ethane (BTBPE), hexabromobenzene (HBB), pentabromoethylbenzene (PBEB), pentabromotoluene (PBT), 2,3,5,6-tetrabromo-p-xylene (p-TBX) and decabromodiphenylethane (DBDPE) were extracted with n-hexane/acetone (1:1, v/v) using accelerated solvent extraction (ASE). The analytes were separated by fractionation on a sulfuric acid-silica column. Purification of the two fractions containing BTBPE and the other flame retardants, respectively, was done on a Florisil®-column and on basic alumina, respectively. After clean-up, samples were analysed by GC-EL-MS. ¹³C-labeled internal standards were used for quantification. Response factors were calculated using a three-point-calibration. Concentrations varied from 0.08 to 0.34 pg m⁻³ (BTBPE), 0.22 to 0.74 pg m⁻³ (HBB), 0.04 to 0.30 pg m⁻³ (PBEB), 0.42 to 1.3 pg m⁻³ (PBT), 0.15 to 0.23 pg m⁻³ (p-TBX) and 1.5 to 3.6 pg m⁻³ (DBDPE). For the purpose of comparison hexabromocyclododecane (sum of α -, β -, γ -HBCD), polybrominated diphenyl ethers (sum of 7 congeners BDE 28, 47, 99, 100, 153, 154, 183) and BDE 209 were also determined. Concentrations ranged from 3.7 to 12 pg m⁻³ (HBCD), 0.72 to 1.5 pg m⁻³ (sum of 7 PBDE congeners) and 0.45 to 9.5 pg m⁻³ (BDE 209). The financial support of the Bavarian State Ministry of the Environment and Public Health (project no. 7403) is gratefully acknowledged.

TU 242

Halogenated flame retardants in the marine atmosphere from Southeast Asia towards Antarctica

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Halogenated flame retardants (HFRs), which are applied to reduce the inflammability of various consumer products have been industrial chemicals of growing worldwide environmental and political concern in the 1990s and 2000s. Polybrominated diphenyl ethers (PBDEs) were the most applied brominated flame retardants (BFRs) in the 1990s and early 2000 which are known to be harmful. Due to their ongoing banishment since the early 2000s, the industry shifts towards alternative non-regulated non-PBDE BFRs. Similar to PBDEs, most of them are semi-volatile and highly persistent leading to a high potential to be transported over the global oceans towards the Polar Regions. In the present study the occurrence, distribution and transport of 10 BDE congeners, 8 non-PBDE BFRs as well as 6 Dechlorane compounds in the atmosphere over the Indian Ocean was studied. High volume air samples were taken during a Polar expedition onboard the R/V Snow Dragon from the East Indian Archipelago towards Western Australia and further to Antarctica in 2010/11. Samples were Soxhlet extracted and analysed via GC-ECNCL-MS/MS. PBDEs were detected in concentrations from 0.08 to 5.1 pg/m³ with BDE-47 and BDE-209 being the predominating congeners. Among the alternative BFRs, pentabromobenzene (PBBz), pentabromotoluene (PBT), 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE), hexabromobenzene (HBB), 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (EHTBB) and bis-(2-ethylhexyl)-tetrabromophthalate (TBPH) were detected. HBB showed the highest concentration ranging from 0.12 to 26 pg/m³, and PBT and PBBz ranged from not detected (n.d.) to 2.8 pg/m³ and from n.d. to 4.3 pg/m³, respectively. Dechlorane Plus (DP) ranged 0.23 to 11 pg/m³ while other Dechloranes remained <0.5 pg/m³. On a spatial scale, the highest concentrations, especially for HBB, PBT and PBBz, were observed over the East Indian Archipelago showing South East Asia to be an important source of alternative BFRs. The approach of Clausius-Clapeyron Plots clearly indicates that the distribution and transport of PBDEs is dominated by long-range transport with little influence of fresh emissions whereas alternative BFRs, especially HBB, are subject to local sources. We present the first data on occurrence, distribution and transport of alternative BFRs and DP over the Indian Ocean.

TU 243

Contamination of the marine biological reserve of atol das Rocas (Brazil) by persistent organic pollutants

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The Biological Reserve of Atol das Rocas shelters the largest seabird population of Brazil and despite be considered a remote area due to its distance from the mainland, this area is not exempt from the influence of anthropogenic agents, such as persistent organic pollutants (POP) that even has caused the decline of populations of several species of seabirds over the globe, due to bioaccumulative and biomagnified effects on typical top predators. This proposal aimed to determine the occurrence and concentrations of those organic contaminants in respective avifauna. One expedition was performed in February, 2010, where 16 liver samples of two species: Anous stolidus (n=14) and Onychoprion fuscatus (n=2) were collected. Samples were analyzed for organochlorine pesticides (OCPs), PCBs and PBDEs. Briefly, samples were extracted in a Soxhlet apparatus, followed by classic adsorption column and GPC column as clean-up stages. OCPs and PCBs/PBDEs were respectively analysed by gas chromatography with electron capture detection and mass spectrometry. Preliminary data showed that PCBs were present in all samples, ranging, in wet weight, from 5.23 - 21.7 ng g⁻¹ for the O. fuscatus species and 20.5-27.9 ng g⁻¹ for the A. stolidus species with predominance of lighters congeners (tri-, tetra- and pentachlorinated groups). The p,p'-DDE occurred in 88% of the samples of O. fuscatus and in the two samples (100%) of A. stolidus with respective values ranging from <0.19-2.45 ng g⁻¹ and 0.50-3.15 ng g⁻¹. HCB was found in 40% of samples of O. fuscatus, with a mean value of 0.35 ng g⁻¹ and in the adult individual of A. stolidus in value of 0.65 ng g⁻¹. PBDE 47, at concentration of 4.70 ng g⁻¹, was found only in the sample of adult individual of A. stolidus. The low levels of contaminants suggest a relative degree of isolation and preservation, however the biggest levels seen on the majority of the pollutants groups on adult individual of A. stolidus could also reflect the bioaccumulative and biomagnified trends. The occurrence and distribution profiles of PCBs supports the hypothesis that the main source of contamination in remote areas is due the long range transport, demonstrating the ubiquity of those pollutants on environment. The ongoing samples and analysis of different species may also assist in understanding of the distribution and in the degree of exposure of POPs in remote environments, such as Biological Reserve of Atol das Rocas.

TU 244

Seasonal variation of specific toxic effects of organic air pollutants from year-long sampling campaign

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Air pollutants were described to produce many toxic effects including endocrine disruption. Evaluation of such types of effect cannot be based only on data from chemical analyses. That shows the importance of incorporation of specific bioassays into evaluation part of the air pollution monitoring programs. The utility of these methods in monitoring of atmospheric pollution levels has been shown previously. It is known that levels of pollutants in atmosphere are closely related to type of pollution source and a season period. In our study, we focused mainly on the latter factor. Air samples were collected for one year at a traffic-burdened urban site in Brno (Czech Republic) and a village site about 8 km from Brno city to address the influence of city agglomeration as an air pollution source on the surrounding area. The samples were analyzed for a wide range of parameters and pollutants. In this work, we show the seasonal variation of aryl hydrocarbon receptor (AhR) mediated effects, anti-/estrogenicity and anti-/androgenicity. These parameters were assessed using in vitro reporter gen assays based on mammalian cell lines. This research was supported by GACR P503/10/P249 and CETOCOEN (CZ.1.05/2.1.00/01.0001).

TU 245

Profiles and cold trapping of polycyclic aromatic hydrocarbons and polychlorinated biphenyls in British Columbian mountain soils

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Soils collected at several elevations on five mountains on and near the Northern Pacific Coast of North America were analyzed for 15 Polycyclic Aromatic Hydrocarbons (PAHs) and 54 Polychlorinated Biphenyls (PCBs). Mount Anderson (MA) is located on the West side of Vancouver Island; Grouse Mountain (GM) is in the city of North Vancouver; Four Brothers Mountain (FB) is 100km further inland in the Fraser Valley; Blue Grouse Mountain (BG) is in a fruit growing region 200km northwest of FB by the city of Kelowna; the Chilko Trail (CT) crosses from the Alaskan Panhandle into northern British Columbia. MA, GM, FB and the ocean side of CT receive copious seasonal precipitation, while BG and the East side of CT are in rain shadows. PAH profiles were similar across soils, with nearly all analytes found in all soils. Principle component analysis was unable to clearly distinguish between mountains, but suggested the soils collected at the summits of mountains are notably different in their PAH profiles. Total PAH concentrations - by dry weight of soil - were an order of magnitude higher in GM soils than the others. PCBs were present in all soils, but the congener profiles varied dramatically between mountains. Many congeners were below the method detection limits at all sites. GM soils had the greatest number of congeners present with 51, FB had 31 congeners and MA had 24 congeners while only 12 and 6 congeners appeared above method detection limits in CT and BG samples, respectively. Mean total PCBs were highest in the GM soils followed by FB, then MA and lowest in the soils of BG and CT. Thus, where precipitation was the same, proximity to sources appears to dictate PCB levels, but where wet deposition is nearly absent, soils have low levels of PCBs. Despite the low precipitation rate the two easternmost samples from CT had relatively high levels of PCBs; the small settlement and train in that area may be a PCB source. After normalization to organic carbon the concentrations of a few congeners exhibited a positive relationship with elevation, indicating orographic cold-trapping, but only for GM soils. Concentrations of two congeners with quite different physicochemical properties - PCBs 42 and 199 - exhibited fairly strong relationships with elevation ($r^2 = 0.94$, $p < 0.01$, and $r^2 = 0.97$, $p < 0.01$ respectively). The generally low PCB concentrations in the soils of remote mountains may mask the phenomenon of cold-trapping.

TU 246

Recommendations for the use of airsheds for interpreting semi-volatile organic compounds in passively sampled air

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To aid in the interpretation of measurements of Semi-Volatile Organic Contaminants (SVOCs) it is common to use Back-Trajectories (BTs), a calculated approximation of the spatial history of the air sampled. The endpoints of each segment of the BTs can be binned into a grid of cells to create an airshed, which indicates the fraction of the sampled air that passed through each cell. Here, to avoid the so-called 'pole-problem,' a 'geodesic' grid of nearly equidistant cells was employed.

The variability of airsheds in time, both year-to-year and season-to-season was calculated as the sum of absolute differences in endpoint counts in each cell from one time to another. For 24 locations for which BTs were available for 2 or more years inter-annual differences ranged from 9% to 21%, and season-to-season differences ranged from 17% to 53%. For one location for which 13 years of BTs were on hand the differences between the first year and subsequent years was seen to increase with time ($r^2 = 0.69$, $p < 0.01$).

To determine a suitable constraint on departure height of endpoints when assigning potential sources a 7 cell departure area was defined as the 'emitting' cell and the six cells adjacent to it. Using forward trajectories it was determined that after four time steps ~87% of trajectory endpoints were outside of the departure area - this includes points from trajectories that double-back into the departure area. At the fourth time step the 95% percentile of height was ~700m. Thus endpoints that are within the departure area but have a height greater than 700m very likely do not represent air masses that received contaminant from emissions in that cell.

Lastly a significance criterion was derived by assuming that when assigning potential source regions to a measurement only departures from a perfectly 'round' airshed - one in which air arrives at the sampler in equal amounts from all directions - are of interest. Based on this a test criterion is proposed, specific to the temporal resolution of the BT endpoints, to determine the significance of any cells in an airshed. It is recommended that airsheds be generated specifically for the deployment time and location of a sampler, that endpoints with heights greater than 700m be removed from the set and that cells failing to meet the test criterion for significance be ignored when assigning potential source areas.

TU 247

The spatial distribution and potential sources of polycyclic aromatic hydrocarbons (PAHs) over the Asian marginal Seas and the Indian and Atlantic Oceans

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Gaseous and particle-bound polycyclic aromatic hydrocarbons (PAHs) were analyzed in air samples taken on an expedition of the Scholar Ship from January 16th to March 14th, 2008. Samples were taken from the East and South China Seas and the Indian and Atlantic Oceans, providing an opportunity to assess spatial trends and potential sources of atmospheric PAHs over those oceans. Continental sources were still the major reason for the high concentrations of PAHs measured over the oceans. The $\Sigma 15$ PAHs in the gaseous phase were elevated on the approach to China and India, while the highest $\Sigma 15$ PAHs in the particulate phase were found at Chennai Harbor and close to Guinea. The high proportion of fluorene over the East and South China Sea could be a marker of coal and coke related combustion emission from Mainland China. The elevated high-molecular-weight PAHs in particles close to Guinea might be related to biomass burning in Africa. These results are consistent with previous PAH emission inventories and highlight the potential impact of continental PAH sources in China, India and Africa on the global marine atmosphere.

TU 248

PAHs along a mountain forest and clearing: the effect of canopy

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1. Introduction

Forest is regarded as a global sink of semi-volatile organic contaminants (SOCs). The so called "Forest filter effect (FFE)" may be an important driver that transfers SOCs from the atmosphere into the forest system (1). Previous study has focused on the atmospheric deposition and atmospheric-surface exchange of SOCs alone the mountain forest and clearing (2-4). This study aimed to compare atmospheric concentration of PAHs under the forest canopy and nearby clearing the Lys Valley, Aosta, Italy, on the slopes of Mont Mars.

2. Materials and methods

Passive atmospheric sampling (PAS) was performed both in the forest and clearing sites at 700 m, 1000 m, 1400 m, 1800 m, and 2400 m (a.s.l.) along the Lys Valley, Aosta, Italy, on the slopes of Mont Mars. 2 high-volume air samplers were used to collect air samples at 1400m for 4 sampling periods.

3. Results and discussion

3.1. Concentrations

The average value of the total PAHs (Σ PAHs) concentrations of gaseous and particle phase was 1860 and 176.1 pg m⁻³ for the forest, and 1230 and 123 pg m⁻³ for the clearing. Seasonality, the PAH concentrations in the mountain atmosphere decrease dramatically from R1 to R4 at a factor of 3800 for gaseous phase and 5.3 for particle phase, respectively, which indicated that the forest filter effect (FFE) was much higher for particle associated PAHs than gaseous phase PAHs.

3.2. Altitudinal Distribution

PAH amounts in the PAS samples (ng PAS⁻¹) were generally declining with the increasing of altitude along the slopes of Mont Mars. The declining rate for the forest was much lower than that at the clearing. Take round 1 for an example, the rate was 0.003 for forest and 0.006 ng PAS⁻¹ m⁻¹ for clearing, respectively. This may be due to strong forest filter effect at lower sites where corresponding to high PAH concentrations in the atmosphere.

3.3. Effect of canopy

Most of the F/C values in the atmosphere were >1 from active samples, however, F/C values were generally <1 in the PAS samples, which could be responsible for a lower sampling rate of PAS in the forest because of lower wind speed. As shown in Figure 2, log F/C and log KOA showed different correlations in the gaseous (green line) and particle phases (black line).

4. Conclusion

The filter effect of canopy was observed along the Mont Mars and higher factors were related to low altitudinal sites and atmospheric particle. K_{OA} values can influence the effect of forest canopy.

TU 249

Size-specific particle-gas distribution of the atmospheric polyaromatic hydrocarbons (PAHs) on the spatial and temporal scales

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Although polycyclic aromatic hydrocarbons (PAHs) are usually not included amongst the group of compounds defined as 'persistent organic pollutants (POPs)' due to their higher reactivity, they are a subject to long-range atmospheric transport (LRAT) and can pose significant health risks. As such, they are among the pollutants most commonly monitored in the atmosphere.

The objective of this study was to examine the temporal variability of the size-specific particle-gas distribution of atmospheric PAHs in order to understand the physical and chemical parameters affecting the gas-particle partitioning and consequently the ambient concentrations and fate of selected PAHs.

Atmospheric samples from urban and rural sites in the Czech Republic were collected between October 2009 and October 2010 using a high volume air sampler equipped with a cascade impactor (separating particles into 6 size fractions: < 0.49 μ m; 0.49-0.95; 0.95-1.5; 1.5-3.0; 3.0-7.2; 7.2-10 μ m). All filters and polyurethane foam (PUF) plugs collecting the gas phase were exchanged weekly, and the individual samples of each fraction were combined into 4 composite seasonal samples. PAHs in gas and particulate fraction were determined using automatic solvent extraction, silica-gel clean-up and fractionation techniques and GC-MS instrumental analysis.

Interpretation of the data suggests that proximity to primary sources drives PAH concentrations as the concentrations measured in the urban site were significantly higher than those measured in the rural site. Seasonality of concentrations levels (higher winter than summer values) as well as the gas-particle distribution (more compounds associated with particles in winter) was well pronounced at both sites for most of selected chemicals. It has been shown before that PAHs are mostly associated with the finest fraction of atmospheric particles, however, seasonal variability of this size-specific distribution is presented for the first time here.

TU 250

Size-specific particle-gas distribution of the atmospheric POPs on the spatial and temporal scales

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A presence of the particulate matter in ambient air (especially of the particles with less than 10 μ m in diameter) has a negative impact on human health as it can induce various respiratory diseases. The fine and ultrafine particles are of special concern as they can penetrate the lungs to the greatest extent and even enter blood stream.

Another factor that has to be considered when assessing human respiratory risks are persistent chemicals that are associated with the atmospheric particles and represent additional risk when released in human bodies. Size-specific distribution of these chemicals is an important parameter determining their behavior, fate and effects. Size-specific partitioning of organic chemicals between various fractions of the atmospheric particles has been previously studied for polyaromatic hydrocarbons and it has been demonstrated that they are mostly associated with the fine and ultrafine fraction. However, there is not much known about other groups of compounds, especially emerging pollutants. As such distribution affects the fate of compounds not only from the toxicological point of view but also from the point of the long-range transport of pollutants to the pristine environments, new data are desperately needed.

This paper reports on seasonal and spatial variability of size-specific particle-gas partitioning behavior of selected groups of legacy (dioxins and furans, pesticides) and emerging (brominated and fluorinated) pollutants. Samples have been collected from the urban and rural sites in the Czech Republic for the period of one year (October 2009 - October 2010) using a high volume air sampler equipped with a six-stage (< 0,49 µm; 0,49-0,95; 0,95-1,5; 1,5-3,0; 3,0-7,2; 7,2-10 µm) cascade impactor collecting particulate phase and polyurethane foam (PUF) plugs collecting the gas phase.

TU 251

Potential input of organic pollutants to the Mar Menor lagoon: estimation of seasonal air concentrations using passive and active air samplers

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A significant fraction of organic pollutants access directly to air and can be transported far from their sources associated to particulate material or dissolved. Pesticides are widely applied for agricultural treatments and polycyclic aromatic hydrocarbons are formed as consequence of urban, transport and industrial activities. Mar Menor lagoon is the ending point of drainages from a large agricultural area where a variety of pesticides are used. This lagoon is surrounded by several villages and that have, especially in summer, an intense touristic activity. There is also a military airport close to the lagoon and this area is influenced by a very industrialized pole, which is located at 20 km south.

There are not previous data of pesticides and PAHs concentrations in air of in this area. In order to estimate the levels of organic pollutants in air and the potential atmospheric input in Mar Menor lagoon, passive samplers consisting of polyurethane foam (PUF) disks housed in chambers were deployed at six sites around the basin of this lagoon. Replicates at each point and calibration with a DIGITEL active sampler (at one point) equipped with a PUF module are used to improve the confidence of the determinations and to estimate the sampling ratios for the different detected species.

PUF samples have been extracted with hexane using a pressurized liquid extractor. The extract was concentrated and was analyzed gas chromatography with mass spectrometry. The quantification limits were lower than 10 ng/L for the majority of the considered analytes. This method has been validated for more than 80 organic pollutants (pesticides and polycyclic aromatic hydrocarbons) and up to 23 have been identified in PUF samples from active and passive samplers. The range of estimated air concentrations is very wide (10pg/m³-2ng/m³), and depends on the specific compound, as consequence of the proximity of sources and environmental variables. Seasonal and spatial patterns have been found for different groups of organic pollutants associated to the main local sources in each case. The larger air levels of anthracene, fluorene and phenanthrene were detected in the proximity to the airport, while pesticides have a more homogeneous spatial pattern, with lower air concentrations by the sea side. Also a clear seasonal pattern has been detected in the case of some specific pesticides, such as chlorpyrifos, with higher concentrations in spring and autumn.

TU 252

Biomonitoring of polycyclic aromatic hydrocarbons by pine needles - analytical alternatives and levels in Europe

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The monitoring of pollutants is a crucial step to assess their environmental exposure to organisms. In general, this task is performed in their own habitat (sediments, soil, water or air), but biomonitoring offers the possibility to estimate the multi-route uptake of contaminants. Given its worldwide presence, adequate characteristics and low availability costs, vegetation has been chosen as a valuable matrix since the 1960s, allowing the passive sampling of a wide range of compounds (especially of atmospheric origin). PAHs are no exception since by their hydrophobic and lipophilic nature, they are prone to partition into the waxy layers of plants and trees (mostly on the leaves) or, in the case of the heavier PAHs, to be deposited as particles in the surface. PAHs are widespread carcinogenic and mutagenic contaminants with natural and anthropogenic sources mainly associated to combustion processes.

The complexity of plant matrices makes the separation of the target compounds from their lipidic structure a hard task and led to the development of multiresidue extraction methods in the last 20 years. This means that biomonitoring studies using these matrices demand for reliable extraction and clean-up procedures, which have been suffering continuous development to reach increasingly lower limits of detection associated with sharper chromatographic resolution.

The efficiency and validity of several extraction methodologies (Sohxlet, ultrasonic extraction, microwave-assisted extraction, accelerated solvent extraction, solid-phase microextraction and hollow-fibre liquid-phase microextraction) prior to a complementary clean-up step when needed were studied for 16 PAHs in pine needles. In concomitance, the levels, patterns and sources of contamination were assessed and compared in sampling sites from Portugal, Spain and Greece.

TU 253

Linear and cyclic volatile methyl siloxanes in Swedish air

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The concentrations of linear and cyclic volatile methyl siloxanes (IVMS and cVMS) were determined in air sampled close to the Swedish background monitoring station Aspvreten, some 100 km south of Stockholm. Parallel samples were collected with daily resolution over one month during November 2011. The sampling rate was 16 m³ d⁻¹. The method was based on an existing method for decamethylcyclopentasiloxane (D5). Modifications were made to account for the strong sorption of hexamethylcyclotrisiloxane (D3). The method was shown to be applicable to the IVMS, whereby L3, L4, L5 and L6 were analysed using tetrakis(trimethylsilyl) ester as the surrogate standard. The concentrations were of the order of 3 ng m⁻³ for D3, 1 ng m⁻³ for D6, 5 ng m⁻³ for D4, and 15 ng m⁻³ for D5. The concentrations of the IVMS were markedly lower, of the order of 0.2 ng m⁻³ for L3, 0.04 ng m⁻³ for L6, and 0.02 ng m⁻³ for L4 and L5. The difference between the concentrations measured in the parallel samples was in almost all cases < 10%. The influence of air mass origin on the temporal variability of the concentrations of the different analytes will be explored.

TU 254

Analysis of cyclic and linear volatile dimethylsiloxanes in aqueous samples and biota

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As a result of their wide use, siloxanes are spread into the environment both via point sources and via atmospheric long range transport and may be found everywhere in the environment. Recent studies have suggested that siloxanes may have direct or indirect toxic effects on various biological processes. Therefore, there is a need of fast, reliable and sensitive methods for the ultratrace analysis of siloxanes in environmental samples. However the routine analysis of these compounds is still challenging because of the low concentration at which they occur (especially in, their high volatility and because of their common occurrence in laboratory instrumentation, including gas chromatography columns, septa and sorbent phases). In this work, different analytical schemes for the analysis of cVMS and IVMS in wastewater and biota is presented. These methods are based on ultrasounds assisted extraction for biota samples, and liquid-liquid extraction for water and wastewater samples followed by gas-chromatography coupled to tandem mass spectrometry (GC-MS/MS). The new analytical methods were validated for their application to the analysis of water, wastewater and biota samples, offering excellent reproducibility, recoveries 63-113% and 40-112% and limits of detection 400 and 1600 pg/g and 0.5 and 7.5 pg/mL in biota and wastewater, respectively. Finally, the occurrence of siloxanes in more than 30 wastewater treatment plants in Catalonia (NE, Spain) was assessed, and the results confirmed the presence of the selected siloxanes at concentrations of ng/L in the final effluents. On the other hand, the analysis of biota samples using an extraction step by ultrasound-assisted extraction was applied for the determination of these compounds in different biota samples from the Llobregat River, the Mediterranean Sea and krill (*Euphasia Superba*) samples from the Antarctic ocean will be presented and discussed.

TU 255

Exchange and partitioning of POPs between the atmosphere-vegetation-soil compartments

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Even though the production and usage of many POPs have been banned decades ago, due to their persistence, they can be still found in the global environment, where in terrestrial environment they cycle between soil, vegetation and atmosphere. POPs can be transferred from the atmosphere to vegetation and soil surfaces by diffusive exchange, dry or wet deposition. A number of studies have shown that POP burdens in vegetation and soil generally reflect spatial trends of concentrations in the air. Vegetation can also be used to identify temporal changes of POP concentration in the air over a range of years whereas bulk soil responds much slower. However, POPs attached to soil/vegetation surfaces may revitalize delivering POPs to the air, enabling them to be transported in the atmosphere or taken up by organisms at different levels of the food chain. The objective of this work is to assess the role that vegetation plays in the overall air-soil-vegetation partitioning and as a control of POP concentrations in the atmosphere and soils. To study the exchange and partitioning of POPs between soil-vegetation-atmosphere, different sampling campaigns were carried out in temperate (sampling sites at northwest UK) and polar environments (Antarctica Peninsula). Results obtained in this study are close to the those predicted by McLachlan, 1999. Higher concentrations of PCBs, pesticides and PAHs have been found in the air sampled close to vegetation surface in comparison to ambient air concentrations, suggesting that vegetation compartment can also deliver POPs, especially the less volatile ones to the atmosphere, even though soil and vegetation seem to be in close equilibrium. The influence of surface temperature, lipid content in the POPs accumulation and on the magnitude of re-emission were also studied. Results from temperate and polar environments will be compared.

TU 256

Endosulfan transport from agricultural fields to Everglades and Biscayne National Parks in South Florida (USA)

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The environmental health decline and the loss of organism diversity of South Florida (USA) ecosystems have been attributed to nutrient inputs from nearby urban and agricultural areas. Intensive agricultural pesticide use may also challenge these ecosystems. One possible mechanism is pesticide release to the atmosphere after application which can be enhanced in this region due to the calcareous soils, frequent rainfall, and high humidity and temperatures. A study was conducted to examine the atmospheric fate of the widely-used insecticide endosulfan. Air samples were collected over a five-year period (2001 to 2006) at a site within the agricultural community of Homestead, Florida and at sites located in nearby Biscayne and Everglades National Parks (NPs). Endosulfan emissions from agricultural areas around Homestead appeared to influence air concentration observations at the NP sites. During an intensive sampling campaign, the highest total endosulfan concentrations at the NP sites were observed on days when air parcels were predicted to move from Homestead towards the sampling locations. The α -endosulfan fraction ($\alpha/(\alpha+\beta)$) was used to examine the contribution of pesticide drift versus volatilization to the overall residue level. The formulated product has an α fraction of approximately 0.7, whereas volatilization is predicted to have an α fraction of 0.9. The median fraction observed at Homestead during periods of high and low agricultural activity was 0.84 and 0.86, respectively, indicating that drift contributes to the atmospheric concentrations. The median fraction at Everglades NP was 0.88 and 1.0 during high and low agricultural activity, respectively, and at Biscayne NP was 1.0 year round indicating air concentrations are primarily influenced by regional volatilization. The near-irreversible isomerization after application of β -endosulfan to α -endosulfan also influences the value of the α fraction.

TU 257

Raman microscopy as a tool to examine agricultural sources of PM₁₀

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Emissions of particulate matter (PM) from animal feeding operations (AFOs) pose a potential threat to the health of humans and livestock and can lead to decreased air quality. Current efforts to characterize PM emissions from AFOs generally examine variations in mass concentration and particle size distributions over time and space, but these methods do not provide information on the sources of the PM captured. Raman microscopy was employed to characterize the distribution of sources present in PM₁₀ emitted from a large cattle feedlot. Spectra from potential source materials (dust from unpaved roads, manure from pen surface, and cattle feeds) were processed and compiled to create a spectral library. Principal component analysis followed by cluster analysis was used to classify the source library spectra into specific groups. A multivariate statistical analysis approach was developed to identify the source of particles collected on PM₁₀ sample filters. Source characterization results from samples collected at a cattle feedlot over a typical hot summer two-day period indicate that materials from the cattle pen surface contributed more than 50% of the total PM₁₀ particles captured followed by unpaved roads at approximately 20% and then feed materials. Results indicate this approach could be used in a number of different agricultural emission characterization scenarios.

TU 258

A langmuir-hinshelwood fit of atmospheric reactions of OH radicals with semivolatile, aerosol-borne compounds in chamber experiments

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A number of semivolatile compounds and proxies of environmental compounds, such as oleic acid, long-chain alkanes, polycyclic aromatic hydrocarbons, plasticizers, PCBs, brominated flame retardants and various pesticides have been investigated for their atmospheric degradation rates by OH radicals and/or ozone. Some of them have been examined in the gas phase and some of them in the adsorbed state as thin films or sub-monolayers on appropriate solid materials in either flow reactors or aerosol chambers, by exposing them to OH radicals or ozone at known levels.

In the last decades, numerous studies were performed to investigate the reaction of OH radicals with compounds in the gas phase. However, due to its complex mechanisms, aerosol-borne reactions with OH radicals are poorly understood. Only recently, the Langmuir-Hinshelwood mechanism and the Eley-Rideal mechanism have been applied on the reaction of semivolatile compounds with O₃, considering different adsorption processes of reactants and the gas-particle equilibrium characteristics.

In this work, we applied both mechanisms in the analysis of the reaction of aerosol-borne terbutylazine with OH radicals in our simulation glass-smog chamber. Home made silica particles were used as carrier material. Compared to previous <10 nm particles (Aerosol 200), these larger particles (diameter about 160 nm) have less tendency to agglomerate. Compared to previous studies, a negative correlation with OH concentration was found for the second order reaction rate constant. At relatively low OH concentrations, the reaction could be explained by the Eley-Rideal mechanism, while at higher OH concentration, the Langmuir-Hinshelwood mechanism is more appropriate to explain concentration-time dependencies. Because the adsorption could be affected by gas-particle equilibria, results from different experimental setups (e.g. Chamber experiments and flow tube experiments) are assumed to be different.

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TU 259

Analysing PBDE in house dust samples with the TSQ Quantum Ultra XLS GC-MSMS in EI- SRM mode and GC-MS negative chemical ionization in SIM; a comparison of two analyzing techniques

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Polybrominated diphenyl ethers are a class of compounds used as flameretardants in a wide variety of household apparatus and furnitures.

Certain congeners have been banned completely and are currently on the list of the Stockholm convention on persistent organic pollutants. (1,2)

There is an ever growing concern about the health risks being exposed to these class of compounds and one of the sources of human intake is next to nutrition, inhalation of indoor air and house dust.

In this application note, a comparison is made between two analytical techniques; GC-MS-MS and GC-NCI-SIM, evaluating detection limits, repeatability and selectivity in matrix.

A broad range of PBDE was analysed, ranging from tri- up to deca BDE, together with another class of brominated flame retardants such as decabromodiphenylethane (DBDPE), tetrabromethylcyclohexane (TBECH), and 1,2,5,6,9,10-Hexabromocyclododecane (HBCDD).

TU 260

Human exposure to flame retardants in different occupational settings from Pakistan

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Flame retardants (FRs) are widely used in a variety of consumer's products, such as plastics, textile coatings, electrical appliances and printed circuit boards to inhibit the development of fires. Studies have shown that these chemicals can leach out into the environment. In order to investigate the presence of FRs in the specific occupational setting and human exposure to FRs, we collected dust, serum, and urine samples from individuals working at electronics and textile market in Punjab, Pakistan.

Polybrominated diphenyl ethers (PBDEs), novel brominated FRs (NBFRs), hexabromocyclododecanes (HBCDs) and organophosphate FRs (OPFRs) were quantified in dust, serum, and urine samples. FRs were extracted from dust, serum, and urine using solid-phase extraction (SPE). In dust and serum extracts, PBDEs and NBFRs, were analysed by gas chromatography (GC) coupled to mass spectrometer (MS) operated in electron capture negative ionization (ECNI), while OPFRs in dust were analysed by GC/MS in electron impact (EI) mode. The separation and determination of α -, β -, and γ -HBCD isomers in dust and serum was achieved using liquid chromatograph (LC) coupled to triple quadrupole MS system operated in the negative electrospray ionization (ESI) mode. OPFR metabolites were analysed in urine using LC-MS/MS in negative ESI mode. Generally, large variations in the individual concentrations were found within and between the investigated groups. The present work indicates that the workers in the studied area are occupationally exposed to various FRs, with probably indoor dust ingestion and air inhalation as a major source of exposure. Correlations were performed between the levels of FRs in dust and serum or between dust and urine samples. For workers, using the 5th, median and 95th percentile concentrations in dust samples different exposure scenarios were calculated via dust ingestion. Exposure scenarios were calculated assuming 100% absorption of intake dust, a 10 hr/day working shift, 70 kg body weight (bw), mean dust ingestion (1.25 mg/hr) and high dust ingestion (3.13 mg/hr). Exposure assessment values were lower than RfD values. Human exposure to FRs seems to originate from a combination of different sources, including indoor sources and diet; however, further studies investigating serum and urine samples from a larger number of occupationally exposed workers are necessary for a more complete assessment of human exposure pathways to these environmental contaminants.

TU 261

Use of volatile organic compounds (VOC) in consumer products and comparison between the European and US reactivity models for assessment

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Use of Volatile Organic Compounds in consumer products and comparison between the European and US reactivity model for assessment:

The environmental impact of Volatile Organic Compounds (VOC's) is their ability to react with NO_x in the lower atmosphere in the presence of sunlight and heat to produce ground-level ozone, also called summer-smog. Summer conditions favour the formation of ground-level ozone primarily because of increased ultraviolet radiation, temperature and low wind speeds. The reactivity potential of VOC's in Europe is defined as Photochemical Ozone Creation Potential (POCP); developed by Derwent et al (1998). In USA / California the concept describing the reactivity potential of VOC's is the Maximum Incremental Reactivity (MIR); developed by Carter et al (2006). MIR's and POCP's both measure the reactivity towards ozone production of a given VOC. The MIR scale is reported as the mass of additional ozone produced per mass of addition VOC reacted. POCP's report the additional ppb of ozone produced by an additional emission of that VOC, expressed relative to that produced by the same emission of ethylene as reference.

International consumer companies with global brands have the challenge to meet different aspects of regulatory requirements to reduce their impact of VOC's. Some examples

are discussed within the poster presentation.

TU 262

Distribution of linear and cyclic volatile methyl siloxanes in indoor air samples and implications for human exposure

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Cyclic (D3, D4, D5, D6) and linear (L2-L5) volatile methyl siloxanes are widely used in personal care products and cosmetics, as well as in industrial applications (biomedical products, surface treatment agents, plasticizers and construction materials). These compounds are of environmental concern due to their volatility, persistence and tendency to bioaccumulate.

The purpose of this study was to investigate their occurrence and distribution in indoor air environments, including domestic (i.e. bathrooms, living rooms, boys/girls rooms) and non domestic (supermarkets, offices, schools, museums) environments. An extensive air sampling campaign was performed contemporarily in the UK and Italy, between May and August 2011. Indoor air samples (n = 100) were collected on adsorption Tenax GR cartridges (60/80 mesh, Markes) using conventional portable air sampling pumps (i.e. GilAir3, Gillian- Sensodyne), operating at a flow of about 120 mL min⁻¹. Sampling cartridges were desorbed using the Automatic Thermal Desorber UNITY2 coupled to a GC/MS-system. Concentrations of total siloxanes ranged from 20.6 to 467 µg/m³ and from 55.7 to 424 µg/m³ in Italian and UK samples, respectively. Cyclic volatile methyl siloxanes (in particular D3 and D5) accounted for ~90% of total siloxane concentrations in all samples.

The highest siloxanes concentrations were found in bathrooms; in particular, D5 represented the principal compound probably due to its dominant presence in most of hair-care products, skin lotions, cosmetics, household products and cleaning agents. Personal lifestyles and everyday-life behaviours (e.g., selection of products, frequency of application, amount applied) had a determinant effect on the amount of total siloxanes found in both Italian and UK adult and living rooms. No significantly differences in concentrations were found in other indoor domestic environments. The daily inhalation exposure (DIE) to cyclic and linear volatile methyl siloxanes was also evaluated.

TU 263

Source contribution of atmospheric PAHs at urban and industrial locations in the city of Novi Sad, Serbia

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Data on polycyclic aromatic hydrocarbons (PAHs) in ambient air accessed at urban and industrial locations in the city of Novi Sad, Serbia, have been analyzed in order to determine emission sources and their contributions to the concentrations of particle-bound PAHs. Previous studies have demonstrated that the major contributors of PAHs in urban areas are the emissions from vehicle exhaust, and emissions releases from industrial processes like aluminium production, creosote and wood preservation, waste incineration, cement manufacture, petrochemical and related industries, commercial heat/power production etc. The sampling campaigns have been conducted at three sampling sites, during the two 14-day periods. The first site was situated near industrial area, with a refinery, power plant and heavy-traffic road in the vicinity. The second site was located nearby the heavy traffic area, especially busy during the rush hour. The third site was residential district. Non-heating sampling period lasted from June 26th to July 9th 2008, while sampling of ambient air during the heating season was undertaken from January 22nd to February 4th 2009. 104 air samples were collected using a high volume air sampler TCR Tecora H0649010/ECHO HiVol with quartz fiber filters (QFFs). 16 US EPA polycyclic aromatic hydrocarbons were determined in all samples using a gas chromatographer Shimadzu GC-2014 with a flame ionization detector. The total average concentrations of PAHs ranged from 0.4 to 3.16 ng/m³ during the non-heating period and from 0.05 to 36.81 ng/m³ in the heating period. Various techniques, including diagnostic ratio (DR) and principal component analysis (PCA), have been used to define and evaluate dominant emission sources of PAHs. Diagnostic ratio analysis indicated that vehicles, diesel or/and gasoline, industrial and combustion emissions were potential sources of PAHs in Novi Sad. Additionally, principal component analysis was used to constrain the potential sources. The results show that vehicular emissions are the predominant source of particle-bound PAHs in Novi Sad, Serbia. Less dominant emission of PAHs comes from stationary combustion sources such as home heating during the winter and oil refinery processes during the whole year.

TU 264

Polybrominated diphenyl ethers (PBDEs) and other flame retardants in adult *Pipistrellus* bats (*Pipistrellus* spp) from an urban area of NW Britain

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Bat (*Pipistrellus* spp) livers of 30 adult males (M) and 23 adult females (F) from an urban area of the NW Britain, were analysed for 23 PBDEs congeners (Tri to hexa-BDEs) as well as for other flame retardant (pentabromomethylbenzene, pentabromoethylbenzene, hexabromobenzene, hexabromocyclododecane (HBCD) and the two isomers of Decchlorane Plus (DP).

The dominant PBDE congeners were BDEs 47, 99, 100, 153, 154 and 183 in both M and F, which are the same dominant congeners present in the penta technical mixtures. However, bats have a lower proportion of PBDE 47 than the technical mixtures, suggesting degradation of this compound. Total BDEs were dominated by penta-BDEs (65% of the total), followed by hexa-BDEs (with just over 20%) for both males and F.

The sum of the PBDEs analysed showed a wide range (10.4 - 8191 ng g⁻¹ wet weight (ww)) of concentrations, with a mean of 961 ng g⁻¹ ww. Males had on average twice the concentration of PBDEs than F (M: 1182 ng g⁻¹ ww F: 673 ng g⁻¹ ww), although this difference was not statistically significant, possibly a result of the small sample size.

Pentabromomethylbenzene was not detected in any sample and pentabromoethylbenzene and hexabromobenzene were only found in a few individuals. In our samples we only could detect the anti isomer of DP, with concentrations between 1.6-38.4 ng g⁻¹ ww. This may have been expected because this is the dominant isomer in the technical mixture. However, we only found DP in M, this may be the result of F offloading accumulated residues to the foetus and to juveniles via milk, as DP is highly lipophilic. The lower residues of PBDEs in F than M is likewise consistent with transfer of residues from mothers to offspring. In contrast however, HBCD (sum of the 3 diastereomeric pairs of enantiomers) was found in higher concentrations in the F (mean= 176.9 ng g⁻¹ ww) than in the M (76.0 ng g⁻¹ ww), and this suggests that there may be sex differences in exposure to or metabolism of this compound. In conclusion, this is the first study that we are aware of to report tissue concentrations of PBDEs and some of their emergent replacement compounds in bats from Europe.

TU 265

Atmospheric pollution due to methane seepage from surface rock layers

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Methane emission contributes to the growing background concentration of ozone, an air pollutant that impacts on air quality and, hence, human health. In particular, methane seepage from surface rock layers into the atmosphere is a problem in many parts of the world. The ground surface is the zone where the processes of weathering are very active. In the presence of gas-bearing rock layers (gas sources) located at a shallow depth below the ground surface, weathering-induced fractures in surface rocks can conduct gas from gas sources into the atmosphere. In the southern part of Donetsk city (Ukraine), weathering-induced fractures in very weak rock (sandy shale) are interconnected and form a system of through channels. The values of gas flow through fractures and intensity of fractures were measured in situ. Observations show that number of fractures per meter measured along traces of through channels is between 34 and 69, and total fracture length of through channels per unit area ranges from 19.5 to 21.6 m/squared meter. It is established that fracture aperture width along trace of through channel can reach 7 mm, and value of the fracture aperture tends to decrease with increasing depth below the ground surface. In situ study shows that role of the system of weathering-induced through channels in the process of gas emission to the atmosphere is much more significant than the role of rock matrix. Indeed, gas flow from through channels is 10.4-19.8 times larger than gas emission from rock matrix, and weathering-induced fractures conduct large portions (91-95 %) of gas emitted from ground surface. The gas flow from individual through channel ranges from 0.00069 to 0.00136 cubic meter/s. The study of impact of methane seepage on human health shows that there are cases of methane poisoning of people living on the ground floor. The frequency (F) of the methane poisonings was statistically analysed. As a result, it is established that frequency F is dependent on average value of gas volumetric flow rate from individual through channel (q), number of through gas-conducting channels (k) located within a 20 m radius around each dwelling house. It is defined that value of F increases according to exponential law, where a variable exponent is the gas flow (q) multiplied by logarithm of the number (k) of gas-conducting channels.

TU 266

Environmental impact of implementing alternative fuels in a Spanish cement plant

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Cement industry produce around 5% of the whole world anthropogenic CO₂ emissions, either as direct (decarboxylation of calcium carbonate -raw material-) or indirect (fuel combustion) emissions. In order to reduce the amount of CO₂ emitted by the cement industry, new environmental policies promote the use of alternative fuels such as sewage sludge and refuse derived fuel, among others. Because a reduction of CO₂ and costs (fossil fuel saves) may be expected, this activity has resulted to be very environmentally and economically beneficial. On the other hand, the population living closest to the cement plants are generally concerned about the possible effects on the environment and health derived from the use of these alternative fuels. In some cement plants of Catalonia (Spain), the concern of the neighbouring population is especially important, as there is a short distance between cement plant and homes. This short distance is sometimes due to poor (or inexistent) urban planning, illegal construction and the lack of legislation in the past, the distance between populated centres and industrial facilities have been notably shortened.

In Catalonia (Spain), in the last years, the cement industry has started to use different kinds of alternative fuels for partial replacement of the energy, which traditionally comes from fossil fuel. To ensure that this fuel partial substitution will not cause a risk to the environment and health of residents around the cement plant, various environmental monitoring campaigns have been conducted in the surroundings of facilities of different characteristics, in terms of used fuel (sewage sludge, refuse-derived fuel, etc.) or zone characteristics (urban, suburban or rural). In each campaign, several samples of environmental monitors (air herbage and soil) were collected before and after the alternative fuel implementation, and the content of metals and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) was determined.

The findings showed no significant differences between campaigns regardless of the use of alternative fuels. In conclusion, the current results support and encourage the

option of using alternative fuels in cement plants working with the Best Available Technologies (BAT).

TU 267

Should the neighbourhood of cement plants be concerned when alternative fuels are used?

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In recent years, co-combustion of alternative fuels in cement plants has become an increasing practice in many countries. The benefits associated to the replacement of fossil by alternative fuels such as sewage sludge or refuse-derived fuel (RDF), are both economically (fossil fuel saving) and environmentally (e.g., reduction of CO₂ emissions, reuse of by-products) evident. These benefits are more important taking into account that cement production is one of the main CO₂ emitting manufacture industries, but also due to the important rising of fossil fuel prices. Furthermore, considering the notable consumption of fossil fuels by the cement industries, the European Union is encouraging the enhancement in the amount of alternative fuels used in cement kilns.

In the present work, the result of a 3-year (2008-2011) environmental monitoring campaign around a cement plant located in the metropolitan area of Barcelona (Catalonia, Spain) was presented. The aim of this study was to evaluate the potential changes in the environmental levels metals and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in the vicinity of a cement plant before and after using sewage sludge as alternative fuel. The concentrations of PCDD/Fs and metals were determined in soil, herbage and air samples around this facility before and after the partial fuel substitution. The potential changes on the health risks for the population living around the facility were also assessed.

In general terms, no statistically significant differences in the pollutant values were found between the 2 scenarios (before and after the partial fuel replacement), with the exception of a few significant decreases in some metals. Besides, the carcinogenic and non-carcinogenic risks due to the exposure to metals and PCDD/Fs were below the national and international safety limits.

TU 268

Environmental impact of mechanical-biological treatment systems. Human health risks of chemical and microbiological pollution

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Due to legislative, environmental, economic, and social limitations, the identification of sustainable disposal pathways for the management of municipal solid waste (MSW) is a very important matter. The mechanical-biological treatment (MBT) approach presents many advantages in comparison to other waste management possibilities. However, adverse health effects related to this practice are not well known, as a varied typology of microbiological, such as bacteria and fungi, and chemical pollutants, such as volatile organic compounds (VOCs), are generated during waste composting and similar processes. Some of these contaminants can be the cause of a variety of infectious diseases, as well as allergies and toxic effects or generating malodorous and hazardous properties. In 2010, a program was initiated to monitor air levels of VOCs and microbiological pollutants near a MBT plant (Ecoparc-2) in Montcada i Reixac, (Barcelona, Catalonia, Spain). To investigate the temporal and seasonal trends of chemical and microbiological pollutants, four 6-monthly campaigns were performed. Air samples were collected at different directions and distances from the facility. The levels of total bacteria ranged from 160 to 1054 cfu/m³ (colony-forming units per cubic meter) with a high increase in the last survey (summer 2011) compared to the initial (winter 2010). In fact, the highest concentrations were detected in the summer campaigns. Fungi at 37°C were also important in the hot seasons with values of 716 and 106 cfu/m³ (summer 2010 and 2011, respectively) versus 60 and 61 cfu/m³ (winter 2010 and 2011, respectively). The concentration of the remaining microbiological agents (gram-negative bacteria and the more specific *Aspergillus fumigatus*) was rather low, with a lack of temporal/seasonal differences. On the other hand, the highest mean concentration of VOCs was found in the third campaign (45.7 µg/m³), with values between 4.55 and 120.9 µg/m³. For these chemical compounds, the greatest concentrations were found in two winter campaigns. The current exposure to those chemicals, estimated from the environmental burdens, should not mean additional non-carcinogenic or carcinogenic health risks for the population living nearby. Finally, a fate and transport model was also executed to validate the acquisition of data from outdoors, as well as comparing modelled and experimental results.

TU 269

Environmental risk management in confined spaces. A study case of an archive like a model of indoor environment involved with biodeterioration

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Proper management of specific indoor environments, such as those that preserve Cultural Heritage, cannot fail to take into account the interactions they may have on human health. The Historical Archives, where cellulolytic microfungi and bacteria find a comfortable environment for their development, are of particular interest in this sense.

In fact, one of the major causes of paper degradation is the biodeterioration, which develops with specific environmental conditions. Therefore, it is necessary to conduct research linking aerobiology with environmental Archives, to understand the conservation state of the documents and, also, the possible risks for the Archive's operators and for people who work within these environments.

The study case involves the Contemporary Political History Archives sites in Ca' Tron, Treviso (Italy) in the Veneto countryside. A specific seasonal aerobiological protocol was developed to monitor the presence of microorganisms both in the air and on the documents stored, the temperature, humidity and light conditions, and the detection of dust (PM₁₀ and PM_{2.5}). For the aerobiology analysis, active and passive samplings were performed. Volumetric samplers were used to measure the concentration of microbes in air (expressed by CFU/m³), while Petri dishes with different culture media (Nutrient Agar, Malt Extract Agar and Malt Extract Agar plus Chloramphenicol) were used for qualitative microbiological sampling (expressed by IMA, CFU/dm²/h). In addition, two parameters were considered for the surface contamination, using nitrocellulose membranes: the Microbial Buildup (MB, the total number of microorganisms collected on a surface in 30 second) and the Hourly Microbial Fallout (HMF, the number of microorganisms collected during 1 hour). In this way, it was possible to:

- define the conservative situation of the Archive;
- identify microbial species present both in the air and on the documents and then identify those potentially cellulolytic and those dangerous for human health;
- to propose measures to improve archive's conservation and thus prevent degenerative risk situations, like the Sick Building Syndrome.

TU 270

Persistent organic pollutants (POPs) in ambient air, human breast milk and edible fish - a potential for health risk in Ghana

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Levels of persistent organic pollutants (POPs) in rural and urban background air of southern Ghana were measured in 2008 using polyurethane foam (PUF) disks passive air samplers (PAS) (referred to as PUF-disk samplers). The objective of this study was to quantify the levels of POPs in air and to establish their baseline information for future monitoring and trend studies. This is the first comprehensive study reporting on levels of POPs in background or ambient air in Ghana covering the coastal zone and the middle belt. POPs in pooled and individual human breast milk samples collected from lactating mothers countrywide were also determined using a high-resolution gas chromatography interfaced with a high-resolution mass spectrometer (HRGC-HRMS). This constitutes the first comprehensive nationwide human breast milk study of assessing risks of HCHs for the general population of Ghana. The results of the study showed that the general population of Ghana is widely exposed to POPs although the current levels are generally low. POPs measured in selected edible fish from three freshwater bodies, namely Lake Volta, Lake Bosomtwi and Weija Lake in Ghana shows that there is a potential health risk from POPs for the general population of Ghana because fish is one of their important protein sources.

TU 271

Toxicity screening of fine particles by a novel microbial test battery

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Burning of wood for heating purposes has become extremely popular worldwide due to the increasing costs of fossil fuels. Claimed as renewable energy source, wood has often been ignored as a source for fine particles causing toxic effects. Rapid reproducible and validated biotests for toxicological characterisation of such particles exist neither for cells nor for micro-organisms. Thus there is a need for established microscale toxicity screening of unknown combustion born particles.

Hence a test battery consisting of two ISO standardised bacterial contact assays and one yeast biotest were modified for the use with fine dust. Having different endpoints these tests provide a toxicological "fingerprint". In order to account for bioavailability of contaminants adsorbed on surfaces of fine particles, such were used as a whole not as extracts.

Particulate matter was gained electrostatically from different furnaces in the flue gas channel. Suspended in water, vortexed for 1 min, and ultrasonicated for 15 min samples were applied in a concentration range of 0.001 - 1.0 mg/mL. Particle sizes (5-10 µm) in these suspensions were measured by dynamic light scattering. The control contained no particles, solely deionized water.

Cytotoxicity in the aerobic *Arthrobacter globiformis* contact assay was determined via dehydrogenase activity (DHA). Genotoxicity was determined via Umu-Test using a genetically modified *Salmonella* strain. The Yes-Test was employed to determine potential estrogenicity of particle samples.

Contrary to FP A, FP B exhibited cytotoxicity between 0.1 and 1 mg FP/ mL. High contents of Zn supposedly caused cytotoxicity in B. FP A was slightly genotoxic (+S9) at 1 mg/mL and potentially estrogenic. Besides PAHs, other heterocyclic compounds might have caused this result. Further investigations are under construction.

These preliminary results suggest suitability of endpoints determined in the modified tests for risk assessment of fine particles.

TU 272

Airborne particulate matter induced pro-inflammatory effects and oxidative stress in A549 cells

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Clean air is a basic requirement for human health and well-being. An average daily inhalation of 20 m³ of air is characterized by an exposure to many different pollutants.

Apart from the classic gaseous pollutants airborne particulates continue to pose a significant threat to human health worldwide.

The objective of this study was to compare the toxicological effects of different source-related particles in regard to their chemical composition. In this context we investigate airborne PM from different sites in the Region of Aachen. A549 cells were exposed to increasing PM concentrations followed by analyses of cell viability, pro-inflammatory and oxidative stress response.

The results showed a seasonal and location dependant variability of the PM concentration for both particle fractions. Chemical analysis of these particles indicated the presence of 21 elements, water-soluble ions and a multitude of different PAH. The major inorganic components are the crustal elements (Ca, K, Mg, Na) and the (transition) metals Al, Fe, Zn. In contrast to metals and PAH, the concentration of ionic species is only approx. 50 % higher at the rural site, compared to the urban site. Water-soluble particulate matter extracts induced a concentration- and time-dependent decrease in cell viability and an increase in pro-inflammatory and oxidative stress markers. The samples of the urban traffic location, characterized by a high concentration of elemental/organic carbon and metals, induced the highest pro-inflammatory and oxidative activity. Due to the combination of chemical-analytical and toxicological methods a characterization of PM induced cause and effects are possible.

The response of A549 cells to ambient PM was markedly different comparing specific samples from various sampling sites and emission sources in the region of Aachen. Our results support other investigations regarding the importance of the chemical compositions and there PM induced toxicity in vitro.

The study is embedded in the project City2020+ which is part of the interdisciplinary Project House HumTec (Human Technology Center) at RWTH Aachen University funded by the Excellence Initiative of the German federal and state governments through the German Research Foundation (Deutsche Forschungsgemeinschaft, DFG).

TU 273

Selectivity of PCBs at low level with high precision using GC-MS/MS Triple Quadrupole

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The low level analysis of polychlorinated biphenyls at levels required by EPA method 1668 has required the need of a high resolution GC/MS system. Using triple quadrupole GC-MS/MS can achieve the needed detection limits plus offer specificity for confident identification and confirmation. This application will explore the relationship between resolution, selected reaction monitoring (SRM) and speed of acquisition to achieve precise quantification and identification of the chlorinated compounds. The studies were performed in matrix, demonstrating that this isolation can readily remove matrix interference at low target compound concentrations. In addition, this method will demonstrate that the quality control criteria of EPA Method 1668 can be met using a GC/MS triple quadrupole system. Advantages for the laboratory are the reduction in cost of the equipment relative to high-resolution GC-MS systems, along with the ability to use the system for more than just specialized applications. In addition, a library of transitions for the PCBs and other compounds simplifies method development and verification, simplifying the adoption of GC-triple quadrupole MS for this analysis.

TU 274

Analysis of pesticides in water samples based on the combination of solid phase extraction and solid phase microextraction coupled to gas chromatography mass detection

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The increase of the agricultural surface area in Argentina has triggered the generalized use of pesticides, which are spread across soils contaminating both surface and groundwaters. The aim of this study was: (a) develop a sensitive Gas Chromatography tandem Mass Spectrometry (GC-MS) method for the quantification of the most widely used pesticides at environmentally relevant concentrations and (b) test this method with surface waters collected from the Suquia River basin, Córdoba, Argentina.

Sample pretreatment involves a combination of Solid Phase Extraction (SPE) and Solid Phase Microextraction (SPME) affording highly effective enrichment of six pesticides, which can be analyzed at ppb levels. We tested the effect on SPE of cartridges and elution solvents as well as type of fiber, absorption-desorption temperature and time, head-space or immersion for SPME. A fractional factorial design was applied to obtain optimal conditions. GC and MS operational issues were also optimized to afford LODs in the same range that ECD detectors. The optimized method was validated in terms of linearity, precision, recovery and repeatability, showing that the proposed procedure is sensitive (LODs ranging 0.2 to 3.5 ng L⁻¹), precise and robust (recoveries ranged 61 - 104%, RSD varied from 4.0% to 22.6 %), with a linear analytical range from 0.1 to 10 µg L⁻¹.

This method was successfully applied to natural water samples, collected at five monitoring sites along Suquia River, considering pre and post-application of agricultural pesticides. Most studied pesticides were present throughout the entire period. Nevertheless, post-application period showed concentrations of atrazine, acetochlor, endosulfan, endosulfan sulfate and cypermethrin 1.5 to 5 fold higher than the corresponding to pre-application. As expected, highest levels of pesticides were observed in areas with intensive agricultural practices, being atrazine, cypermethrin and endosulfan sulfate predominant. In urban or recreational areas the prevalent pesticide was cypermethrin. Some values surpass the National Guidelines for pesticides in freshwaters, pointing out the need of controls in addition to the evaluation of damage to aquatic biota.

EC06P - Sorption and bioavailability in sustainable remediation of organic chemicals

TU 278

Simulating the bioavailability of mineral hydrocarbons for earthworms using different extraction methods

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In the context of assessing the habitat function of contaminated sites only the bioavailable contaminant fraction is of relevance. Information on the habitat function, therefore, can be obtained in ecotoxicological tests performed with representative soil organisms. Depending on the test organism the duration of effect measurements varies between several weeks and several months. In order to obtain results focusing on bioavailability faster, chemical methods are being developed to predict the amount of contaminant that can be taken up by a specific organism. Suitable methods have to simulate the respective exposure pathways and represent the bioavailable fraction of the contaminant. Extraction procedures for mineral hydrocarbons were regarded with respect to their suitability for simulating the bioavailable fraction for soil organisms. As effect parameter the reproduction activity according to OECD Test Guideline 222 was selected. The test was performed in eight sandy soils that had been contaminated with mineral oil for many years. The results obtained in the earthworm studies were compared with the concentration of the mineral hydrocarbon contamination using different extraction procedures: (I) total content using exhaustive extraction (C10 - C40 fraction); (II) fraction of C10-C22 performing exhaustive extraction; (III) water based extraction - shaking procedure (IV) water based extraction - column procedure (V) water based extraction - use of an extra solid phase: HPCD.

The best relation between an increased concentration of contamination and a reduced habitat function was detected for the C10-C22 fraction of the HPCD-extraction. Relations with the C10-C40 fraction of the HPCD-extraction and with the C10-C22 fraction of the exhaustive extraction were less pronounced. No relation was observed for the water based extractions using shaking and column procedures.

Earthworms are soft bodied organisms and exposed to contaminants mainly via soil pore water and skin. For mineral hydrocarbons the mobile fraction of C10-C22 of the HPCD-extraction seems to be a good indicator for the available fraction. A value of 300 mg/kg may be a suitable threshold value regarding the habitat function of soils. Values exceeding this content may indicate that there is concern for a reduced habitat function.

TU 279

Impact of geosorbents on bioavailability of polycyclic aromatic hydrocarbons to humans by oral uptake

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To assess the health risk resulting from human uptake of contaminated soil from hand-to-mouth activity, it is prerequisite to know the oral bioavailability of the contaminants. Soil-bound contaminants can be mobilized by digestion juices and hence become available for absorption through the intestinal wall [1]. With a physiologically based extraction model (PBET) that is simulating the conditions in the human gastrointestinal tract, it is possible to determine the bioaccessible fraction of soil-bound contaminants.

Previous studies revealed strong variabilities of the bioaccessible fraction (23-68 %) of polycyclic aromatic hydrocarbons (PAH) in natural site samples [2]. Reasons for these variations are unknown. Apart from worldwide use of varying PBET models, we assume that quality and quantity of present geosorbents in the samples lead to variabilities. It is generally known that sorption of PAH in the aquatic environment depends on the type and amount of organic carbon present [3], however, it is unclear if this is also the case in a digestion juice system. The aim of the study is to show differences in bioaccessibility of PAH from single commonly occurring geosorbents. The results are expected to lead to a better understanding of variations in PAH bioaccessibility in natural site samples.

In this study, the geosorbents sand, clay, peat and char coal, which had been characterized in various previous studies, spiked with 16 deuterated EPA-PAH, were extracted with a PBET-model [4]. The bioaccessible fraction of contaminants was analyzed by gas chromatography-mass spectrometry.

The results show that the different geosorbents impact bioaccessibility as expected from the aqueous phase. Pyrene-d10 often showed highest bioaccessibilities: 45.5% ±11.1 (sand), 10.2% ±5.4 (clay), 3.5% ±3.2 (peat) and 0.2% ±0.1 (char coal). As it is known from aqueous sorption-desorption experiments, here also char coal significantly reduced desorption into the human digestion juice. We conclude, that currently bioavailability of PAH from soils and sediments rich in coal, char coal and soot may be significantly overestimated.

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TU 280

Bioavailability measurements in risk assessment - conformity of methods

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Bed sediments often act as a sink for nonpolar organic pollutants released into the aquatic environment. When sources of contamination cease or when emissions are strongly

reduced (e.g. historical pollution), sediments can become a secondary source to their surrounding environment. As a result of the challenges encountered when attempting to predict contaminant bioavailability and mobility, gauging the risk posed by these contaminated sediments remains challenging. In an effort to control and reduce the impact of contaminated sediment on ecosystems and human health, a risk assessment guideline tool for contaminated marine sediments was recently developed for Norway. In many cases, the comparison of total sediment concentrations with environmental quality standards overestimates the risk posed by these sediments as a result of much stronger partitioning to organic phases in the sediment than commonly assumed. Therefore tools to estimate contaminant bioavailability in sediments, bioaccumulation potential and risk of transfer to the overlying water phase are needed. This study aimed to assess (predict the potential for) the in vivo bioaccumulation of contaminants from historically polluted sediments using simple generic sorption models and from freely dissolved pore water concentrations measured with passive sampling. Freely dissolved pore water concentrations were measured in batch experiments in the laboratory using low density polyethylene (LDPE). In vivo exposures were carried out using *Nereis virens* and *Hinia reticulata*.

TU 281

Bringing (bio)accessibility extractions to the next level - combining mobilisation medium and infinite absorption sink

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Soil bioaccessibility extraction techniques are generally simple dissolution experiments, where the fraction of compounds that is transferred to the medium is measured and considered to be bioaccessible. However, such techniques can lead to an underestimation of bioaccessibility, since they do not account for the consumption of contaminants by either degradation or absorption. It is therefore crucial to develop practical bioaccessibility extraction approaches that combine both mobilisation and consumption processes. The mobilisation medium can be chosen to either maximize desorption without attacking the matrix or even to simulate the relevant organism conditions. A sorbent can act as an infinite diffusion sink for continuously removing the mobilized contaminants from the medium.

Initially, we combined cyclodextrin extraction with a polymer of poly(dimethylsiloxane) and activated carbon which lead to the contaminant trap method¹. This contaminant trap is a practical and simple approach for the isolation and quantification of the desorption resistant contaminants in soils, its main limitation being that it is not possible to back extract from the polymer. The next step was to find a polymer material and format that can act as infinite sink and allow simple back extraction. Silicone rods were chosen, which are already used in silicone rod extraction and passive sampling. This resulted in a better and more relevant bioaccessibility extraction approach and simplified the analytical procedures. The silicone rods were applied to both cyclodextrin solutions and artificial digestive fluids, using PAHs as model compounds. Passive dosing² was applied to determine the free fraction and partitioning of PAHs in cyclodextrin and digestive fluids, which in turn was used for sink dimensioning. Validation experiments with spiked cyclodextrin and digestive fluids confirmed an efficient absorption of PAHs by the silicone rod. Finally, determination of the readily desorbing PAHs from a wood soot sample with and without the absorption sink clearly showed that a sink is needed and makes a substantial difference.

References

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TU 282

Is there sufficient 'sink' in current bioaccessibility determinations of organic pollutants in soils?

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Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental organic pollutants, a number of which are known carcinogens. Soils are the major environmental sink for PAHs and it has been estimated that over 90 % of the U.K. PAH burden resides in soil. Total pollutant concentration is frequently used in the assessment of risk posed by contaminated land to human health. However, it has been widely established that such an approach may significantly overestimate the amount of pollutant absorbed by humans resulting in an overestimation of risk. One of the main pathways for human exposure to contaminated soils is direct ingestion as a result of hand-to-mouth activity. To address a number several in vitro physiologically-based extraction tests have been proposed for metal and organic pollutants. In recent work we developed a colon extended physiologically-based extraction test (CEPBET), this enhanced the bioaccessibility of PAH compared to a two compartment, i.e. stomach and small intestine, model. The aim of the current work was to combine the CEPBET bioaccessibility test with a charcoal contaminant trap in order to maintain a full diffusion gradient for the desorption process, thereby enhancing the bioaccessibility extraction. This was believed to be necessary because the gastrointestinal tract (GIT) provides a considerable sorptive sink for PAH due to its large surface area and lipophilic nature.

The integration of the contaminant trap significantly enhanced PAH desorption from incubated soils, reducing gut medium concentrations to <5% of the no trap values within the appropriate physiological timescales. This clearly identifies the need for a sink in bioaccessibility extractions. The complete configuration of the CEPBET system is required as the COL significantly increases pollutant desorption. A configuration of CEPBET is now required that combines the 'trap' with the ability to actually measure the bioaccessible fraction. This seems not possible with the present contaminant trap, since quantitative back extraction from the carbon silicone composite is difficult if not impossible. Once an appropriate extractable 'trap' has been found there is the requirement for validation of CEPBET with animal studies.

TU 283

Bioavailability studies: the last available tools for evaluating PAH risks realistically

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Polycyclic aromatic hydrocarbon (PAH) risk assessment is currently overly conservative in the U.S., with Screening Levels that are orders-of-magnitude below anthropogenic background in most urban areas. Additionally, the United States Environmental Protection Agency (USEPA) is proposing to increase the Relative Potency Factors (RPFs) for 5 PAH and to increase the number of carcinogenic PAHs with RPFs from 6 to 26. Several proposed RPFs are 10x or greater including dibenz[a,h]anthracene (10x), benzo[c]fluorene (20x), dibenzo[a,l]pyrene (30x), and benzo[j]aceanthrylene (60x). If this approach is adopted, human health risks from exposure to PAHs will increase considerably, and risk-based clean-up levels will drop to below background even for commercial/industrial receptors and a 1x10⁻⁴ risk level. It is clear from the literature that the mammalian bioavailability of PAH from weathered soils/sediments is considerably less than 100%. Agencies in the U.S. have increasingly rejected the use of bioavailability adjustments derived from the literature. USEPA has recently supported a policy of performing in vivo bioavailability studies of site-specific media impacted by complex organic compounds. The authors have designed such an in vivo study and are seeking its regulatory approval for execution in 2012. This paper will summarize literature bioavailability results for PAHs and discuss methodological issues regarding the on-going study, which is a robust, internally consistent animal bioavailability study with site aged soils. Issues that will be discussed include type of controls (internal versus external), target PAHs, animal species and strain, sampled biological media (blood, urine, feces, other tissues), pharmacokinetic issues (single time point versus area under the curve), and analytes of interest (parent PAHs versus PAH metabolites vs DNA or protein adducts).

TU 284

Desorption-resistant fraction in PAH-contaminated soils: aged spiked soils can not resemble historically contaminated soils

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Polycyclic aromatic hydrocarbons (PAH) are priority pollutants of soil and groundwater in many countries. Prolonged contact time of PAH and soil constituents may lead to their sequestration, rendering a fraction of contaminants inaccessible for biological processes and diminishing the potential efficacy of bioremediation measures.

The aim of the present study was to evaluate the significance of commonly used spiking protocols for reflecting field conditions in terms of bioaccessibility assessment.

Therefore, 25 Austrian soils were collected and spiked with four selected priority polycyclic aromatic hydrocarbons (Phenanthrene, Fluoranthene, Benzo(a)pyrene and Benzo(g,h,i)perylene). PAH desorption behavior from freshly contaminated and aged soils was monitored and compared with PAH desorption from three historically contaminated soils. The non-bioaccessible PAH fraction in the soils was determined using a passive sampling device, the "contaminant trap" recently described by P. Mayer et al [1]. In addition, the soils' infrared spectra and desorption data were incorporated into a multivariate statistical approach (partial least squares regression, PLS) to determine specific soil organic matter regions responsible for PAH sorption.

In the analysis of the non-accessible PAH fraction, the absence of pronounced ageing effects in PAH-spiked, aged soils was noted. After 56 days of desorption time, a distinct desorption resistant fraction occurring in all soils was observed for Benzo(g,h,i)perylene only. Several experimental boundary conditions may serve to explain the lack of an ageing effect, including influences by the contaminant matrix, the extent of sorption site coverage and the contamination's age. Moreover, industrial activity is often adjoined by the enrichment of soils with highly sorbing constituents (e.g. soot, cf. MGP soils). In the present study, weak binding of PAH in spiked soils (aged and freshly contaminated) in connection with the identification of humic acids as possible binding sites via PLS indicate the absence of high-affinity sites (e.g., black carbon) for PAH sorption. This was found for at least 23 of the 25 soils, which were all collected from non-industrialized areas.

The present results indicate that the weight of accessibility data obtained with single-substance spiked soils may have to be re-evaluated.

TU 285

Highways versus pipelines - modelling the contributions of two fungal transport mechanisms to efficient bioremediation

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Based on experimental studies, two fungus-mediated transport mechanisms have been suggested to facilitate the bacterial degradation of organic soil contaminants: bacteria may use liquid films around fungal hyphae for quick dispersal ('fungal highways'), and fungi may take up and translocate contaminants through their mycelial network ('fungal pipelines'). Both mechanisms promise to enhance the bioavailability of contaminants to degrading bacteria. However, a comparative study of their respective

efficiency in increasing biodegradation performance, and its dependence on environmental conditions, has hitherto not been conducted. Using a microbial simulation model, we therefore investigate bacterial degradation performance in response to networks that either act as bacterial dispersal vectors ('highways') or as contaminant translocation vectors ('pipelines') or as a combination of both. We analyse biodegradation improvements compared to the situation without networks, and systematically test a variety of spatially homogeneous and heterogeneous environmental scenarios. Our results suggest that each mechanism can improve biodegradation performance. The degree of improvement, however, may vary distinctly depending on the environmental conditions, and may also be negligible under certain conditions. Particularly, networks acting as 'highways' allow bacteria to overcome motility restrictions and reach remote areas, whereas networks acting as 'pipelines' may initiate degradation by bringing remote contaminants to bacteria. As a consequence, highest biodegradation improvements often emerge from the combination of both mechanisms. We therefore conclude that 'fungal highways' as well as 'fungal pipelines' should be considered for developing novel bioremediation strategies based on fungus-mediated transport. Future experimental studies should focus on detection and appropriate stimulation of the two mechanisms in typical bacteria-fungi associations in contaminated soils.

TU 286

Influence of rhizosphere oomycete mycelia on bacterial biodegradation of phenanthrene present in nonaqueous-phase liquids (NAPLs)

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Two rhizosphere oomycete species (*Pythium aphanidermatum* and *Pythium oligandrum*) were tested for their influence on biodegradation of phenanthrene present in NAPLs by the soil bacterium *Mycobacterium gilvum* VM552. The selected oomycetes showed no antagonistic effects on the bacterium in co-culture assays. An optimized production protocol was established for oomycete mycelia using a direct scrape-out method with solid agar, which prevented the contamination by carbon-rich medium components. Mineralization of ¹⁴C-phenanthrene present in hexadecane/fuel-NAPL mixtures by *M. gilvum* VM552 was increased in the presence of *P. oligandrum* mycelia. The fungus shortened the lag phase for mineralization and delayed the mineralization plateau. However, there was no stimulation of mineralization when the NAPL was composed of a heptamethylnonane/fuel mixture. We suggest that fungal growth on biodegradable NAPL mixtures may promote the bacterial colonization of the NAPL/water interface, possibly by promoting cell adhesion and/or causing interface fertilization, thus increasing the bioaccessibility of PAHs for bacteria.

TU 287

Sunflowers in rhizoremediation: a possible alternative for improving the bioavailability of PAHs in soils

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The success of the rhizoremediation depends largely on the ability of degraders of contaminants in soil and plant growth that drive organisms to colonize roots efficiently. In our work, we present the effect of sunflower rhizosphere on the biodegradation of PAHs in soil with creosote (21.75 mg·kg⁻¹ containing 6 HAPs). We conducted two experiments of biodegradation: one of them in greenhouse with cultivated sunflower and other in the laboratory with suspensions of soil and root exudates of sunflower. The sunflower root exudates were extracted using *in vitro* culture. The presence of the plants caused in greenhouse conditions a selective increase in PAH-degrading populations and a decrease in the concentration of five indicator PAHs. In turn, the root exudates of sunflower had under laboratory conditions a similar stimulatory effect on the degradation of PAHs in soil slurries. The stimulation of the indigenous bacterial population resulted in both situations in a similar residual concentration of PAHs. We can conclude that the effects of plants on the residual concentrations of PAHs could be reproduced under laboratory conditions by slurring, shaking and the addition of root exudates.

TU 288

Combining synchronous fluorescence, liquid scintillation and passive dosing for studying the bioavailability of pyrene in the presence of dissolved humic substances

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Degrading bacteria usually take up dissolved polycyclic aromatic hydrocarbons such as pyrene, by diffusion from the surrounding aqueous phase. Here, the dissolved concentrations determine bioavailability, and low freely dissolved concentrations, sometimes, can cause slow biodegradation rates. When dissolved humic substances (HS) are present, it is important to know if the association with HS affects the concentration of freely dissolved pyrene and how this affects biodegradation rates. We have applied a new combination of analytical techniques (synchronous fluorescence spectrometry, scintillation counting) to establish the dynamics of ¹⁴C-pyrene in the aqueous phase during biodegradation, in the presence and absence of HS isolated from soil. We also used a technique based on the principle of passive dosing to control the dissolved concentrations of pyrene by partitioning from a preloaded polymer (polydimethylsiloxane). The results indicate that the establishment of an appropriate chemical activity gradient is critical for a positive or negative influence of HS on biodegradation.

TU 289

Biosurfactants and sustainable bioremediation: effects on slow desorption PAHs

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Slow desorption from soil and sediments is a critical limiting factor that controls biodegradation rates of hydrophobic pollutants such as PAHs, resulting in a long-term persistence of these compounds in the environment. The use of biosurfactants is a promising alternative for enhancing desorption of soil-sorbed PAHs and their bioavailability for microbial degradation. It is already known that biosurfactants can promote dissolution of solid PAHs and also enhance desorption when they are present as rapidly desorbing fractions (> 0.1 h⁻¹). However, not much is known about the effect of biosurfactants on bioavailability of slowly-desorbing hydrophobic compounds, as PAHs. In this study, we focus on the capacity of rhamnolipid biosurfactants, produced by *Pseudomonas aeruginosa* 19SJ, to enhance the bioavailability of different soil-sorbed ¹⁴C-labeled PAHs in soil-aqueous systems. In a well-controlled batch system, we studied the effect of biosurfactants, at concentrations above the critical micellar concentration (CMC), on the biodegradation of fast and slowly-desorbing fractions of ¹⁴C-labeled PAHs present in soil. Desorption kinetics of ¹⁴C-PAHs from soil were performed by Tenax solid-phase extraction and compared to mineralization kinetics by a PAH-degrading soil bacterium (*Mycobacterium gilvum* VM552) in the presence of biosurfactants. Results indicated that the promoting effects of biosurfactants on biodegradation were accentuated in soils exhibiting a slow-desorption profile. Given the biodegradable and non-toxic nature of biosurfactants, their use constitutes a promising alternative for promoting bioavailability of this 'resistant' fraction in a sustainable way.

TU 290

Bacterial taxis and sustainable remediation: effects on bacterial dispersal

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We focus in modulating the deposition and motility of degraders in porous media as a bioavailability-promoting strategy. The positive effects of - often energy-consuming - mobilization approaches in bioremediation depend on the efficiency of bacterial movement in porous media, which is often restricted by high deposition rates and adhesion to soil surfaces. Besides, adhesion to surfaces may be sometimes beneficial in promoting biodegradation of slowly-desorbing chemicals. In well-controlled column systems, we assessed the influence of different chemoeffectors on deposition of a chemotactic, naphthalene-degrading bacterium (*Pseudomonas putida* G7). We also characterized the motility behavior by capillary assays and by analyzing the movement of individual cells through computer-assisted motion analysis, determining parameters like linear speed and rate of change of direction. The cell interaction with the column packing material, and subsequently deposition, depended on the motile behaviour (hypermotility, attraction or repellence). For example, positive chemotaxis caused smooth trajectories and reduced the affinity of the cells for surfaces, thus promoting transport due to a decreased deposition, whereas repellence caused the opposite effect. We propose that, by promoting the right motility behaviour, we can adapt cell deposition to a given bioremediation scenario, thus achieving an enhanced and sustainable bioremediation performance.

TU 291

Behavioural responses of *Tetrahymena pyriformis* exposed to microgradients of hydrophobic organic chemicals

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Behavioural changes of microorganisms may reveal sublethal adverse effects of hydrophobic organic chemicals. Using them as an endpoint requires, however, that the organisms can be observed microscopically during exposure. We therefore applied passive dosing on microscope slides as a new experimental platform to study the effect of selected polyaromatic hydrocarbons (PAHs) on the behaviour of the ciliate *Tetrahymena pyriformis*. Motile cells and organisms that are able to sense chemical signals may direct their movement along concentration gradients of a chemical, a behaviour known as chemotaxis. *T. pyriformis* was therefore exposed to various PAH-gradients and the swimming behaviour of the cells was then recorded under infra-red light illumination. Trajectories of the cells were obtained using an automated cell tracking software and were subsequently analyzed for chemotaxis by statistical measures. The Taylor model, which mathematically describes the scale-dependent transition from ballistic to diffusive movements of particles in suspension, was applied here for the first time in an ecotoxicological context to obtain motility parameters such as the tumbling frequency and effective swimming velocity to evaluate changes in the swimming behaviour of the cells. The results did not reveal chemotactic behaviour of *T. pyriformis* in the concentrations gradients of the compounds tested. However, motility behaviour was affected at levels that were about two orders of magnitude below the reported effective chemical activity causing 50% lethality, emphasizing that behavioural responses are a sensitive endpoint in toxicity testing.

TU 292

Sorption of ionized and polar organic contaminants to natural dissolved organic matter

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Concentrations of dissolved organic matter (DOM) have been rising in the environment over the past decades due to climate change and decreases in acid rain. The transport potential of organic contaminants can be affected by increased DOM concentrations in surface water. It is therefore relevant to gain more insight into the mechanism of organic contaminant sorption to DOM. In this study, the interactions between polar organic contaminants (neutral and ionized forms) and DOM were studied to assess the

influence of charge and molecular structure on the sorption process. Representative emerging contaminants were selected, covering hormones, pharmaceuticals, personal care products, and pesticides. Batch sorption studies were conducted with natural DOM-containing water samples obtained from Finland, United Kingdom, and the Netherlands and by using polyacrylate passive samplers to determine aqueous contaminant concentrations. The DOM samples were characterized by chemical and physical fractionation methods. The results indicated that charge and molecular size of ionized compounds dominate sorption to both DOM and polyacrylate. Positively charged compounds showed a strong pH dependent sorption to DOM, whereas the neutral forms were the most important species responsible for sorption to polyacrylate. Negatively charged compounds showed the lowest affinity to both DOM and polyacrylate. For most neutral compounds investigated, the water phase represented a more favorable medium compared to DOM. The obtained results may increase our knowledge on the sorption of polar organic contaminants to DOM, which is currently based on only limited data.

TU 293

Binding can increase the mobility and uptake of hydrophobic organic compounds

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The binding of hydrophobic organic compounds (HOCs) is usually considered to be a risk reduction process. However, if the bound form becomes mobile then it can in fact enhance uptake and risk. This phenomenon can be seen in 1) colloid facilitated transport 2) the use of chemical agents to enhance soil remediation and 3) in biology, where body fluids can enhance the release of HOCs from soil and consequently increase their bioaccessibility.

In the current work we studied the above examples using “enhanced capacity” as a new measurement endpoint. Enhanced capacity (E) has some similarities to the well-established concept of “solubility enhancement”, but should not be confused with it. Solubility enhancement is determined at, and applies only to, the saturation level of the HOCs, whereas E can be determined at or below the saturation, and for defined levels of each mixture component. This means it refers to a more relevant range of environmental concentrations and mixture compositions. Passive dosing method¹ was applied to determine E for HOCs of 1) soil leachates collected from tile-drains of sandy clay loam and sandy loam fields of the Danish Pesticide Leaching Assessment Programme 2) sodium dodecyl sulfate and hydroxylpropyl- β -cyclodextrin that are used as chemicals in soil remediation technology and 3) artificial digestive fluids used in in-vitro test for assessing bioaccessibility of soil-bound PAHs². The use of passive dosing circumvented artefacts associated with adding crystals of HOCs into an aqueous solution, and due to the low relative standard deviation of the method even small enhancements could be measured with high precision.

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TU 294

Dynamic passive dosing for studying microbial PAH degradation: a comparison of experimental and model results

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Biodegradation plays a key role in PAH fate, and understanding kinetics as a function of (bio)availability is critical for elucidating their persistence. However, biodegradation mainly occurs in an aqueous environment, posing technical challenges for producing reliable kinetic data because of low PAH solubilities and sorptive losses. To overcome these, an experimental approach based on dynamic passive dosing is presented which: avoids using co-solvent for introducing the PAHs, buffers substrate depletion so biotransformation is measured within a narrow and defined concentration range and enables high compound turnover to simplify biodegradation measurements even at realistically low concentrations. The biodegradation kinetics of two PAHs by the bacterium *Sphingomonas paucimobilis* EPA505 were measured at defined dissolved concentrations ranging over 4 orders of magnitude, from 0.115 to 865 $\mu\text{g L}^{-1}$ for phenanthrene and 0.028 to 141 $\mu\text{g L}^{-1}$ for fluoranthene. Both compounds had similar biodegradation rates, and these increased in line with higher dissolved concentrations. First-order biodegradation rate constants were also similar for both, but these decreased at higher dissolved concentrations. The experimental results were compared to those obtained using different modelling approaches, including simulations based on the Best equation. These show a good agreement at the lower dissolved concentrations, whereas at higher concentrations the experimental measurements were lower than predicted, and indicate that other factors such as PAH toxicity or essential nutrient availability play a role. Therefore, combining measurements of PAH biodegradation kinetics at defined concentrations using dynamic passive dosing, and their interpretation by modelling is a useful tool to further understand their bioavailability, biodegradation and persistence.

TU 295

Enhanced mass transfer of hydrophobic organic compounds from NAPLs into the aqueous phase by dissolved organic carbon

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Hydrophobic organic compounds (HOCs) are often found as mixtures in the form of non-aqueous phase liquids (NAPLs). Due to their hydrophobic nature, the HOCs preferentially remain in the NAPL, with slow mass fluxes into the aqueous phase. However, since water dissolved HOCs play a key role in diffusive uptake into organisms, microorganisms using HOCs as a source of carbon and energy face a large reservoir of inaccessible food in the NAPL which is often reflected in slow bioremediation of NAPL contaminated sites. Interestingly, mobile “colloidal-like” phases can contribute to diffusive mass exchange processes between surfaces and the bulk aqueous phase, a phenomenon termed enhanced or facilitated diffusion. Therefore, this study investigated the role of dissolved organic carbon (DOC) in enhancing the mass transfer of hydrophobic organic compounds from NAPLs into the aqueous phase above that attributable to dissolved molecular diffusion alone. In controlled experiments, mass transfer rates of five NAPL-phase PAHs (log KOW 4.15 - 5.39) into the aqueous phase containing different concentrations of DOC were measured. Mass transfer rates were increased by up to a factor of four in the presence of DOC, with the greatest enhancement being observed for more hydrophobic compounds and highest DOC concentrations. These increases could not be explained by dissolved molecular diffusion alone, and point to a parallel DOC-mediated diffusive pathway. The nature of the DOC-mediated diffusion pathway as a function of the DOC concentration and PAH sorption behaviour to the DOC was investigated using diffusion-based models, and found to increase with DOC concentration and compound sorption. Therefore, for “super”hydrophobic compounds this pathway could both dominate but also increase mass transfer rates by orders of magnitude, even at environmental DOC concentrations. This has important implications for their bioremediation, as well as bioconcentration and toxicity.

TU 296

On the effect of vitamins and nutrients on the solubilization of petrodiesel/biodiesel blends in water

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The effect of mineral salts on the aqueous solubility of nonelectrolytes is largely assumed to exhibit the “salting out” or “salting in” effects in which the aqueous solubility of nonelectrolytes is decreased or increased upon the addition of salts. Pioneering investigations by Setschenow [1] and later by Carter and Hardy [2] among others, resulted in semi-empirical relationships that describe the dependence of the solubility of nonelectrolyte solutes on salts concentrations. We conducted equilibration experiments of petrodiesel/biodiesel blends (B0, B20, B40, B60, B80, and B100, where B100 is 100% biodiesel) in water in the presence and absence of standard vitamins and nutrients used for bacterial growth studies. The solubility of aromatic compounds was found to be in agreement with the salting out effect in the presence of the added nutrients. The solubility of the C10 - C21 n-alkanes, however, exhibited very odd and complex behavior and was found to be significantly enhanced ($p < 0.0005$), up to 40-fold in the presence of the fatty acid methyl esters (FAMES) and the vitamins and nutrients medium, compared to FAMES and deionized water alone. A similar observation was made by Baker [3] regarding the effect of salts on solubilization of the alkanes and aromatic compounds in the presence of fatty acid soap solution. They reported that, in the presence of sodium chloride in dilute fatty acid soap solutions, the solubility of paraffin hydrocarbons is markedly higher and that of aromatic hydrocarbons is lower. Those observations are found to contribute significantly to the bioavailability of the n-alkanes in the water column. Biodegradation studies conducted in our laboratory showed significant enhancement for the microbial utilization rates of the n-alkanes in the petrodiesel/biodiesel blends, which we interpret to be due to the increase in aqueous concentrations of the n-alkanes in the presence of the FAMES and nutrients.

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TU 297

Photo-transformation of 2,3,7,8-TCDD in presence of natural organic matter studied by in vitro bioassay

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2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) as a representative of hydrophobic organic compounds (HOCs, frequent anthropogenic environmental pollutants comprising also various pesticides), can cause many adverse effects in organisms. Activation of arylhydrocarbon receptor (AhR) can be very important mechanism of toxicity for so called “dioxin-like” HOCs. Natural organic matter (NOM), being naturally occurring compounds (up to 50 mg/L in waters), occurs together with HOCs in contaminated water. Beside other important ecological properties, NOM serves as a natural source of reactive oxygen species that are formed after HS irradiation. Direct photolysis of HOCs is a very important way of their degradation in the aquatic environment. Nevertheless, oxygen species, formed after HS irradiation, can theoretically enhance the photochemical degradation of HOCs. In present study, we have assessed the ability of various NOM concentrations to enhance photo-degradation of TCDD. Aqueous TCDD+NOM

solutions were irradiated by sun light in quartz tubes for up to 3 days. Photo-degradation of TCDD studied by in vitro assay (based on the H4IIE-luc transgenic cell line) was observed, but both TCDD alone and TCDD in mixtures with low concentrations of NOM (up to 10 mg/L) were photo-degraded in almost the same half-lives. High NOM concentrations (25-150 mg/L) have significantly extended the half-lives of TCDD photo-degradation, probably due to filter effect of more coloured solutions. Supported by projec CETOCOEN (European Regional Development Fund no. CZ.1.05/2.1.00/01.0001).

TU 298

Influence of sorption on bioavailability and biodegradation of secondary alkane sulfonates (SAS) in marine sediments

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Coastal marine ecosystems are often influenced by wastewater discharges from surrounding populations. Surfactants, with a worldwide production over 10 million tons per year, are among the organic contaminants showing highest concentrations in wastewater. Most available studies on this topic deal with the distribution and fate of alkylphenol polyethoxylates (APEO) in aquatic systems, as some of their degradation intermediates are endocrine disruptor compounds (EDC). Linear alkylbenzene sulfonates (LAS) have also been extensively studied as they show the highest worldwide production volumes. Environmental data on many other surfactants, however, are still scarce. This is the case of secondary alkane sulfonates (SAS), one of the major anionic surfactants used in the market of dishwashing, laundry and cleaning products. Although this compound is easily removed during wastewater treatment, previous studies have shown that SAS and other anionic surfactants such as LAS accumulate in sludge as they are hardly biodegraded during anaerobic digestion. Recently, anaerobic degradation of LAS was confirmed in marine sediments due to the presence of sulfate reducing bacteria. This research has focused on determining whether SAS are biodegradable or not in absence of oxygen in the marine environment, and, if that happens, on the role of sorption on the speed of the biodegradation. First, sorption experiments were performed using several amounts of sediments (0.5-5 g) and SAS concentrations (1-10 mg/kg). Distribution coefficients ranged from 54 to 1505 L/Kg depending on the SAS homologue considered. Thus, we observed that the sorption capacity was much higher for those homologues having longer alkyl chains (e.g., C17-SAS) rather than for more polar homologues such as C14-SAS. Later, biodegradation experiments using anoxic marine sediments and seawater were conducted. SAS anaerobic biodegradation was observed for the first time, reaching overall values up to 98% in 166 days. Half-life values ranged from 20 days (C14-SAS) to 37 days (C17-SAS), showing that the speed of this process significantly depends on the sorption capacity and, therefore, bioavailability, of each SAS homologue.

TU 299

Remediation of PCB- and PAH- contaminated soil with modified clays

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Contamination of soils and sediments by polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) is a major concern worldwide. This is connected with their persistence and the threat they pose to the environment and human health. Development of efficient and reliable strategies to reduce PCB and PAH concentrations in contaminated soils has been a crucial task for scientists. Immobilization of these organic contaminants seems to be preferred since total removal of pollutants is hardly possible. This study is therefore aimed at evaluating the potential of modified clay minerals (with surfactants, humic acid and papaya seed) in the retention of PAHs and PCBs in soils.

Sodium dodecyl sulphate (SDS) and humic acid were used to modify bentonite. Kaolinite was modified by Papaya Seed. Different percentages of the modified clays were mixed with the contaminated soil and suspended in 0.01M CaCl₂ in the dark for twenty days. After equilibration analysis of PCBs and PAHs in solution was carried out using SPME coupled with GC/MS.

Presented are the sorption capacities of the modified clays and results of remediation. The results of this study indicate a promising potential of the modified clays to immobilize PCBs and PAHs in soils.

TU 300

Fate of iodinated X-ray contrast media in a soil column percolation experiment simulating elevated dissolved organic carbon (DOC) by amendment of saccharose

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Iodinated X-ray contrast media (X-RCM) occur in concentration ranges of several hundred ng/L in many surface waters used for drinking water production. To clean up surface water, sustainable water treatment techniques like river bank filtration or artificial groundwater recharge (AGR) are used, but some X-RCM like amidotrizoic acid are known to be very persistent. Due to low the sorption tendency of X-RCM [1], their fate is rather related to biodegradation, as proved by the detection of metabolites reported in literature [2].

To simulate the process of AGR, the fate of six iodinated X-ray contrast media ioxthalamic acid, iohexol, iomeprol, iopamidol, amidotrizoic acid and iopromide was studied during percolation of a sandy gravelly substrate in a column percolation experiment using filtrated surface water from the Rhine. The question of interest was, whether elevated DOC would improve or impair the removal of X-RCM during the column passage. Therefore, filtrated Rhine water used for column percolation was spiked with concentrated aqueous saccharose solution to achieve elevated DOC and to simulate the pollution of the Rhine with low molecular organic compounds, e.g. from food industry or accidental spills. Time weighted composite samples of column influent and effluent were analysed for X-RCM and DOC and treatments with and without saccharose amendment were compared.

Moderately elevated DOC (1.3-fold of background) stimulated iohexol removal, but for most X-RCM there was no effect. In contrast, twenty-fold elevated background DOC impaired the removal of most X-RCM, while there was a tendency towards elevated iopamidol removal. The latter stimulation is supposed to be related to the observed changes in redox settings in the column induced by the high DOC load, as the fate of iopamidol is reported to depend on site-specific redox conditions at several field sites in literature [1].

Regarding the removal of X-RCM, moderate pollution of surface waters with low molecular organic compounds can thus be compensated by natural water treatment like AGR, but excess pollution will reduce the cleaning capacity of these systems and must be avoided.

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TU 301

Effect of pH and electrolyte concentration on soil adsorption of pesticides

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The Japanese Positive List System for Agricultural Chemicals was implemented in 2006. Under this system, the uniform limit was set at 0.01 mg/kg for agricultural chemicals for which maximum residue limits (MRLs) have not been established. Since then, some agricultural chemicals have been detected in some vegetable crops at levels above the uniform limit, and the distribution of these crops has been prohibited. One of the reasons why the limit has been exceeded is that succeeding crops are contaminated by pesticides remaining in the soil. Assuming that these pesticides in the soil are taken up by plants via the soil solution, an understanding of the soil-water distribution of the pesticides is important to predict crop pesticide residues. Soil adsorption of nonionic pesticides is strongly influenced by soil organic matter content. However, there is little information available on the influence of soil pH and electrolyte concentrations, changed by chemical fertilizer treatment, on pesticide adsorption. We therefore used batch experiments to investigate the effects of these factors on the distribution coefficient for adsorption (K_d) of about 30 pesticides. We used buffer solutions (pH 3, 5, 7.5, and 9) and CaCl₂ solutions (0.01, 0.1, and 1 mol/L) and five soils with different organic matter contents. Each soil was shaken with an aqueous solution containing 0.1 mg/L of pesticide at a soil-to-solution ratio of 1 to 5, with a 24-h equilibration period for all experiments. Increasing the CaCl₂ concentration from 0.01 to 0.1 mol/L had no significant effect on soil adsorption of pesticides, but the K_d values with 1 mol/L CaCl₂ solution were slightly higher than those at the other two concentrations. Pesticide adsorption was greatly affected by pH level. In general, K_d values were negatively correlated with pH levels, and the rate of K_d change, calculated by dividing the K_d values at pH 3 by those at pH 9, was greater in the case of soils with higher organic carbon contents and of pesticides with higher octanol/water partition coefficient (log K_{ow}). The results suggest that the hydrophobic interactions between pesticides and soil organic matter are influenced by pH levels. Further, it is implicated that crops can take up the pesticide from soil at high pH compared with at low pH.

TU 302

Relationships between soil adsorption of pesticides and pesticide/soil properties

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The positive list system for pesticide residue in food, such as crops, was applied in Japan in 2006. For pesticides for which maximum residue levels were not set, the residue levels in food must not exceed 0.01 mg/kg (uniform limit). Pesticides, which are applied to foliage and soil at the crop cultivation, remain in soil after harvest, and pesticides remaining in soil may contaminate the succeeding crop. Crops, for which growing cycle is short, are rotated in Japan. Therefore, the residue levels of some pesticides in succeeding crops have exceeded 0.01 mg/kg in Japan. To prevent excess of the residue level 0.01 mg/kg in the succeeding crop, the succeeding crop must be cultivated in consideration of (1) the physicochemical properties of the pesticides applied to the preceding crop, (2) the soil properties affecting the pesticide residue in soil, and (3) the pesticide's uptake into the succeeding crop.

The succeeding crop may take up the pesticide in soil solution from the root or the stem as a kind of a pesticide's uptake into the crop. Data on the distribution of a pesticide in soil/solution (pesticide soil adsorption) is important for assessing the risk of pesticide contamination of the succeeding crop. Soil adsorption of nonionic pesticides

is influenced by organic carbon content in soil; however, there are few investigations on influence of pesticide and soil properties except organic carbon content on the adsorption of pesticide in soil. The present study conducted batch experiments with approximately 30 pesticides and 7 soils in Japan to determine their soil adsorption coefficients (K_d values). The results of relationships between K_d values and pesticide/soil properties will be present.

TU 303

Leaching of aged DDTs and current use pesticide residues in undisturbed soil columns: non-ionic surfactant and carboxylic acids effects

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Soil pollution by Persistent Organic Pollutants such as DDTs (p,p' -DDT and its metabolites p,p' -DDE and p,p' -DDD) represents a continuous source to the environment. As a consequence of their high persistence DDTs occur up to 60 cm on agricultural soils. The Current Use Pesticide (CUP) endosulfan is characterized by its high lixiviation potential due to its relatively low K_{ow} value. Introduction of riverine water on polluted soils during irrigation or flooding events as well as plant growing may modify pesticide availability and leaching. Surface waters receive discharges from industrial, urban and agricultural uses that will account for a variety of compounds such as surfactants, colloids and dissolved organic carbon. This work studies the vertical distribution and movement of aged DDTs and endosulfan in undisturbed soil columns irrigated with: 1-control (riverine water), 2-Tw-80 (riverine water + Tween 80 0.5 cmc), and 3-acids (riverine water + sodium citrate and sodium oxalate, 0.05 M). Experiments were carried out with 13 cm x 30 cm soil columns taken from Aridisol soils from a fruit field in Patagonian, Argentina. Columns were saturated with distilled water and solutions (4L) were added under saturated condition. One L leachates ($n=4$) were collected for pesticide residues analysis. After elution, columns were dried (24 h), opened and sampling on 5 cm sections. Pesticide desorption was performed by batch technique and residues analysis on soil and water by GC-ECD. p,p' -DDE > p,p' -DDT > α -endosulfan > β -endosulfan pattern was found in all soil sections. p,p' -DDE levels ranged between 7-250, 8-70, and 13-200 ng g⁻¹ dry weight in control, Tw-80 and Acids, respectively. Under all conditions pesticide levels decreased concomitantly with depth and sand increase. In control and Acids, p,p' -DDE and p,p' -DDT desorption from soil was correlated with their levels in soil ($r>0.8$ $p<0.02$). Total pesticide levels in elution waters followed the order Tw-80 > Acids > Control. Under control or Acids, α -endosulfan was the main compound on the first elution volume and p,p' -DDE started to lixiviate after 2 L while for Tw-80, DDE represents the main residue in all elution waters. Lixiviation of aged p,p' -DDT residues or endosulfan effectively occurs under irrigation with riverine water and is increased by adding non-ionic surfactant or carboxylic acids. Tw-80 had a washing effect of pesticides leading to lower levels on soil while carboxylic acids enhance pesticides availability.

TU 304

Sorption of cationic organic compounds to soil as the sum of soil components

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Many emerging contaminants are strong bases that under environmental conditions mostly occur as cationic species. The environmental fate of these compounds depends on sorption from water to particulate matter, such as soil, sediment and dissolved colloids. Since both natural organic matter and clay minerals are negatively charged substrates, both sorbent types are well known to bind organic cations, but it is not clear to what extent each type contributes in natural soils. Using a customized dynamic HPLC column retention method, we studied the sorption of a set of organic cations to individual soil components, such as natural organic matter (NOM, Pahokee peat) and pure clay minerals (kaolinite, illite, bentonite). In addition, sorption affinity to five different Eurosoils was tested, which ranged in organic carbon content (fOC) from 1.3 - 9.2%, and in clay content from 6 - 75%. We examined to what extent sorption to natural soil can be predicted based on sorption to individual soil components alone. In general, sorption of primary amines to clay minerals was weak relative to quaternary ammonium compounds (quats), whereas sorption of primary amines to NOM was strong relative to quats. Such relative sorption affinity differences between test compounds between NOM and clay minerals were used to evaluate soil sorption.

TU 305

Degradation of UV filters in sewage sludge by the ligninolytic fungus *Trametes versicolor* and study of the 4MBC degradation process in liquid medium

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Sunscreens agents, also known as UV filters, have become very popular chemicals since they were shown to have a protective role against photoaging, photocarcinogenesis and photoimmunosuppression promoted by UV sun radiation. These compounds are extensively used in personal care products but are also present in a wide variety of industrial goods such as textiles, paints, or plastics to prevent photodegradation of polymers and pigments. UV filters can enter the environment through the liquid effluent of wastewater treatment plants (WWTPs) but also absorbed in the sludge due to their high hydrophobic character, and later spread on agricultural fields. A solid-state treatment of WWTP sludge with the white-rot fungi *Trametes versicolor* is reported in the present work as feasible to degrade them in a range from 87 to 100%. This experiment is the first step of any future fungal treatment, essential to prove that the elimination is only due to the action of the fungus and not of other microorganisms as the sludge was previously sterilized.

Degradation studies of 3-(4'-methylbenzylidene) camphor (4MBC) in liquid media were also done in particular and complete removal was achieved in less than 24 h. The main metabolites were identified and the first steps of the transformation pathway were elucidated: a mono- or di- hydroxylation by cytochrome P450 and the subsequent conjugation with a pentose. These results support the extended hypothesis that conjugation processes constitute one of the defensive mechanisms that fungi activate against toxic hydroxylated compounds.

TU 306

Concentration levels of phthalates in water and sediment samples from Nadela river basin, Vojvodina region

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Phthalates belong to the group of dominant industrial pollutants and are ubiquitous overspread class of compounds with low water solubility/high fat solubility and low volatility. Due to their low water solubility, the phthalates hydrolyse relatively slowly, but the actual dynamic rate varies according to temperature, concentration and solubility. The physical and chemical properties of the phthalates have made them suitable as plasticisers in polymers such as plastic and rubber. Among the phthalates diethylhexyl phthalate (DEHP) predominates and has many possible applications, especially for PVC. The highest concentrations of DEHP are found in products for flooring, foil and plastic-coated fabrics. Phthalates are also included as plasticisers for binders in different kinds of paint and adhesives. Within the Project the concentration levels of phthalates in water and sediment from Nadela River were determined. Nadela basin is situated near city of Pancevo, Vojvodina region, and is covered by numerous industrial sites and can represent the significant source of these pollutants. The field survey was done in 9 sampling sites in the catchment area of the Nadela in the early spring 2009. The laboratory analysis were conducted in Environmental Institute, Kos, Slovak Republic and in the laboratories of Institute for Public Health of Vojvodina. All water samples were positive on presence of four of the six selected phthalates: di-n-ethyl phthalate (DEP), diisobutyl phthalate (DIBP), di-n-butyl phthalate (DBP), and DEHP. The concentrations varied in the range: for DEP from 0.01 µg/l till 0.2 µg/l, DIBP from 0.08 µg/l till 0.95 µg/l, DBP from 0.3 µg/l till 2.5 µg/l and DEHP from 0.7 µg/l till 2.987 µg/l. In sediment, three of selected phthalates were determined in all sampling sites. All tested samples were positive on DIBP in the range of 24 - 80 µg/kg, DBP in the range of 215 - 996 µg/kg and DEHP in the range of 124 - 779 µg/kg. On 5 of 9 sampling sites the concentration levels of DEHP exceeded EQS and this phthalate is listed to be a human carcinogen. In the case of sediments concentrations of standards have not been exceeded in any sample. Based on the analytical results and compared with the concentration levels obtained for other priority substances, it can be concluded, that DEHP is the most problematic priority substance in the Nadela hydro-system basin.

The work was supported by Ministry of Education and Science, Republic of Serbia (III46009 and TR34014).

TU 307

Increasing levels of PBDEs, PCBs and organochlorine pesticides in fish following a mega-flooding episode in the Negro River basin, Argentinean Patagonia

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A mega-flooding event in the Negro River basin, Argentinean Patagonia, caused a significant outflow of contaminants from the surrounding soils. Agriculture, hydroelectric centrals and industrial-urban conglomerates are the most important activities in the basin. This study evaluates the effects of flooding event on polybrominated diphenyl ethers (PBDEs), endosulfans, DDTs and polychlorinated biphenyls (PCBs) levels in females of silverside (*Odontesthes hatcheri*) tissues. Muscle, liver, gills, gonads and stomach content were analyzed by GC-MS. Levels in water, suspended particulate matter (SMP) and sediments (SS) were also measured. Post- and pre-flood fish showed the following pattern: DDTs > PCBs > endosulfans > PBDEs. Post-flood fish showed the highest contaminants levels (ng/g lipid), at expenses of PBDEs and PCBs. PBDEs showed the greatest difference between post- and pre-flood fish (up more than 240 times). BDE-47 was the predominant congener in all tissues; however BDE-100 and BDE-99 showed the highest increase in post-flood liver (up to 400 times). The dominance of BDEs 47, 99 and 100 was consistent with the general pattern found in abiotic samples and fish of the same area, as well as denote the use of technical mixtures of penta-PBDEs. PCBs levels in post-flood fish increase until 23 times (from 63.2 to 1,478.6 ng/g lipid in liver), which were dominated by penta- (PCB-110, 118) and hexa- (PCB-153, 138) congeners. These results could have stemmed from historical usage of Arochlor 1254 and 1260 in Argentina. In post- and pre-flood fish, endosulfan sulfate presented the highest levels suggesting metabolic activity in addition to direct metabolite uptake, while an a-/b- ratio >1 similar to the technical mixture which is widely used in the region. In post-flood muscle, DDTs levels increased 5-fold than pre-flood. The metabolite p,p' -DDE represented about 80% of DDTs. This pattern would be a result of the intensive use of this insecticide during long time on agricultural practices. Contaminant profiles observed in pre- and post-flood silverside were according to water, SPM and SS, showing that this specie is a good biomonitor of aquatic pollution of Negro River. The presence of pollutants in this river was modified and enhanced by the flooding, increasing their bioavailability to fish. Additionally, pollutant occurrence deserves more attention, and monitoring programs are recommended in order to diminish their incorporation to aquatic ecosystem.

TU 308

Polychloro-dioxins, furans and biphenyls in fish, crabs and clams from the San Jacinto River Waste Pits, TX

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Fish, crabs, and clams were collected from the San Jacinto River waste pits, a superfund site in Houston, TX and analyzed for polychlorodibenzo-p-dioxins, polychlorodibenzofurans (PCDD/Fs) and dioxin like polychlorobiphenyls (dl-PCBs). Sample preparations comprised of tissue homogenization, enhanced pressurized liquid extraction and cleanup, and concentration. Isotopically labeled surrogates were spiked prior to extraction and were used to correct for target analytes loss during sample preparation. Samples were analyzed employing gas chromatography negative chemical ionization mass spectrometry. The method detection limits ranged from 2.0 to 10 pg/g ww in fish tissues. Ten out of twelve high priority dl-PCBs (Identified by World Health Organization, 2005) were measured with concentrations ranged from 2.0 to 948 pg/g ww. Average dl-PCBs concentrations were at least an order of magnitude higher than that of PCDD/Fs. Average PCDD/Fs fish tissue concentrations were at least an order of magnitude than that of crabs and clams. TEQclam was found to be at least 25X higher than TEQfish. Biomagnifications factor and Biota Sedimentation Accumulation Factors were also calculated.

TU 309

Effect of humic substances on remediation of soil- phosphogypsum mixtures

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Phosphogypsum (PG) is waste material in industrial production of phosphoric acid. Due to high content of nutrients (calcium, phosphorus and sulfur) it is sometimes used as a fertilizer for poor unfertile soils. But besides nutrients, PG also contains toxic impurities, such as fluorine, strontium and some others. One of contemporary tools to reduce the ecotoxicity of polluted soils and soil-PG mixtures is an application of humic substances (HS). The objective of this study was to evaluate the detoxifying ability of a number of humates towards PG applied to model soil mixtures.

Model soil (MS) contained (wt, %) 20% of kaoline, 10% of peat and 70% of sand with particle size 0.2-0.4 mm (ISO 11268-1). PG was carefully mixed with MS at concentration 3.3 and 7.5 wt, %. Humates from peat - Pe-FlexK, Pe-EcoK, coal - BC-EnK, BC-HumNa and lignosulphonate - OW-LhK introduced into the MS in the form of an aqueous suspension (distilled water) to achieve a concentration in soils 0.005 and 0.020 wt, %. The ecotoxicity was evaluated in water extracts using three species of test-organisms from different taxonomic groups: microalgae *Scenedesmus quadricauda* (decrease of cells population growth, 72h), crustaceans *Daphnia magna* (mortality, 96h), and higher plants *Sinapis alba* (decrease of root elongation, 120h). Toxicometric parameters (EC₅₀ - the median PG concentration that caused a 50% test-reaction reduction, and NOEL - no observed effect level - the PG concentration that caused test-reaction reduction below the toxic level) and detoxification indexes *D* were calculated.

Analyses of average detoxification indexes *DAV* (averaged over three test-organisms) showed that influence of humates depended both on PG concentration and humates' nature. Thus, application of PG at concentration 7.5 wt, % was toxic for all the test-cultures at all treatments and humates were not able to decrease the toxicity. At lower rate of PG (3.3 wt, %) some humates decreased the toxicity (*DAV* is 0.5-0.8), whether other did not. Among all humates tested the highest detoxifying ability was observed for two samples: Pe-FlexK from peat and BC-EnK from coal. For these humates EC₅₀ and NOEL values increased in 1.3-1.8 times for *S. Quadricauda*, in 1.1-1.4 times for *D. Magna*, in 1.6-2 times for *S. alba* for 0.005 wt, % humates content.

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ET01P - A Systems Biology approach to predictive Ecotoxicology

TU 312

Birds species versus crops: a GIS-based procedure to identify specific combinations

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Regulation (EC) No 1107/2009, applied from 14 June 2011, requires new data protection rules for both active substance and product data and, for the first time, includes data protection for extension of authorisations to minor uses. Moreover, rules for either avoiding duplication or sharing tests and studies involving vertebrate data have been included. Article 62, in particular, introduces new vertebrate data sharing provisions which allows Member States to use vertebrate studies on behalf of prospective applicants if an agreement with the holder(s) of the authorisation cannot be reached.

Considering birds, the species currently used in risk assessment are the ones considered the most sensitive to all pesticides. This approach, which can be considered a good worst case scenario at broad scale, could fail at detail scale where sensitive species living on specific crops could be exposed to pesticides.

In this work a realistic approach has been developed to correlate the avian population at local level, derived from a monitoring survey, with the land-use (Corine land-cover) and the relative pesticide treatments.

A GIS analysis was performed to identify peculiar combinations crop/bird species in order to determine which species is most likely to be exposed to a specific active substance.

The area considered was the North of Italy characterised by an intensive agriculture and a consequently high load of pesticides.

These results could be an useful tool to address the uncertainty associated to the bird risk assessment.

TU 313

Toxicity of sludge water treatment station for aquatic invertebrates

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The water treatment stations (WTP) were worldwide operating in order to treat and deliver potable water from untreated river water. A large quantity of sludge is generated in the water treatment process, which contains suspended solids, bacteria, heavy metals and others toxic substances present in the untreated water. The amount of sludge generated in treatment is directly proportional to the amount of treated water, a WTP that treat 1500 L/s of water generates approximately one ton of sludge/day, which returns to the origin river. The aim of the present study was to evaluation the sludge toxic potential located in Piracicaba, São Paulo, Brazil. The samples were collected in two phases of treatment called: flocculation (first phase of treatment) and decanter (second phase), in July and October 2009 and September 2011. The toxicity tests were carried out with the bioindicators: *Pseudokirchneriella subcapitata* and *Hydra attenuata*, and the dates were compared to the physical and chemical determinations, such as: pH, electrical conductivity and dissolved oxygen (DO). For *P. subcapitata* and *H. attenuata* tests were prepared an elutriate solution mixing the sample with distilled water (1:4 ratio). The elutriate samples was used as 100% and diluted to 75, 50, 25, 12.5 and 6.25% with specific culture medium. The samples collected in October 2009 were slightly more toxic for *P. subcapitata* and *H. attenuata*. Both samples were high toxicity for *P. subcapitata*, showed IC₅₀ of 11%. However for *H. attenuata* showed EC₅₀ of 15.4 and 19.1% and LC₅₀ of 17.7 and 64.6% (flocculation and decanter, respectively) which correlated with the highest electrical conductivity determinations (660 and 404 mS cm⁻¹) and DO (0.79 and 3.87 mg L⁻¹). The proximity of the EC₅₀ and LC₅₀ results for *H. attenuata* indicate the predominance of acute affects, or lethal stages (tulip and crumpled), showing the effective power of the toxic sludge, mainly the one collected in the flocculation tank. It's possible to conclude that both species tested were adequate to characterize the toxicity of the sludge generated by WTP. Both sludge were very toxic to these organisms and the toxicities were strictly correlated with the increase of electric conductivity, the high concentration of heavy metals and the lowest concentration dissolved oxygen in elutriate.

TU 314

Toxicity of copper on the freshwater snail *Physa acuta*: reproduction output and biomarkers of effect

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Copper constantly enters aquatic environments through several paths such as industrial effluents, agriculture runoffs and domestic wastewaters. Trace amounts of copper are needed for metabolic and biochemical processes in several organisms but, like for every chemical compound, in excess it becomes toxic. This study aimed to assess effects on reproduction and detect stress responses using biomarkers of effect in the freshwater pulmonate snail *Physa acuta* exposed to copper sulfate in laboratory conditions. A 14/28 days chronic test was conducted using adult snails in 5 different copper concentrations showing that reproduction was impaired as the concentrations increased. Biomarkers activity (e.g. AChE, GST and CAT) and energy reserves were determined and correlated with reproduction. As expected the energy cost allocated to the detoxification process was one of the reproduction impairment causes.

Aquatic snails, like *Physa acuta*, have proved to be good test organisms to assess the presence of chemicals in the environment as they have proven to be very sensitive to exposure.

TU 315

Inhibition of the fatty acid synthesis in chlorophytes by triclosan - a metabolomics approach

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Triclosan is an antimicrobial substance which is used in many pharmaceutical and personal care products such as soaps, toothpaste and mouthwash. -Because of this broad application it has been detected in streams, seawater and sediments in concentrations up to 0.007 µmol L⁻¹ [Singer et al., Environ. Sci. Technol., 2002]. In a prior study Franz et al. [Aquatic Toxicology, 2008] observed that chlorophytes are one of the most sensitive organisms affected by triclosan in the range of environmental concentrations. This raises the question of metabolic pathways affected by triclosan in chlorophytes. We used an established metabolic approach to analyse exposed green algae *Scenedesmus vacuolatus* [Kluender et al., Metabolomics, 2009]. Therefore we exposed a synchronised culture of *S. vacuolatus* with different concentrations of triclosan (range from 0.002 to 0.071 µmol L⁻¹) for 14 hours to derive concentration-dependent changes in metabolites and to be able to compare them to phenotypic observations (inhibition of cell growth and photosynthetic activity). After harvest and derivatization hydrophilic and lipophilic metabolite extracts were analysed with GC-MS and evaluated by multivariate

statistics (PCA). Two concentration dependent response patterns could be identified. Metabolites from the hydrophilic phase showed comparable sensitivity as phenotypic observations (EC50 algal growth: 0.02 $\mu\text{mol L}^{-1}$). In contrast the lipophilic metabolites of the green algae were affected significantly at the lowest tested concentration. Identification of lipophilic metabolites with the NIST and Golm library for GC-MS data revealed many fatty acids (e.g. hexadecanoic acid, oleic acid, octadecanoic acid) changed at low concentrations. These findings will be discussed in the perspective of the connectivity of response pathways to the known mode-of-action of the toxicant in bacteria, the inhibition of the Enoyl-ACP-reductase during the fatty acid elongation [McMurry et al., Nature, 1998].

TU 316

Morphometric and biochemical evaluation in gills of *Lepomis gibbosus*, after acute exposure to several xenobiotics (pesticides, detergents and pharmaceuticals)

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In recent decades, research and awareness of issues related to environmental exposure to anthropogenic xenobiotics has increased. These compounds can affect non-target organisms, altering their physiology, and consequently endangering the balance of ecosystems. Some studies reported the evaluation of morphometric and biochemical changes in fishes, after exposure to several compounds, including pesticides, detergents and pharmaceuticals, which can be quantified using appropriate analytical tools. The gills are important organs in fish since they perform numerous functions. Their multifunctionality, the vast surface of exposure to the external media and their location in the fish body, justify their importance as key organs for the direct action of pollutants in the aquatic environment. This work aimed to assess biochemical changes in gills, in terms of oxidative stress/phase II conjugation isoenzymes glutathione S-transferase - (GSTs) and gill morphometric analysis (secondary lamellar length -SLL and width - SLW, interlamellar distance - ID, and basal epithelial thickness - BET), after acute exposure to the pesticide chlorfenvinphos, the detergent SDS and anticholinesterase pharmaceuticals (neostigmine and pyridostigmine). The results of this study revealed that only neostigmine significantly decreased the activity of GSTs at concentrations of 1 and 100 mg/L. Considering the morphometric analysis of the gills, the data obtained showed that chlorfenvinphos increased values of BET and decreased SLL at a concentration of 0.75 mg/L. SDS increased values of SLL in fish exposed to a concentration of 3.9 mg/L. The drug neostigmine was responsible for an increase in SLL values at the highest tested concentration, and pyridostigmine increased SLW values at the concentration of 100 mg/L. The potential action of neostigmine in exposed fish, with inhibition of GSTs activity, is a noteworthy result, since it establishes a reduction in the effectiveness of conjugation and elimination of other xenobiotics, enhancing toxicity. Gill morphometrical indices showed evidence of a surface respiratory disturbance as result of exposure to several xenobiotics, which could be advantageous for fish by reducing toxicant absorption.

TU 317

Oxidative stress and higher level effect of engineered nanomaterials in the nematode *Caenorhabditis elegans* : functional toxicogenomic approach

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Toxicogenomics have been increasingly used in ecotoxicology but the challenges of these technologies are inferring biological importance from the data. To deal with this problem, integrated pathway and network analysis are being applied as they give a better understanding of biological effects at multiple levels. This systemic approach is particularly interesting for investigation of chemicals of which mode of actions are less characterized, such as, nanomaterials. Despite the recent increase in research on the toxicity of engineered nanomaterials (ENM) serious deficiencies in the knowledge relating to this area still exist, especially, the relationship between higher level effect and the mechanism of toxicity. Current mechanistic studies on ENM have reported oxidative stress as one of the most important mechanism of toxicity. In this study, to understand the relationship between higher level effect of ENM and mechanism of toxicity, functional toxicogenomic analysis was conducted in the nematode *Caenorhabditis elegans*. Survival and reproduction were first examined in worms exposed to silver nanoparticles (AgNPs) and multi-wall carbon nanotube (MWCNT). Subsequently, mechanism of toxicity was investigated using microarray followed by gene ontology (GO) and pathway analysis. Finally higher level effect of altered genes and pathways selected as mechanisms of toxicity was investigated using functional genomics tools such as, loss of function mutant or RNAi of genes involved in these pathways. Their responses were compared with those of wildtypes.

TU 318

Biomarkers evaluation on the pearl oyster *Pteria sterna* (Gould, 1851) in The Ensenada de la Paz B.C.S. México

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The pearl oyster is an important resource in the State of Baja California Sur Mexico, due to a lack of studies related to determine their response to xenobiotics in this study an evaluation of 4 biomarkers: respiration rate, O:N index, oxidative stress and AchE activity were carried out in the gill tissue of pearl oyster, from the cultivation vivero, to detect presence of toxic and genotoxic substances, and evaluating the use this biomarkers as reliable tool in environmental biomonitoring studies. Adults oysters (5.15 $\pm$ 0.58 cm) were collected in winter (feb 2010) and summer (sep 2010) in the bed, located near to the Pichilingue harbour. Samples tissue gill, was disintegrated; oxidative stress was determined as malondialdehyde (MDA) concentrations; and AchE inhibition by means of the Ellman technique. The results indicated that not significant differences in index O: N, the degree of oxidative stress and AchE activity among the organisms collected in summer and winter were detected, but significant differences exist in the oxidative stress grade and AchE inhibition being the pearl oyster collected in Pichilingue and the organisms exposed to toxic metals. It is evident that these biomarkers are a good tool in environmental biomonitoring studies.

TU 319

Physiological and biochemical biomarkers in the catarina scallop *Argopecten ventricosus* (Sowerby, 1842), as tools to Monitor in the Ensenada de la Paz B.C.S., México.

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The catarina scallop constitutes an important fishing resource in B.C.S., Mexico state, due their production to decreased 86%, in this paper, an evaluation of 5 biomarkers: respiration rate, feeding rate, O: N index, oxidative stress and genetic damage in the scallops, obtained from the in the cultivation nursery of UABCS and CICIMAR was done, to detect the presence of compounds with toxic and genotoxic effects and evaluate the use of these biomarkers as a reliable tool in environmental monitoring studies. Adult clams were collected (15 to 20 organisms) (5.0 $\pm$ 0.5 cm) during the summer and winter for 3 years for the evaluation of biomarkers. The biomarker data were integrated for calculation of BRI (Biomarker response index) to establish the biological health of the organisms collected at each site. Significant differences in feeding rate, index O: N, the degree of oxidative stress and genetic damage among the organisms collected in summer and winter were detected. The organisms collected in summer had higher levels of lipid peroxidation (42.7 nM MDA g-1) and a higher degree of cells with genetic damage (24%) compared with the results obtained in winter (9.07 nM MDA g-1 and 15.8% damaged cells). According to the mean values of BRI most degraded site was CICIMAR-IPN. The previous results are consistent with the levels of pollutants recorded in areas where clams were collected. It is clear that the battery of biomarkers used is a useful tool for environmental biomonitoring studies.

TU 320

Relationship between exposure duration, mortality, bioaccumulation and physiological parameters in glochidia (larvae) and juvenile freshwater mussels exposed to copper

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Species richness and population density of freshwater mussels Unionidae have declined substantially throughout North America. Surveys have revealed that environmental pollution was one of the top five stressors to these species and that the early life stages are one of the most sensitive to copper pollution. In this respect, this study aimed to characterize the acute (96 h) and chronic (28 days) sensitivity in newly released glochidia larvae (*L. cardium*) and 6 months old juvenile (*L. siliquioidea*) freshwater mussels exposed to copper and understand the toxic mechanism(s) of action. Dissolved EC50 and EC20 for glochidia larvae was 22.7 and 9.7 $\mu\text{g Cu/L}$, respectively. These values indicate that *L. cardium* are protected by the BLM-based acute WQC 2.3 $\mu\text{g Cu/L}$ but under protected by the hardness-based WQC 11.3 $\mu\text{g Cu/L}$. Glochidia copper uptake and accumulation indicate that there is a bioadsorption process based on physicochemical interactions between metal and functional groups of cell membrane which can cause damage at the cell surface. Our results also showed a decrease in whole body sodium concentration. Moreover, we observed an increase in the amount of Reactive Oxygen Species followed by a decrease in Total Oxyradical Scavenging Capacity and an increase in Lipid Peroxidation, characterizing a situation of oxidative stress. For the chronic toxicity test with juvenile mussels, the EC50 was 10.3 $\mu\text{g Cu/L}$ and when they were exposed to 2 $\mu\text{g Cu/L}$, 21.3 % mortality was observed. The survival data indicated that *L. siliquioidea* is barely protected by the BLM-based chronic WQC of 1.45 $\mu\text{g Cu/L}$ but significantly under protected by the hardness-based WQC 7.8 $\mu\text{g Cu/L}$. Results from copper bioaccumulation suggesting that copper toxicity is not associated with its bioaccumulation. Most striking in the chronic experiment was the reduction of whole body sodium content and inhibition of Na^+ K^+ ATPase, indicating that copper toxicity for mussels chronically exposed is a consequence of an ionoregulatory disturbance. In contrast to the acute experiment, redox parameters did not show significant differences between control and copper exposed mussels, but a lipid peroxidation could be observed in both tests acute and chronic. Take into account our results, mechanisms of copper acute toxicity for these sensitive mussels seems to be different to the chronic ones. This difference can be related to the time of exposure and/or to physiological specific aspects of different life stages.

TU 321

Cryptic lineages - are they comparable in their sensitivity towards chemical stress?

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The term "cryptic lineages" covers taxa that exhibit genetic differences but an at least superficial morphological equality and the respective specimens thus are misleadingly classified as a single nominal species. Although a well-known concept in evolutionary genetics since several decades, its relevance in an ecotoxicological context has only poorly been investigated. However, there is an urgent need for an ecotoxicological assessment of cryptic complexes as the genetic differentiation between lineages often reaches magnitudes similar to those between species. Thus, deviations regarding behavioural and physiological endpoints within cryptic lineage complexes are conceivable.

Accordingly, the present study investigated two cryptic *Gammarus fossarum* lineages - namely type A and type B - for differences in their sensitivity towards chemical stress. Two size classes of each cryptic lineage type were exposed to different concentrations of the fungicide tebuconazole or the insecticide thiacloprid in six independent experiments. The feeding rate on leaf discs was determined after seven days of exposure as a measure of sensitivity. The subsequently conducted meta-analysis revealed a statistically significant higher overall sensitivity of the cryptic lineage type A compared to type B ($n=16$; $p<0.0001$), by combining the results of all bioassays. Discrete meta-analyses for each of the substances tested, showed a similar tendency, however, results were statistically significant only for tebuconazole ($n=8$; $p<0.0001$). This deviation in sensitivity between lineages is new due to the minor consideration of the cryptic lineages concept in the field of ecotoxicology, however, mechanistically yet not explainable. Hence, further studies should address the sensitivity to other stressors, by also considering ecological aspects like the physiological fitness, of a broader range of cryptic complexes. Nonetheless, genetically distinct lineages may confound the results of ecotoxicological tests, as indicated in the present study, and should thus be interpreted with caution.

TU 322

Status of PAHs in greater Johannesburg area and possible sources

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The presentation gives an overview of the status of the presence and levels of PAHs in greater Johannesburg area. Although many studies of persistent organic pollutants such as pesticides have been studied in South Africa, the study on PAHs is still having a lot of gaps. The presentation gives an overview of modern extraction techniques that have been used for the extraction of the PAHs in water and sediments in rivers and dams in greater Johannesburg area. The common PAHs found and their concentration levels are presented. The levels of PAHs in water are compared to those in sediments. Detailed discussion of the possible sources of PAHs is given and how these sources impacts aquatic ecosystems in the studied area. Finally, the overview of PAH status in South Africa urban environment is given.

TU 323

Linking transcriptome to phenotype: response of a mouse liver cell line to Benzo(a)pyrene

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The aim of the systems biology initiative, "From contaminant molecules to cellular response: system quantification and predictive model development", is to build a model of interactions between cells and the toxic chemical Benzo-a-Pyrene (BaP). As part of this initiative, we aim to identify the regulation of genes by BaP in a time and concentration-dependent manner and relate the regulation to cellular distribution of BaP and the physiologic or toxicologic response. Exposure to BaP has been linked to carcinogenic, immunosuppressive and pro-inflammatory effects but there is still limited knowledge on BaP interactions with cells on a systems level. The well characterized Hepa1c1c7 was chosen as a cellular model to identify cell-BaP interactions and advance mechanistic understanding and predictability of the multiple toxic responses. In a first step, two concentrations (50 nM and 5 μ M) and four time points (2, 4, 12 and 24 h) were used for comprehensive transcriptome analysis. The results show a small number of genes being regulated at the early time points for both concentrations and a high number of genes being regulated at later time points for the high concentration. Genes like Cyp1a1, Tiparp and Nqo1 are regulated early for both concentrations whereas Aldh3a1, well known to act against oxidative stress, is only regulated at the higher concentration. Chemical toxicity, cell viability, detection of ROS formation and lipid droplets were determined. There was a significant reduction of the cell viability but only after 48 hours at 5 μ M BaP. Hypoxia and oxidative stress is one process network strongly regulated after 12 hours of BaP exposure. Using H2DCFDA staining, ROS production is already observed after 4 hours of exposure to high BaP whereas no response is observed for the low BaP concentration. However, at 24 hours, ROS appears to be produced even for the low BaP concentration. Gene response after 12 hours of 5 μ M BaP exposure moreover shows genes related to lipid metabolisms. Indeed, lipid body staining using Nile red revealed significant changes in lipid droplet morphology already after 4 hours. We conclude that the low BaP concentration induces a small gene response, that, together with biochemical defense mechanisms, helps the cell to return to its initial state. However, the high concentration causes strong pathway regulation and cellular dysfunction accompanied by early ROS production and Lipid droplet modification.

TU 324

Insights into the effects of silver by transcriptomics and proteomics in *Chlamydomonas reinhardtii*

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Ecotoxicology aims to identify effects that toxicants elicit upon the environment. A major challenge is to understand the toxic mechanisms at a molecular level and how these relate to functional changes at the organism and population level. Integration across various levels of biological organization, molecular, cellular and organismal, is required to draw conclusions on impacts in populations, communities and ecosystems (1).

Our research deals with the analyses of silver induced alterations in gene and protein expression in the algae *Chlamydomonas reinhardtii* and anchoring of these changes to specific phenotypes. This study is multidisciplinary and involves conventional toxicology analysis, transcriptomics, proteomics, metabolomics and bioinformatics. The transcriptome of silver exposed algae were analysed using *C. reinhardtii* whole genome microarray. The global protein profile of silver exposed *C. reinhardtii* (5h exposure time point) was done by Multidimensional Protein Identification Technology (MudPIT) which consists of 2D-LC and mass spectroscopy. The transcriptome analysis determined that the most significantly regulated transcripts are those of metabolic pathways of photosynthesis, tetrapyrrole synthesis, mitochondrial electron transport, protein transport and oxidative stress response. Similarly, the proteome profile showed these biological processes being significantly regulated indicating that silver affects key functional pathways in *C. reinhardtii*. The regulation of several transcripts/proteins involved in the photosynthesis and ATP synthesis could be corroborated with inhibition of photosynthesis and total ATP content at the physiological level. Also, the oxidative stress response could be linked to the physiological endpoint of lipid membrane peroxidation and synthesis of storage lipid bodies. Global analysis of the transcriptome and proteome not only gives an insight into the mechanism of toxicity of silver but also, by linking them to ecologically relevant phenotypes, a better ability to predict environmental risk.

TU 325

An integrative approach to understanding the response of *Caenorhabditis elegans* to valproate exposure

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C.elegans has been extensively used to study the effects of chemical exposure, aging, pathogenicity and reproductive development. With a well annotated genome, well characterized cell lineage maps, ease of making knock-outs, availability of mutants and short life span, it is a good model system to study the effect of chemical exposure on biological systems. In this study, wild type N2 *C.elegans* were treated with sodium valproate, a known teratogen and a histone deacetylase inhibitor used as a first line drug in epilepsy, manic depression, bipolar disorder and schizophrenia. Computational biology and omics technology was utilized to develop gene signatures associated with valproate exposure. We employed a systems biology approach by mapping transcriptomic data to known biological pathways to identify possible pathways affected by valproate treatment. The network models generated with the genes significantly associated with valproate indicates an overall downregulation of nuclear proteins. Metabolite signatures associated with valproate treatment were identified by Nuclear Magnetic Resonance (NMR) spectroscopy on formalin fixed worms.

Functional analysis was done using the DAVID gene ontology web tool. Genes were mapped to known pathways using Ingenuity Pathway Analysis software (Ingenuity® Systems, www.ingenuity.com). Networks derived from the genes highly correlated to valproate exposure were analysed by the same software. Metabolomic analysis was performed on the aqueous fraction of methanol:chloroform extracted samples on Bruker Avance 500MHz spectrometer at HWB-NMR Center, University of Birmingham. *C.elegans* were fixed prior to extraction, by adding 37% formalin directly on to plates.

Treatment with valproate resulted in an increase in lipid level and a decrease in egg laying in a dose dependent manner. The genes with positively correlated expression to valproate were enriched with functional terms such as structural molecule activity (cuticle/collagen), detoxification, phosphorylation and lipid metabolism. Genes related to embryonic/larval development/reproductive development, cell cycle/growth, transcription and chromosome organization are negatively correlated to valproate levels. This could be mainly due to the down regulation of the components of the histone deacetylase complex, DNA replication initiation complex, spliceosome and some translation initiation factors by valproate.

TU 326

Development of a resource for the collection, analysis and integration of ecotoxicological-related high throughput data

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In environment, the presence of chemical pollutants or climate changes can induce organism responses at the cellular and molecular level. Beside more traditional analysis system like evaluation of biomarkers, researchers can nowadays benefit of high throughput technologies (i.e. microarray, deep sequencing, 2-DE) that allow very large scale investigation. Data deriving from these technologies offer snapshots of the changes induced by the presence of environmental variations.

Management, analysis and interpretation of the massive data obtained from high throughput technologies can be quite challenging and requires bioinformatics skills still beyond the possibilities of many laboratories focused on "wet biology". Moreover all these results are poorly informative if considered separately and do not offer an exhaustive overview of the real cellular response and of changes in gene expression. Indeed they do not consider the relation between mRNA level, protein concentration and metabolite levels which are known to poorly correlate. Finally it would be very useful to compare the biological effects induced by the same environmental stress on different organisms through comparative studies.

Here we propose a web based system that works as a centralized resource for the collection, analysis and integration of ecotoxicology related high throughput data.

'Aggregated' information is accessible by means of simple web based data mining tools and is supported by effective and simple graphic representations to facilitate the understanding of biological response mechanisms.

We have benchmarked our platform with data obtained by high throughput analysis techniques- microarray and 2-DE - applied on the social amoebae *Dictyostelium discoideum* after treatment with several doses of mercury.

We show the advantages of using our system which allows the comparison of the results obtained from the two techniques, effective tools for the visual inspection of data and integrates data from external resources such as specialized databases (ie. genomic databases, metabolic pathways, Gene Ontology).

TU 329

On the appropriateness of using the common mixture toxicity models CA and RA on species sensitivity distributions: a theoretical approach

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Environmental species are not only exposed to single substances but typically to mixtures. Therefore it is of great importance to assess the ecological risk not only substance by substance but also for mixtures of compounds. Two models are generally used to predict mixture toxicity: the concentration addition (CA) (or dose addition) and the response addition (RA) (or independent action) models. Their adequacy to predict mixture toxicity of similarly acting compounds (CA) and dissimilarly acting compounds (RA) for single species was already shown during the last decades. To assess the risk of chemical mixtures to ecosystems, these two models have also been proposed to be applied directly on species sensitivity distribution (SSD) curves to derive a multi substances potentially affected fraction of species (msPAF). Usually, this is done in two steps: (i) the CA model is applied on individual SSDs of similarly acting compounds and then (ii) the RA model is applied on the predicted SSDs by CA between groups of similarly acting compound. However, as mentioned above the validity of mixture models were shown only for single species when applied on single dose-response curves and not on multiple species SSDs. For a validation of this approach, one should therefore first apply the mixture models for each species, then construct the mixture SSD to calculate the msPAF and compare it with a msPAF derived by applying the mixture models directly on the SSDs. This approach is however rarely done because the information on the whole dose-response curves of each single species for all substances is needed and this information is poorly available.

In this study, we highlight the difference between the two methodologies (SSD combination with CA/RA models or single species mixture assessment followed by SSD construction) to derive a msPAF. To compare the two methods we calculate msPAF with different cases, varying the EC50s and the slopes of the individual dose-response curves.

The results show that the predictions with CA are quite similar between the two methods and small differences can be explained by the goodness of fit of SSDs and the species tolerance correlation among substances. However, the application of RA may result in a differing msPAF between the two methods. Finally, we define cases in which the two methodologies give similar results and for which the use of CA and RA directly on SSDs to predict the risk of mixture compounds is appropriate.

TU 330

Estimating inter-individual sensitivity from survival data using a mechanistic model

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In the literature, there are two main alternatives to model mechanistically dose-survival relationship in ecotoxicity tests. Effects are related to a concentration of concern, for instance body concentration, and either individuals have different concentration thresholds for death, or individuals have equal probability to show an effect, with dying organisms just being the “unlucky” ones. A general framework to unify both approaches has recently been proposed but only special cases could be confronted to actual data. We used such a unified model to analyse four datasets. We showed the possibility to estimate properly the toxicity parameters together with inter-organisms differences of sensitivity for at least one of these parameters (here the threshold for effect). For all datasets, the model we propose outperformed the standard approach without accounting for differences of sensitivity. More accurate parameters’ estimations would be obtained through the incorporation of prior knowledge, in particular relative to background mortality. We also pointed that the standard threshold estimate is close to the medium value of the distribution (which would induce the death of half of the organisms), and that more relevant parameters; such as the concentration protecting 95% of the population would be 2 to 4 times inferior to this standard threshold. We believe that our approach is easy to understand and to apply by non-experts in modeling. Moreover, it permits to relate two fundamental concepts in understanding the response in ecotoxicity tests: the link between internal concentration and effects and the inter-individual variability regarding sensitivity.

TU 331

Incorporating data quality scores and acute-to-chronic ratios in the construction of SSDs

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The species sensitivity distribution (SSD) approach is in principle an alternative to the assessment factor approach for PNEC calculation. However, due to requirements on data quantity, its use is currently restrained to a small number of compounds. One central problem is that commonly many available data are discarded from the analysis for reason of limited validity, endpoint (exclusion of EC₅₀ type data) and exposure time (exclusion of acute data).

The objective of the present study was to assess how the SSD approach can be extended to the use of the entire data sets of complex heterogeneous ecotoxicological information (data of different data quality, acute and chronic values, EC₅₀, EC_x and NOEC values). More precisely, we aimed to provide a method which provides reliable and robust PNEC assessment, which is applicable to wider area of compounds.

For this purpose, we replaced the dichotomic classification of data as either valid or not valid by a system of quality scores which integrate on the inherent experimental and statistical reliability of data and on their biological relevance (organism and endpoint). This system serves as weighting factors of individual data in the SSD analysis. Furthermore, we studied different options of how to incorporate acute values. Different options (use of probabilistic Acute-to-Chronic ratios and weighting) for data treatment were integrated in a tool, which allows a generation of SSD functions and estimate uncertainties.

As test cases, we calculated the standard SSD function (based on chronic NOECs only) for several real ecotoxicological data sets on compounds fulfilling the strict TGD/REACH requirements for SSD application. Subsequently, we applied the developed method using weighting scores and acute to chronic extrapolation to the entire data set and bootstrap subsamples. SSD function obtained for subsamples of the entire data set using different options for data treatment were compared to the SSD based on chronic NOECs. Influences of different options for data treatment and sample size will be discussed with respect to the robustness and reliability of the SSD function and the associated HC₅ value.

TU 332

Investigating the replacement of NOEC and LOEC values with regression-based ECx values

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Data from chronic toxicity tests have traditionally been analysed by hypothesis test based statistical analysis to obtain a No Observed Effect Concentration (NOEC) and a Lowest Observed Effect Concentration (LOEC). Although a firmly entrenched paradigm, much recent debate has questioned whether these methods are the most appropriate way to analyse data from eco-toxicology studies. As part of this debate the use of ECx values and associated confidence intervals, determined by regression analysis and other modelling techniques, have been investigated by the OECD and others as possible replacement or complementary endpoints and are now included as a requirement in some test guidelines.

From the analysis of a number of data sets, the work described has shown that it is possible to apply a number of different static regression models to results arising from ecotoxicity tests, and to derive ECx values. The work indicates that, although not consistent for all datasets examined, it is possible to derive ECx values broadly similar to the respective NOEC and LOEC values.

However, a number of issues remain which will be of importance when considering the possible replacement of NOEC and LOEC values with ECx values, including experimental design. It is thought that current test designs may not necessarily be suitable for data analysis using regression based methods, and that modifications to current protocols may be required to ensure that regression analysis of test data yields reliable and significant results.

The work described has applied regression analysis to a number of simulated data sets which were developed according to differing test designs. This has shown that the experimental design of an ecotoxicity study can have implications for the results obtained by statistical analysis using regression techniques. In particular, both the estimated ECx values and the associated confidence intervals have been shown to be impacted by experimental design. This could have implications for both the ethical and practical aspects of ecotoxicological testing, when seeking an optimum test design.

TU 333

Statistical approach to select soil bioindicators for soil monitoring, risk assessment and soil characterization. Results from the French national programme ‘Bioindicators’

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The Random Forests statistical approach (Breiman, 2001), is used to classify, predict and select the best set of explanatory variables in large fields of research. Yet, only few applications were made in environmental research, especially in soil ecotoxicology.

The analytical strategy of the French “Bioindicators Programme” (2006-2012) is to (i) measure a large number of bioindicators in contrasted pedo-geo-climatic contexts covering French area, using similar sampling protocols, (ii) manage high numbers of biological and meta-data in a database enhancing data analysis, (iii) develop a data mining of collected data during two years, with the help of a biostatistician team. The main objective is to develop a generalised method for selecting relevant bioindicators with regard to the targets (characterisation, monitoring, risks[3DOTS]), that may assess risks of soil contamination to ecosystems.

More than 170 biological variables are screened, including microorganisms (e.g. biomasses of fungi and bacterial communities, bacterial diversity, enzymatic activities), mesofauna (e.g. nematodes and collembola), macrofauna (e.g. earthworm at community levels and metal accumulation in snails). Such bioindicator variables were measured in soil surface horizons, at 13 sites, producing 47 different contexts in terms of land use historic and origin of contamination (PAH, ETM, pesticides).

As a first step of statistical analyses, our results showed the importance of correlations between biological variables in intra and inter-groups. Then discriminated analyses using Random forests revealed sets of relevant indicators for land use (crops, pastures, woods and forests), levels of metal contamination (high, medium and low) as well as

TU 334

Advanced non-linear regression methods for concentration-response curves in R

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A recent editorial in IEAM has suggested to ban the use of traditional no-observed-effect levels (NOELs) and lowest-observed-effect levels (LOELs) as basis for risk assessment and decision making, and instead use curve-fitting for estimation of concentration-response relationships. This should motivate an evaluation of current concentration-response modelling approaches in ecotoxicology, and adoption of new methodologies for curve-fitting that are used in other fields of environmental science. The European Water Framework Directive (WFD) has triggered much research on non-linear relationships between physico-chemical stressors and ecological responses during the last decade. For example, thresholds in stressor-response relationships are particularly relevant for assessment of ecological status of water bodies. Basic statistical methods such as linear regression or ANOVA are not suitable for estimating such relationships. The response may also be more complicated than e.g. a simple sigmoid curve, therefore flexible regression methods have been used for exploring the shape of the response curve without a priori assumptions. Moreover, we may be interested in estimating an extreme part of the response rather than the average. In this presentation, I give examples of ecological responses to eutrophication and acidification stress in lakes. The ecological responses are univariate indices representing community composition of phytoplankton, macrophytes, macroinvertebrates and fish. Building upon basic linear regression, I describe three statistical 'tricks' which enable more flexible curve-fitting: (1) Using a non-linear link to the response variable (generalised linear model); (2) Using a piece-wise linear function for the stressor variable (generalised additive model); (3) Analysing a quantile of the response variable instead of the average (e.g. 90%; quantile regression). All analyses are carried out in the free statistical programming software R.

TU 335

An interfaced R-package to fit SSDsE. Billoir¹, D.R. Fox², C. Charles³, M.L. Delignette-Muller³¹Plateforme de Recherche de Rovaltain, Valence, France²Environmetrics, Melbourne, Australia³Université Lyon 1, Lyon, France

Species Sensitivity Distributions (SSDs) rely on the assumption that aquatic species of a community or assemblage differ in their sensitivity to a hazardous chemical. Toxicity values are used as indicators of the sensitivity and are assumed to follow a theoretical distribution accounting for the inter-species variability. A Hazardous Concentration (HC) is then derived using a low-order percentile of the SSD fitted to a limited (and often small) set of toxicity values, the latter being assumed to be a representative sample of species sensitivity among the community to be protected.

The SSD approach is an element of most regulatory guidance documents for environmental risk assessment (e.g. ANZECC, ECHA, USEPA). Yet, the approach to be used is still debated, including: (1) whether parametric or non-parametric methods should be preferred - the applicability of the latter being limited by the small size of toxicity data sets available and (2) in the parametric cases: (2a) the choice of the distribution shape - this is often decided on the basis of mathematical properties, especially on the existence of analytical results, and (2b) the choice of the method for estimating HCs and their confidence interval.

Several softwares have been provided through environmental protection agencies for estimating HCs and their companion uncertainty, such as Burrlioz in Australia and New Zealand, ETX in The Netherlands or Web-ICE in the U.S. Those three softwares have been developed as turn-key tools for the application of regional regulatory recommendations and rely on different choices regarding the three points aforementioned. Besides, it appears that none of these programs allows censored data to be accounted for. For users interested in examining the impact of the methodological choices made in existing softwares (and in the corresponding regional guidance documents), we are currently developing a new and open source tool enabling the comparison of several distributions and also the use of both pointwise and interval-censored data (or a mix of both).

TU 336

A probabilistic model for species sensitivity distributions taking into account inherent uncertainty and variability of effects to estimate environmental risk

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Empa, St. Gallen, Switzerland

TU 337

Toxicokinetics-Toxicodynamics survival model, from theory to practiceL. Mariani¹, E. Billoir², C. Forfait¹, S. Charles³, M.L. Delignette-Muller¹¹Université Lyon 1, Villeurbanne cedex, France²Plateforme de recherche de ROVALTAIN, Valence, France

Toxicokinetics-toxicodynamics (TKTD) models simulate the time course of processes leading to toxic effects on organisms. Even for an apparently simple endpoint such as survival, a large number of TKTD models exist. They have been reviewed by Jager et al (2011) who proposed a unified theory (GUTS) for TKTD threshold models and the use of a likelihood approach for estimating model parameters. Here, we focus on a TKTD threshold model in the GUTS framework called DEBtox (Dynamic Energy Budgets in toxicology) survival model. This model assumes an effect above a threshold concentration. We examine a simple question: is it possible to estimate parameters of this model by maximum likelihood approach from standard survival data sets?

We analyzed survival data sets of the freshwater invertebrate *Daphnia magna* exposed during at least 21 days to nine different contaminants. We tried to fit 4 nested DEBtox survival models to each data set by maximizing the log-likelihood function to estimate model parameters.

We illustrated two structural problems linked to the model itself or to the likelihood function. (1) We cannot simplify the model by fixing the natural mortality to 0 even if data did not show natural mortality and (2) because of discontinuities in the log-likelihood cross sections when the threshold parameters is equal to one of the tested concentrations, there are many local minima. This is due to the structure of the threshold model and especially to its non-derivability at the threshold value. We will investigate various solutions to this issue, including adaptation of optimization algorithm and modification of models with fully derivable functions.

TU 338

The IBR revisited: optimization to avoid misuseS. Devin¹, C. Cossu-Leguille², A. Geffard³, L. Giamberini¹, L. Minguez¹, F. Rodius¹, T. Burgeot⁴, S. Pain-Devin¹¹LIEBE - CNRS UMR 7146, Metz, France²LIEBE - CNRS - UMR 7146 - Université de Lorraine (UdL), Metz, France³Laboratoire d'Eco-Toxicologie, Université Reims Champagne Ardenne, Reims, France⁴Département Biogéochimie et Ecotoxicologie, IFREMER, Nantes, France

Multibiomarker approaches are widely used for in situ assessment of ecotoxicological effects of contaminants and to understand the relationships (1) between biomarkers and (2) between biomarkers and contamination status of studied sites. Several tools have been proposed to integrate these responses in a single and simple measure. Among them, the Integrated Biomarker Response (IBR) is a promising method that provides both a graphical synthesis of the different biomarker responses and a numeric value that integrates all these responses at once. The IBR is the sum of the area defined by the k biomarkers arranged in a radar diagram. However, in their initial publication, the authors provide two calculation methods: the first one is a complicated formula that works whatever the number of biomarker is, while the second one is a simplified formula that works only when 4 biomarkers are used. The attractiveness for simplicity led to frequent misuse of the IBR (40% of the 38 publications using it). Moreover, the final outcome of the calculation process highly depends on the sequential organisation of the biomarkers. We aim to propose a new procedure to resolve these main problems in the IBR application.

We first go back to trigonometry basics to propose a new formula for the IBR, that is far more simple than the original one. Then we wrote a procedure that creates all the possible circular permutations of k biomarkers. It results on a (k-1)! matrix of IBR value that allows to calculate the mean IBR for a site and to prioritize IBR values among sites in a more confident way.

A case study using this method is presented, based on the results of a survey of 8 sites presenting contrasted levels of sediment contamination. The permutation is performed for a set of 7 biomarkers in the bivalve *Dreissena polymorpha*, resulting in a matrix of 720 IBR values. It evidenced the variability of the IBR, that is not our main concern.

Indeed, the consequence is a different prioritization of contaminants effects depending on the biomarker sequence, that could lead to misunderstanding of contamination consequences on biota. Considering the need of multibiomarker approaches to understand the complexity and the variability of biological responses, our study provides an efficient and robust tool to integrate these data and avoid subjectivity in the final outcome of the method.

TU 339

Statistics Service - a new tool for efficient data analysisA. Wedemeier¹, T. Bierz², N.A. Hernandez Vargas³, B. Erzgraeber²¹BASE, Limburgerhof, Germany²BASF SE, Limburgerhof, Germany³Heidelberg University, Heidelberg, Germany

In this talk we present a newly built statistics tool which allows a highly convenient and efficient data analysis. The tool combines Excel (a Microsoft Office product) and R (a cost-free available statistics programming language) via a specific server.

This Excel-R connection significantly broadens the spectrum of available statistical methods in Excel. Moreover, the user (e.g. lab technician) can easily conduct complex statistical analyses:

The user selects the desired statistical method in Excel after highlighting the input data for the computational analysis. The data is then transferred to the server, the computation starts and the results are automatically transferred back to Excel. Besides the results (e.g. graphical plots) the user can also receive an interpretation help (txt file) of the result output.

In addition to a short introduction of the Statistics Service technical background, we will demonstrate its functionality using examples from the environmental fate modeling for plant protection products, e.g. the analysis of environmental fate data and selection of relevant input parameters to exposure models, in order to provide a general overview of how this tool works.

TU 341

Long term effects of an early exposure to PAHs on zebrafish behavioural responsesC. Vignet¹, K. Le Menach², S. Péan¹, L. Lyphout¹, D. Leguay¹, H. Budzinski², M.L. Bégout¹, X. Cousin³¹IFREMER, L'houmeau, France²EPOC - LPTC, Université Bordeaux 1, Bordeaux, France³IFREMER/INRA, L'houmeau, France

Polycyclic aromatic hydrocarbons (PAH) emission in the environment is constantly increasing with human activity. The goal of this study was to assess long term consequences of fish early exposure to PAH on behaviour. Eggs were collected from AB zebrafish strain and were placed at 4 hours post fertilization (hpf) until 96 hpf in a 3 cm diameter Petri dish containing 3 g reference sediment either plain (control) or spiked with a mixture of Benzo[a]pyrene, Pyrene and Phenanthrene at environmental concentrations based on values measured in the Seine Estuary (France), a highly polluted site. Thereafter, larvae were transferred in clean water and raised until adulthood. Behavioural tests such as locomotion during 24-hrs, sudden dark change challenge and T-maze exploration were performed at adults' stage (F0) and sudden dark change on larvae produced by F0 adults. In adults, during night or dark period, contaminated fish were significantly less active than control fish. A similar observation was made for contaminated F0 in T-maze safe area. Contaminated F1 larvae were more active when light was on than control and less active when the light was off. This study indicates that contamination of zebrafish using environmentally relevant concentration during the very first stage of development with a PAH mixture of 3 molecules induced late behavioural effects measured at the adult stage. Further, effect was transmitted to the next generation and behavioural responses of F1 larvae were different to that of F1 control larvae. Consequences on fish abilities (e.g. predator escape, food search, courtship) will be discussed.

TU 342

Long-term food-exposure to PCB mixtures induces reproductive and behavioural disruptions in zebrafishX. Cousin¹, T. Daouk¹, S. Péan¹, C. Vignet², T. Larcher³, F. Rouspard⁴, L. Lyphout¹, D. Leguay¹, V. Loizeau⁴, M.L. Bégout¹¹Ifremer, L'houmeau, France²IFREMER, L'houmeau, France³INRA, APEX, UMR703, ONIRIS, Nantes, France⁴IFREMER Laboratoire de Biogéochimie des Contaminants Organiques, Brest, France

Although the use of polychlorinated biphenyls (PCBs) has been banned for several decades, they are still present in the environment. Field analyses have established correlations between exposure to PCBs and alterations in fish physiology including reproductive function and behaviour. In the present study, we performed a life-cycle exposure using zebrafish and mixtures representative of some environmental situations in terms of doses, composition and containing mainly non dioxin-like congeners. Exposure was performed through diet which is the main contamination route in the wild. We demonstrated a bioaccumulation of PCBs in males and females as well as a maternal transfer to the eggs.

Several reproductive traits were altered after exposure to a PCB-contaminated diet, including a reduction in the number of fertilized eggs per spawn as well as an increase in the number of poorly fertilized spawns. This was related to modifications of ovary histology revealing a decrease of maturing follicles and an increase of atretic follicles in the ovaries of females exposed to PCBs.

In addition, several behavioural traits were monitored. Fish exposed to the highest dose (equivalent to that found in the Seine Estuary) displayed an increased swimming activity during the period at the end of the night. Fish exposed to an intermediate dose (equivalent to that found in the Loire estuary) displayed behavioural disruption analogous to hyperactivity that showed some similarities to a human behavioural deficit syndrome known as attention deficit / hyperactivity disorder (ADHD), which can be observed in humans after exposure to PCBs.

These results indicated that exposure to PCBs mixtures mimicking some environmental situations, can lead to a dramatic reduction in the number of offspring produced by a female over a lifetime and disrupt behaviour. Potential ecological consequences are discussed with regard to reproduction, fitness and survival.

TU 343

Indirect estimation of population-level effect of pollutants based on tolerance evolution and fitness cost of toleranceY. Tanaka¹, H. Mano¹, H. Tatsuta²¹National Institute for Environmental Studies, Tsukuba, Japan²Department of Ecology and Environmental Science, Graduate School of Agriculture, Nishihara, Japan

Evolution of tolerance to a pollutant chemical in a natural population of organisms gives an evidence of the examined population to have suffered adverse effect by the chemical because the tolerance evolution of a population is solely brought about by selection pressure that removes sensitive individuals or genotypes from the population. Despite the advantage in using the tolerance evolution as a monitoring tool of chemical pollution in the field, such approach is not relevant for a quantitative impact evaluation because observed differences in the tolerance between a contaminated and an uncontaminated site are rarely connected to the ecological risk that burdens the population.

Applying the theory of evolutionary ecology and quantitative genetics into the tolerance evolution, we attempted to estimate the selective force that is needed to maintain the observed difference in the tolerance between populations. For this aim, we made a heuristic assumption for the evolutionary analysis of tolerance: the strength of tolerance induced by a genotype to a particular compound is positively associated with fitness cost of the tolerance, and the joint action of selection induced by the cost and selection favoring higher tolerance achieves optimization of the total fitness that takes into account the fitness gain by the tolerance and the fitness cost due to the tolerance. The fitness gain by the tolerance means reduced toxicity of a specific exposure concentration in comparison to sensitive individuals, and can be quantified by the life table toxicity data. As a case study of such an approach, we detected inter-population differences of the fenvalerate tolerance among natural populations of a water flea (*Daphnia galeata*) in Kasumigaura Lake (Japan) and an agricultural reservoir near to this lake. There was a statistically-significant negative association, among isofemale clones, between tolerance values and intrinsic population growth rates under null exposure. With the fitness optimization, we derived an estimate of stationary exposure level of the chemical (all compounds that exhibited co-tolerance with this chemical) as 0.015 ppb, and concluded that the examined population of this species had been suffering the population-level effect that corresponded to 1/4 reduction of the intrinsic population growth rate. The indicated level of the population-level effect may have considerably reduced the probability of persistence of the population.

TU 344

Triclosan toxicity: a multi-generational and demographic assessment using *Daphnia magna*K. Sibley¹, M. McKee¹, J. Lucas²¹University of Guelph, Guelph, Canada²University of Saskatchewan, Saskatoon, Canada

Triclosan is a ubiquitous compound in wastewater treatment plant effluents and biosolids and is commonly detected in surface waters throughout North America and Europe. The acute toxicity of triclosan (TCS) toward aquatic organisms has been well studied but information on long-term, low level exposures at environmentally relevant concentrations is lacking. The objective of the present study was to evaluate the toxicity of triclosan in a multi-generational test with *Daphnia magna* in laboratory exposures. 24-hr old *D. magna* neonates were exposed to TCS (0.5 to 150 µg/L) in six successive 21-d life cycle tests. Brood randomly collected from 5 replicate beakers within each treatment on day 21 were used to seed the next generation. Survival across the 6 generations exceeded 80% in all treatments across all generations. A slight but non-significant delay in time to first brood (7-9 days) occurred in the 150 µg/L treatment in the first generation but not thereafter. Total offspring produced over 21 d and fecundity (average offspring/adult) decreased significantly at concentrations >50 µg/L (NOEC = 10 µg/L) in the first 3 generations but this difference disappeared in the last three generations indicating increased tolerance to TCS. Reproductive demographic data were used to estimate the intrinsic rate of increase (r) and indicated significant declines in population levels at the three highest treatments based on the first 3 generations but not the last 3 generations. In this study, effects were only observed at TCS concentrations approximately one order of magnitude greater than those found in surface waters, which indicates that the long-term risks of TCS are likely minimal.

TU 345

Assessing the chronic aquatic toxicity of phthalate ester plasticizersA. Staples¹, R. Guinn², K. Kramarz³, M. Lampi⁴, N. Scholz⁵¹Assessment Technologies, Inc., Keswick, va, United States of America²Eastman Chemical Company, Kingsport, tn, United States of America³BASF Corporation, Pasadena, tx, United States of America⁴ExxonMobil Biomedical Sciences, Inc., Annandale, nj, United States of America⁵Evonik Oxeno GmbH, Marl, Germany

Phthalate esters are a class of chemicals varying greatly in terms of uses, properties and toxicity. C1 to C4 phthalate esters are used in non-vinyl commercial products and pharmaceuticals. C8 to C10 phthalate esters are additives imparting flexibility to vinyl resins. The purpose of the present study is to assess chronic effects of phthalate esters on aquatic organisms. Studies show that populations of fish and invertebrates may be adversely affected by exposure to C1 to C4 phthalate esters, but are not adversely affected by exposure to C8 or higher phthalate esters. Secondary endpoints, including molecular, biochemical, and/or histological responses to chemical exposure, do not appear to predict effects related to primary endpoints of survival, growth and development, or reproductive fitness. A previously published risk assessment for C1 to C4 phthalate esters demonstrated low risks in North American and Western European surface waters. Risk assessments conducted by authorities in Europe with DEHP, DINP, and DIDP have concluded no risks to aquatic organisms due to aqueous solubility constraints, low expected surface water concentrations, and metabolic biotransformation capabilities of aquatic organisms. Important chronic aquatic toxicity studies that have included transgenerational exposure have been conducted since those assessments and are presented here. The data from the present studies, support the earlier risk assessment conclusions. The data also provide further support for a narcosis-related aqueous solubility cutoff at approximately C6 and higher phthalate esters. Finally, for the C1 to C4 phthalate esters, the present study shows that secondary endpoints (e.g., molecular, biochemical, and/or histopathology) to date, provide limited benefit in practical ecological risk assessment of phthalate esters to aquatic species.

TU 346

Effects of land use and pesticide exposure on hatching characteristics of *Daphnia magna* ephippia

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Daphnia is a well established model organism and standard test species in ecotoxicology. *D. magna* reproduces by cyclical parthenogenesis, where environmental cues associated with unfavourable conditions trigger sexual reproduction. In permanent and temporary standing waters, sexually produced encapsulated dormant eggs (ephippia), build up over the years to form a dormant resting egg bank, which is important for long-term survival of the population. Each year a fraction of the dormant eggs hatch from the sediment. Through this benthic-pelagic coupling, structure and function of resting egg banks have important consequences for ecological and evolutionary dynamics of the active populations and communities. To date however, almost no information is available on the effects of pollution on these dormant egg banks.

Coors et al (2009) used land use patterns as a proxy for historical and current pesticide contamination and demonstrated that the tolerance of *D. magna* populations for the pesticide carbaryl was positively correlated with land use intensity of the area around the ponds. As in standard ecotoxicity tests, these experiments were performed for 48 hours with *D. magna* neonates less than 24 hours old.

In this study we investigate the effects of land use intensity and pesticide exposure on hatching characteristics of *D. magna* ephippia from natural populations. Therefore we sampled dormant egg banks of ponds from different regions in Belgium, in landscapes with varying degrees of agricultural activity (from natural areas to areas with intensive agriculture). In the laboratory hatching experiments were performed, looking specifically at differences in hatching characteristics of *D. magna* ephippia under optimal conditions and after pesticide exposure in relation to historical pesticide exposure.

TU 347

Is there a potential for wild *Daphnia magna* populations to undergo selection at conventionally derived no observed effect concentrations of chemicals?

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The genetic variability within a population exposed to stress governs the micro-evolutionary potential of that population to undergo selection and adapt to the stress. However, adaptation caused by natural selection will by definition reduce overall genetic diversity, a process termed genetic erosion. This may result in a cost of tolerance, whereby populations evolved under a certain chemical stressor, may become less tolerant to novel stressors. It is hypothesized that a population under stress exhibits an increase in genetic variation for life history traits relative to a control population. We used the genetic coefficient of variation (CV_G) and the broad sense heritability (H^2) as measures for genetic variability to assess the potential for micro-evolutionary responses in wild populations exposed to copper and zinc, as model toxicants. Life-table experiments with concentrations of copper and zinc close to the conventionally derived geometric mean NOEC values (50 µg Cu/L, 428 µg Zn/L) were conducted with 8 *Daphnia magna* populations collected from lakes and ponds across Belgium, with non-elevated Cu and Zn levels. Additional full dose-response experiments were carried out with the most and least sensitive populations. Our results indicate that several fitness traits have the potential to respond to natural selection and genetically adapt, but that this depends strongly on the population and the exposure treatment considered. In long-term multi-generational exposures this may result in shifts in genotype frequencies and reduction of genetic diversity.

TU 348

Evolutionary consequences of the loss of genetic diversity in *C. riparius* populations - Introducing the MIDGE project

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Chronic pollution has been shown to decrease genetic variation in populations of several species alongside with adverse effects on the physiology of organisms. This loss of genetic diversity may reduce the potential of populations to adapt to changing environments.

Ecotoxicological studies need to consider not only short term effects of pollution, such as changes in life history traits of organisms, but also its long term effects, such as genetic erosion. This genetic erosion can lead to the loss of evolutionary potential and thus it is also of crucial importance to assess the evolutionary consequences of changes in genetic variability.

There is an urge for evolutionary toxicology studies because only an integrated approach linking contamination, genetic variability, life-history responses and fitness costs associated with genetic adaptation can lead to a more reliable prediction of the anthropogenic impacts on biodiversity.

In the MIDGE project aims to study the Microevolutionary Dynamics and Genetic Erosion in pollution-affected *Chironomus* populations. More specifically we are investigating the genetic variability of *C. riparius* populations from unpolluted and contaminated sites using mitochondrial sequence variation and nuclear microsatellite analyses and we intend to address three main questions:

- Does chronic pollution affect genetic variability of *C. riparius* populations in the field?
- Are *C. riparius* populations in contaminated areas adapted to pollution exposure?
- What are the evolutionary consequences of this altered genetic variability due to pollution in terms of fitness costs?

Measures of genetic erosion have recently been proposed as the ultimate biomarker of effect and by focusing on effects of contaminants on genetic variability in natural populations we aim to aid in developing new bio-monitoring approaches and provide advanced scientific basis for integrative ecological risk assessment methodologies which are essential for effective environmental conservation strategies. The SETAC meeting is an excellent opportunity to share ideas, approaches, methodologies and preliminary results with other researchers working in the field of evolutionary toxicology.

TU 349

Association between increased resistance to copper and salinity in *Daphnia longispina* clonal lineages under short- and long-term multi generational exposures

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Presently, many coastal freshwater ecosystems are already exposed to chemical contamination. Depending on the period and intensity of such exposure, the selective pressure exerted by chemical contamination could have caused the disappearance of the most sensitive genotypes from exposed population, causing its' genetic erosion. Whether such eroded populations will be capable of coping with future environmental perturbations, namely those associated with the predicted climate changes such as the gradual increase of salinity in freshwater coastal lagoons, will largely depend on the association between resistance to the chemical it was exposed to and to the future environmental stressor. Accordingly, this work aimed at assessing if an increased resistance to copper is associated with an increased sensitivity to salinity; or at least if some of the genotypes most resistant to copper are among the most sensitive to salinity, both under (i) short-term exposures and under (ii) long-term generational exposures. To attain these objectives, the lethal and sublethal (growth and reproduction) sensitivity to salinity of six clonal lineages of *Daphnia longispina*, exhibiting different lethal resistances to copper, was assessed. Subsequently, to evaluate its acclimation to salinity, each clonal lineage was acclimated to 0.1g of NaCl for two generations and its lethal and sublethal sensitivity to salinity was re-assessed. The observed results, revealed significant associations between an increased resistance to copper and the lethal or sublethal sensitivity to salinity neither under short-term exposures nor under long-term multigenerational exposures. Also, most of the studied genotypes exhibited fitness costs in reproduction after being exposed to NaCl for more than one generation, and became acclimated to low levels of salinity.

TU 350

Metal tolerance in the earthworm *Eisenia fetida*: Is it a case of adaptation or mere physiological acclimation?

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In a recent study, we showed that the earthworm species *Eisenia fetida*, inhabiting an extremely high metal polluted compost heap on a wine farm, did not have elevated body loads of the metals but exhibited genotoxic tolerance when exposed to Cd in the laboratory (Voua Otomo and Reinecke, 2010). To unravel the mechanism behind the surprisingly low metal body burdens on one hand and genotoxic tolerance on the other hand, we investigated the estimated bioavailability of these metals (Cu, Zn, Pb and Cd) using sequential extraction methods with CaCl₂ and di-ethylene-triamine-pentaacetic acid (DTPA) and allozyme polymorphism in this field population, a laboratory control as well as a long-term Cd exposed population. The amounts of mobile (extracted with CaCl₂) and mobilisable (extracted with DTPA) metals in relation to the total (extracted with nitric acid) metals were all below 0.05% for all four metals, suggesting low availability for uptake. The low availability of these metals could not be explained by physico-chemical properties of soil but by the phenomenon of aging of the metals. There was no difference in allozyme frequency between metal tolerant and non-metal tolerant populations of *E. fetida*. This suggested that the tolerance found in earlier studies could be a mere physiological adaptation.

TU 351

Potential role of biotransformation and antioxidant enzymes during maternal transfer of microcystin resistance in *Daphnia*

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Due to eutrophication and other factors, the frequency of cyanobacterial blooms in freshwater has increased worldwide. They are an important problem to the ecosystem, particularly affecting zooplankton populations which feed on phytoplankton. Among other toxins, some cyanobacteria produce microcystin (MC), MC-LR the most studied variant. MCs inhibit serine/threonine protein phosphatases type 1 and 2A and provoke oxidative stress. The planktonic genus *Daphnia* plays a central position in pelagic food

webs linking primary producers to consumers. The negative effects on Daphnia are well understood; toxic cyanobacteria affect growth, survival and reproduction. However, one possibility to ameliorate toxic effects is to biotransform MC by conjugation to glutathione via glutathione S transferase, thereby reducing toxicity and aiding excretion. This process is thought to underlie the ability to withstand MC in Daphnia and could explain the enhanced tolerance found in the offspring of Daphnia exposed to live toxic cyanobacteria.

In this study, multigeneration exposures were conducted, we exposed the parental generation to MC-LR for 1 or 7 days and determined the enzyme mediated tolerance to MC in their offspring, a) by assessing the acute effect of MC-LR on biotransformation, antioxidant and energy metabolism enzyme activities, and b) through 21 day chronic toxicity tests exposing to the same concentrations of toxin.

Seven day exposure of the parental generation to MC-LR induces higher activity of glutathione S transferase and malate dehydrogenase in the offspring and enables it to increment the catalase activity when challenged with the toxin. In offspring from the 1 day exposed parental generation those effects were less pronounced or not visible. Offspring from non-exposed and 1 day exposed mothers suffered from decreasing survival when exposed to MC-LR whereas the offspring from 7 day exposed mothers show higher survival. Higher survival of the offspring is correlated with the elevated activity of glutathione S transferase, malate dehydrogenase and catalase, suggesting maternal transfer of activation factors.

These results prove transgenerational or maternal effects provoked by a natural toxin in a very important freshwater herbivore which may explain the observed acquirement of enhanced tolerance over generations through a biochemical perspective.

TU 352

Is there a functional role of DNA methylation in the stress response?

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DNA methylation, the addition of methyl groups on the 5' position of cytosines in the DNA plays an important role in the regulation of gene expression of eukaryotic cells. Considerable research has been performed in vertebrate organisms and plants whereas DNA methylation in invertebrates has only recently received attention. In particular for Daphnia magna, a key test organism in aquatic toxicology and an important species in many aquatic ecosystems, knowledge on DNA methylation is very limited. Within our long-term aim of unravelling the potential functional role of DNA methylation in the stress response of D. magna, this study aimed at sequencing the entire cytosine methylome. For the comprehensive profiling of DNA methylation a methylated binding domain (MDB) protein based sample preparation technique was used. Since this technique is very specific and works well with fragments densely methylated, it was decided to use also a less specific technique based on DNA digestion with a novel restriction endonuclease. This endonuclease recognizes the C₅NR (R=G/A) methylated sites and cleaves DNA at fixed distances generating DNA fragments easily identified on gel.

Bioinformatics analysis (i.e. mapping with the most recent D. magna genome assembly) was performed in order to generate the methylome map. Ongoing bioinformatics analyses is now focused on determining structural, functional or evolutionary relationships among the methylated sequences and to determine if methylated sequences occur in genes or pathways that may be relevant for dealing with chemical and ecological stress.

TU 353

Effects of benzo(a)pyrene and vinclozolin on freshwater snails: I) epigenetic and genetic responses of adults and II) offspring embryo toxicity

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Vinclozolin causes transgenerational effects by inducing DNA methylation changes in exposed rats. Benzo(a)pyrene is a clastogen used as a reference chemical for the in vitro mammalian cell micronucleus test. Taking into account that epigenetic and genetic profiles could be altered by these pollutants we studied molecular and cellular responses after adult exposure of freshwater snails (Physa acuta). Additionally, the embryo toxicity in the following generation (F1) after paternal exposure was evaluated. Mature snails exposed for 46 days and deposited egg masses were used for determining parental endpoints (F0) and transgenerational effects, respectively. DNA extracted from mature snails were analysed to evaluate differences in DNA methylation patterns. In order to analyze methylation-sensitive sites we used PCR technique with arbitrary primers (AP-PCR) using HpaII and MspI digested samples. HpaII and MspI are isoschizomers that recognize the tetranucleotide sequence 5'-CCGG-3' but that show differential cleavage sensitivity to cytosine methylation. HpaII cannot cleave if one or both cytosines are fully methylated (in both strands), whereas MspI cleaves C5mCGG but not 5mCCGG sequences. Gel electrophoresis of PCR products and comparative analysis between HpaII and MspI patterns allowed the study of CCGG regions in the genome that are sensitive to methylation and the effect of these pollutants on these sites. Genotoxicity was measured by the micronucleus test in cells isolated from whole adults after homogenization of soft tissues. Finally, embryonic development of offspring was monitored. Eggs masses were recovered and the teratogenic effects of both non-exposed and exposed single egg capsules were measured in multiwell plates. Parental responses at cellular and molecular levels were compared with F1 responses using an ecological relevant endpoint (embryo toxicity). The aim of this work was to establish potential linkages between mechanisms of action and long-term responses at higher levels of biological organisation, which could ultimately have consequences on population dynamics. This study was supported by the Spanish research project CTM2008-03492.

TU 354

Later-life effects of early-life mitochondrial DNA damage

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I will suggest that mitochondrial DNA is a potentially important and understudied target of environmental toxicants, and one that when impacted is likely to have long-term effects. Mitochondrial DNA (mtDNA) is more sensitive than nuclear DNA to many common genotoxins, and lacks some repair pathways that are present in the nucleus. In particular, helix-distorting adducts formed after exposure to environmentally important genotoxins such as ultraviolet C (UVC) radiation and some polycyclic aromatic hydrocarbons and mycotoxins are repaired in the nuclear genome by nucleotide excision repair. However, nucleotide excision repair proteins are not present in the mitochondria, so that the fate of such DNA damage in the mitochondrial genome is unknown. The effects of such persistent damage are also unknown; we hypothesized that the effects of such damage would be particularly important after early life stage exposure since mtDNA copy number is lowest at that time. Using the nematode model Caenorhabditis elegans, we found that UVC-induced photodimers result in lower levels of mtDNA-encoded mRNAs, decreased ATP levels, decreased oxygen consumption, larval developmental arrest, and neurodegeneration. Furthermore, UVC-induced mtDNA damage is slowly removed in a process dependent at least in part on mitochondrial fusion, fission and autophagy. Mutations in mitochondrial fusion and autophagy genes exacerbate the larval arrest, suggesting a potent gene-environment interaction in which the effects of mtDNA damage caused by environmental agents are exacerbated by decreased mitochondrial fusion and autophagy.

TU 355

Is trans-generational PCB transfer a key process for parental detoxification and adaptation in amphipods?

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To date, reproduction success and reproductive disorders have been studied in several marine organisms like fish and amphipods to address the question of the impact of contaminant exposure on wild populations. This study is focussed on the characterisation of the progress of toxic effects from subcellular damage at the individual level of the parental generation to deterioration of the offspring in the amphipod species Monoporeia affinis. Monoporeia affinis have been sampled at differently polluted and reference areas of the Bothnian Bay. Reproduction success in amphipods includes various variables as e.g. fecundity, sex ratio, sexual development, embryo aberrations in terms of malformed and membrane damaged embryos, undifferentiated and dead eggs and broods, and stage of embryo development. In addition, amphipods were sampled for the in situ assessment of lysosomal membrane stability (LMS) as an indicator of toxic damage in the parental generation. These studies were combined with chemical analysis (PCBs, PAHs, and metals) of sediment and organisms to gain information about potential cause-effect relationships. The integrated analysis of the data showed that the nature of assessed correlations between contaminant concentrations in sediment, contaminant concentration in amphipods, parental damage, and reproduction success differ between the contaminants. For PCBs, high sediment concentrations correspond with low concentrations in amphipods and vice versa. The concentration in amphipods is negatively correlated with lysosomal membrane stability and embryo malformation. High membrane stability and low PCB concentrations in the parental generation corresponds with high fecundity and vice versa. Based on these results, a conceptual model on the transfer of PCB during embryogenesis has been developed. It is hypothesized that PCBs are taken up by the amphipods according to the respective bioavailability. During embryogenesis, PCBs are transported to the gonads together with nutrients and yolk. Thus, PCB concentrations in the parental generations are low and membrane stability and fecundity high. The PCBs act toxic on the developing embryo instead. In case of high PCB concentration in the parental generation, fecundity is reduced and embryo malformations low. These results indicate an adaptation to high PCB concentrations possibly mediated by a limited transport of PCBs during embryogenesis.

TU 356

The enzymatic functional stability of zinc-tolerant microbial communities: a new approach to detect terrestrial ecotoxicity of metals

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The soil microbial community exposed to metals over a long period can acclimate or adapt their biogeochemical functions and become metal-tolerant. This tolerance could increase the vulnerability of the microbial community to additional soil disturbances and then decrease the soil microbial functional stability. The functional stability is defined as the ecosystem capacity to recover its biogeochemical functions after a disturbance. This concept is more and more studied because this approach could detect ecotoxic effect which would have not been found out by common microbial bioindicators. A method which assesses soil health by analyzing the functional stability of soil enzymes has been set during the last years and has developed a stability indicator named the Relative Soil Stability Index (RSSI). These indexes were calculated to assess the enzymatic functional stability of 9 zinc-contaminated field soils after a heat disturbance (desiccation at 60°C for 24h). Four enzymes were analyzed: arylsulfatase, acid phosphatase, protease and urease. The RSSI of arylsulfatase and protease were linearly correlated to the labile zinc concentration in the soils (arylsulfatase : R²= 0,65 for n=7 and protease : R²=0,62 for n=6, $\alpha < 0,05$), while the enzymatic activity of these enzymes was not correlated to the labile metal fraction. These results suggest that the sulfur and nitrogen cycles would be unstable in zinc contaminated field soils. This potential ecotoxic effect would not have been detected if only the enzymatic activity has been analyzed.

Does genetic adaptation matters? An hypothesis tested using life-history consequences of adaptation and acclimatization to copper of *Daphnia longispina*C.B. Barata¹, A.R. Agra², A.M.V.M. Soares³¹IDAEA-CSIC, Barcelona, Spain²Universidade de Aveiro, Aveiro, Portugal³University of Aveiro & CESAM, Aveiro, Portugal

The present study was conducted to test the hypothesis that genetically adapted clones of *Daphnia longispina* showed a higher fitness performance under copper exposure than acclimated clones. Genetically tolerant and sensitive clones were selected from a reference and a historically exposed populations to an acid mine drainage from an abandoned pyrite mine. Four sensitive and resistant clones from reference and impacted populations were exposed to a range of copper exposure levels and their life history responses in terms of survival, reproduction and population growth rates compared. In another experiment the most sensitive clone was exposed during four generations to the same range of copper concentrations and its life-history performance compared. The genetic differences between the resistant and sensitive clones were that in the polluted environment individuals of the resistant clone survived 50 % better, reproduced three days earlier, produced three more offspring per day and had population growth rates 45 % greater. In the unpolluted environment, however, individuals from the resistant clone had the lowest reproduction rates. After four generations acclimated individuals from the sensitive clone reproduce earlier, had greater clutches at first reproduction and higher population growth rates than none acclimated ones but apparently there were no fitness costs. Therefore, our results showed that life history changes due to adaptation and acclimatization were comparable under the polluted environment but not under a clean environment. This results support the view that genetic adaptation is ecologically costly but acclimatization not.

Evaluating effects of pollution on *Caenorhabditis elegans*' population dynamic through a bio-energetic approachB. Goussen¹, A. Péry², R. Beaudouin², M. Dutilleul³, C. Lecomte³, J.M. Bonzom³¹IRSN / INERIS, Saint-paul-lez-durance cedex, France²INERIS, Verneuil en halatte, France³IRSN, Saint paul lez durances, France

The assessment of toxic effects at biologically and ecologically relevant scales is an important issue in ecosystem protection. Mathematical models exist to predict effects of pollutant on population dynamics from individually data. Nevertheless there are only a few datasets and models that account for adaptive phenomena which may appear in a stressed population. The selection pressure exerted by a pollutant is known to amplify the phenomenon of natural selection. It is thus essential to understand and quantify the adaptive dynamics governing populations under stress in order to assess ecological risk.

Regarding this background, we adapted a bioenergetic model to study adaptive phenomena in *Caenorhabditis elegans* population dynamic exposed to a heavy radiotoxic metal (uranium). The Dynamic Energy Budget (DEB) (Kooijman, 2010) bioenergetic approach highlights the distribution of energy fluxes between processes such as growth, reproduction, maturation and maintenance. It is a relevant basis to understand and model the links between assimilation disruptions, growth and reproduction fluctuations in organisms exposed to anthropogenic stress (e.g. pollutant, global change) and to assess potential consequences on population over many generations.

We therefore studied the responses of *C. elegans* exposed to six experimental concentration of uranium over several generations. The individual traits followed were growth curve, egg laying curve, survival until end of egg laying. We showed that uranium impacted *C. elegans* growth curve and egg laying over several generations, with, consequently, adverse effects on the population dynamic and variations on DEB parameters. Nevertheless, results also tend to show an evolutionary response throughout the generations.

ET11P - Marine environmental chemistry and ecotoxicology**Biotechnological potential of impacted scenarios for the restoration of TBT contaminated environments**R. Monteiro¹, L. Fonseca², A. Correia³, C. Ana⁴, F.L. Lemos⁵¹Polytechnic Institute of Leiria, Peniche, Portugal²Instituto Politécnico de Leiria, Peniche, Portugal³Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal⁴University of Aveiro, Department of Biology & CESAM, Aveiro, Portugal⁵ESTM and GIRM, Polytechnic Institute of Leiria, Peniche, Portugal

Tributyltin (TBT) is an organotin compound commonly used as an antifouling agent in marine paint formulations. Due to its wide industrial application and its consequent discharge into the environment, TBT pollution is recognized as major environmental problem at a global scale, being recently considered to be the most toxic substance ever introduced into the marine environment. Microorganisms from historically contaminated sites are able to tolerate pollutants and even degrade them, which may be a key factor in the restoration of contaminated environments. Nevertheless, byproducts resulting from the degradation process might be more or less toxic than the parent compound to ecological relevant species. The determination of the substance presence by analytical chemistry, although essential, may not present ecological relevance, as it might not be related to its ecotoxicity.

In this study, TBT-resistant bacteria collected from 7 Portuguese ports (Póvoa de Varzim, Leixões, Aveiro, Figueira da Foz, Peniche, Setúbal and Sines) were isolated in increasing concentrations of the toxicant (0.1, 1, and 3mM of TBT) and those growing at the highest concentration were characterized by genomic fingerprinting (REP-PCR) and tested as potential bioremediation tool in laboratory contaminated media.

The percentage of TBT-resistant isolates varied between 0.08% (Setúbal harbor) and 7.67% (Peniche). REP-PCR analysis revealed a total 111 distinct genetic profiles, being Peniche the location with lower variability while Figueira da Foz had the highest variability. Selected isolates were used to bioremediate waters contaminated waters, and their potential as bioremediation tools was assessed through ecotoxicological testing with the gastropod *Gibbula umbilicalis*. Ecotoxicological testing suggested that some TBT-resistant bacteria are able to reduce the toxicity of TBT contaminated waters. Ecotoxicological testing also revealed that the median lethal concentration (LC50) of TBT in *Gibbula umbilicalis* at 48 and 96 hours was 61.45 µg L⁻¹ and 15.69 µg L⁻¹, respectively.

This study contributed to the understanding of TBT resistance, however more intensive and focused research in the area of TBT bioremediation mediated by marine bacteria is still needed, particularly on the mechanisms behind TBT resistance and on the identification of pathways and genes responsible for TBT degradation.

Bacterial diversity on Portuguese ports: TBT degrading bacteriaR. Monteiro¹, L. Fonseca², I.S.M. Illyane¹, D.M.G. Campos³, A. Alves³, A. Cruz⁴, A. Correia³, A.M.V.M. Soares⁵, J.L.T. Pestana³, C. Ana⁶, F.L. Lemos⁴¹Polytechnic Institute of Leiria, Peniche, Portugal²Instituto Politécnico de Leiria, Peniche, Portugal³Department of Biology and CESAM, University of Aveiro, Aveiro, Portugal⁴ESTM and GIRM, Polytechnic Institute of Leiria, Peniche, Portugal⁵University of Aveiro & CESAM, Aveiro, Portugal⁶University of Aveiro, Department of Biology & CESAM, Aveiro, Portugal

Tributyltin (TBT) is an organotin compound commonly used as an antifouling agent in marine paint formulations. Due to its wide industrial application and discharge into the environment, and severe effects caused by it (e.g. imposex), TBT pollution is recognized as major environmental problem at a global scale.

Microorganisms from historically contaminated sites are due to tolerate pollutants and even degrade them, which can be key factor in the restoration of contaminated environments.

We have isolated 157 TBT-resistant isolates from Portuguese ports and harbours. Bacteria resistant to 3mM TBT were characterised by rep-PCR. Relationships between the PCR-generated patterns were examined by cluster analysis. Among the 157 isolates examined, 111 different banding patterns were identified. We have characterized these isolates in what concerns growth rate in the presence of increasing concentrations of TBT and later identified them by 16S rRNA gene sequencing. Bacterial diversity was also assessed by DGGE.

We were able to detect TBT-resistant bacteria from all the sampling sites. It is possible to observe that with increasing concentrations of TBT, the concentration of isolates decreased and consequently the percentage of resistant bacteria compared to the control was lower. At 0.1mM, Póvoa de Varzim had the highest percentage of resistants' (45.5%) while Setúbal had the lowest (2.56%). Regarding 1mM and 3mM concentrations, Peniche had the highest percentage rate (8.2% and 7.7% respectively) as well the highest number of colony forming units per milliliter values (410 ± 26 cfu mL⁻¹ and 383 ± 91 cfu mL⁻¹ respectively). On the other hand Aveiro (0.26 ± 0.09%) and Setúbal (0.08 ± 0.03%) had the lowest percentage of resistants' for 1mM and 3mM, respectively. Aveiro had the lowest isolates concentration in the control, while Peniche had the highest. Statistically significant differences were observed for all locations between selective mediums and control groups.

This study contributed to the understanding of TBT resistance, namely to get insides into the constitution of TBT-resistant communities in port and harbour waters.

Are TBT sources migrating from harbors to marinas in Latin America?G. Fillmann¹, C.A. Paz-Villarraga², P. Miloslavich², M.A. Fernandez², I.B. Castro¹¹FURG, Rio grande, Brazil²Universidad Simon Bolivar, Caracas, Venezuela³Universidade Estadual do Rio de Janeiro, Rio de Janeiro, Brazil

Tributyltin (TBT) is a very toxic compound that was used as antifouling paints biocide during four decades. Due its environmental toxicity the International Maritime Organization banned TBT based antifouling paints in September 2008. Thereafter, TBT environmental concentrations as well as imposex levels (the most widely used TBT biomarker) declined in several coastal areas worldwide. However, in Latin America some recent observations have shown high TBT and imposex levels in areas close to marinas, despite the general reducing pattern in many areas. The present study reported imposex parameters (imposex %, VDSI and RPLI) in gastropods from Venezuela and Brazil in harbor areas and marinas. In Venezuela, samples with 30 adults of *Purpura patula* were collected in 23 sites (2 located inside marinas). In Brazil, the sampling

collection has been performed for some years at Angra dos Reis (10 out of 33 stations were close to marinas) and Arraial do Cabo (2 out of 10 stations were close to marinas) in Rio de Janeiro state. These studies used 30 adults of *Stramonita haemastoma* per site (whenever found). The results for Venezuela showed that the VDSI ranged from 0.22 to 1.86 in areas under the influence of harbors, whereas VDSI values were 3.77 and 4.56 in the marinas. Similarly, RPLI ranged from 0.07 to 5.58 in the harbor areas and were 49.36 and 54.56 to the marinas. In this case, the main source of organotin pollution is clearly the marinas. In the Angra dos Reis study, imposex was detected in 100% of females close to the marinas, and in 5 out of 10 stations the animals previously inhabiting the rocky shores were no longer found. In Arraial do Cabo, two stations nearby a single marina showed an imposex incidence of 4% and 0 in 2001, increasing to 83% and 47% in 2008. In this region, another marina located nearby a small harbor showed imposex incidence of 100% in three stations in 2001, whereas no gastropods were found during 2008 sampling. Thus, although the international restrictions on TBT use are apparently reducing the impacts from harbors, these three independent studies at Venezuela and Brazil (Angra dos Reis and Arraial do Cabo) clearly pointed out that marinas are becoming an important source of TBT pollution. Based on that, a similar pattern is expected to occur in other Latin America coastal areas.

TU 364

Imposex and organotin (OT) levels in *Nucella lapillus* along the Portuguese coast: a re-survey in 2011

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The use of tributyltin-based antifouling paints (TBT-based AFP) is globally forbidden since the 17th September 2008 by the 'International Convention on the Control of Harmful Antifouling Systems on Ships' (AFS Convention). It is therefore important to ensure the continuous monitoring of organotins (OT) in the environment and the recovery of marine ecosystems to evaluate the effectiveness of legislation in reducing TBT pollution. The current work assessed imposex and OT tissue contamination in *Nucella lapillus* at sixteen sites along the Portuguese coast in 2011. The vas deferens sequence index (VDSI), the female penis length index (FPLI), the relative penis size index (RPSI), the percentage of imposex affected females (%I) and the percentage of sterile females (%S) were determined to assess imposex levels. Additionally, monobutyltin (MBT), dibutyltin (DBT), TBT, diphenyltin (DPT), triphenyltin (TPT), monooctyltin (MOT) and dioctyltin (DOT) were quantified in the soft tissues of whole females by GC-MS. The OSPAR Ecological Quality Objective (EcoQO) set for this species (VDSI < 2) was achieved in 94% of the sampled sites. All surveyed populations presented VDSI values that fall into OSPAR class B (0.3 < VDSI < 2) with one single exception in the South western coast - Zambujeira do Mar (site 15) - also being the only site where sterile females were still recorded. OT concentrations in *N. lapillus* soft tissues varied between <0.2ng Sn/g and 24ng Sn/g dry weight (dw) for MBT, 2.0 and 26ng Sn/g dw for DBT, 2.3 and 30 ng Sn/g dw for TBT and <0.2 ng Sn/g dw for TPT, DPT, MOT and DOT concentrations were all below the detection limit. TBT recent inputs were estimated to occur at four sites by the butyltin degradation index (DBI = ([MBT] + [DBT]) / [TBT]). The temporal trend of *N. lapillus* imposex was analysed for the period 2000-2011 in order to assess the evolution of TBT pollution levels in the Portuguese coast.

TU 365

TBT from anti-fouling paints in the environment - an overview of the situation today

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The Baltic Sea is a sensitive brackish water environment where most organisms live at the edge of their distribution area. Pollutants thus mean an extra stress to these organisms. Traditionally anti-fouling paints are based on leakage of biocides, which prevents the attachments of fouling organisms on underwater structures such as a boat hull. The draw-back is that these biocides are persistent and that they also affect non-target organisms. The environmental authorities have with time implemented more strict regulations, especially for the paints that may be used on the east coast of Sweden. The use of tin-organic substances were prohibited in 1989 for all boats less than 25 m and no paints based on copper leakage were approved from 2001 and up today no copper paints have been on the market for use on the east coast. In spite of these restrictions much toxic substances originating from anti-fouling paints are found in the sediment in both natural harbours and in marinas along both the west coast and the east coast of Sweden. Similarly high concentrations are found in harbours for ships. Our investigations show that the highest concentrations are found in boat yards > uptake areas > marinas > natural harbours. 100 µg TBT/kg DW is considered high in many countries and the highest concentrations in boat yards reached 50 000 µg TBT/kg DW. The reason is under laying paint layer being scraped off.

TU 366

Comparing anadara trapezia exposure, dose and response to metal contaminated estuarine sediments using laboratory and field exposures and resident molluscs

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Establishing relationships between metal exposure, internal dose and associated biological effects for organisms is necessary to understand the fate and effects of metals in the environment.

The accumulation and sequestration of biologically available metals by aquatic organisms, particularly bivalve molluscs, has led to their use as biomonitors of metal contamination, as it is this portion which is of interest in pollution effects assessments. Biomarker measurements can provide evidence that organisms have been exposed to contaminants at levels that exceed their detoxification and repair capacity, thus establishing the link between contaminant exposure and ecologically relevant effects. Laboratory exposures, in which physiochemical factors can be controlled, using previously unexposed organisms are routinely used in the development of toxicological effects tests. Metal accumulation is influenced by metal bioavailability via water, sediment and dietary pathways. These pathways may be influenced by mode of exposure and the history of previous exposure which in turn may affect biological responses.

An approach for establishing the relationships between tissue metal dose and biological response of exposure to copper, zinc, cadmium, lead, and selenium contaminated sediment from Lake Macquarie, NSW, Australia, was evaluated for the sediment dwelling bivalve mollusc *Anadara trapezia*. A comparison of tissue metal accumulation and effects was made between resident organisms, and previously unexposed organisms in laboratory and in situ caged exposures. Organism dose was measured by total metal tissue burden. Subcellular fractionation of whole tissue was used to determine what fraction of the total accumulated tissue metal was in a metabolically available form. Measurements of oxidative stress (total antioxidant scavenging capacity of cells and lipid peroxidation) were used as effects biomarkers of metal exposure. Lysosomal membrane stability was used as a measure of cellular integrity. By identifying relationships between exposure and effects at various levels of biological organisation, using a variety of exposure approaches, a better understanding of the mechanisms of organism stress responses to metals in ecological systems was gained and the predictive capability of ecological risk assessment improved.

TU 367

Imposex occurrence and the genetic changes in Veined Rapa Whelk (*Rapana Venosa*) from Bohai Bay, China

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In order to assess the present status of organotins (OTs) pollution and the potential ecological risk, biological effects in biota and the contents of OTs in biota and sediments were investigated in Bohai bay, north of China. Imposex occurrence was found in veined rapa whelk (*Rapana venosa*) from three sites in Bohai bay (HH, NPH and DST), ranging from 0% to 12.45% in 2010 and from 0% to 12.50% in 2011, respectively. The chemical analysis results showed that TBT and TPT were the main OTs in tissues and sediments, which can account for the occurrence of imposex. Furthermore, the genomic DNA that was amplified with 8 pairs of fluorescence-labeled primer showed varied genetic distances and Nei's genetic identity among the veined rapa whelk from the 3 sites. The present study demonstrated that the biota was contaminated by OTs in the Bohai bay, and this imposed a potential risk to the veined rapa whelk.

TU 368

Clam valve rhythm-based online biomonitoring system to detect waterborne arsenic

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Arsenic (As) usually represents high level in groundwater at southwestern and northeaster coastal areas of Taiwan. The freshwater clam has a high market value and commercially important to Taiwan's aquaculture. Previous studies indicate that biomonitoring is the scientific technique for detecting environmental pollution situations for presenting the harmful of toxic chemical in water. The purpose of this study was to synthesize water chemistry-based bioavailability and valve daily rhythm in *Corbicula fluminea* to design a biomonitoring system for detecting waterborne As. We integrated valve daily rhythm dynamic pattern and water chemistry-based Hill dose-response model to build in a valvometry technique programmatic mechanism, offering a rapid and cost-effective dynamic detection system. We validated the simulated dissolved As concentrations based on valve daily rhythm behavior with published experiment data. The results indicated that the As concentration detection threshold of biomonitoring system should be less than 1 mg L⁻¹ and the detection times are associated with the exposure concentrations. This study presents a *C. fluminea*-based biomonitoring system that can particularly provide the real time transmitted information on the waterborne As activity. This parsimonious *C. fluminea* valve rhythm behavior-based real-time biomonitoring system presents a valuable effort to promote the automated biomonitoring and offers early warnings on the potential ecotoxicological risks in regions with elevated As exposure.

TU 369

A comparative in situ study on ecotoxicological effects of pharmaceuticals in Ireland, using marine mussels (*Mytilus* spp.)

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In recent decades the amount of pharmaceuticals used and released has constantly increased. One of the main sources of this pollution is through the release of wastewater

effluent, both treated and untreated, into the aquatic environment. These novel contaminants can be found now through the developed world, including Ireland. In our field study, we collected blue mussels (*Mytilus* spp.) from a pristine site in the west of Ireland (Lettermullan, Co. Galway). They were caged and exposed at three sites around Ireland for three months in 2010. Cages at site one were based above the wastewater diffuser pipe of Mutton Island wastewater treatment plant (WWTP), a secondary treatment facility serving Galway city with a population equivalent (p.e.) of 73,000. This WWTP treats both municipal and industrial waste. The second location was at the North Bank Lighthouse in Dublin Bay. This lighthouse is based 700m downstream of the Ringsend WWTP, which is a tertiary treatment facility. Wastewater from homes, industries, commercial premises as well as rainfall water is treated in Ringsend, which serves a p.e. of 1,7 million. Omev Island, Co. Galway was the control site for this study. It is a remote island on the Galway coast, with little anthropogenic input. The protein expression signature and other general stress responses were determined by measuring changes in a suite of biomarkers comprising glutathione S-transferase, lipid peroxidation, DNA damage and vitellin-like proteins. Our results contribute to the understanding of whether chronic exposure of pharmaceuticals causes a real threat to aquatic organisms, their habitat and, ultimately, to humans health.

TU 370

Chronic exposure of young spat of the Pacific oyster (*Crassostrea gigas*) to zinc

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The marine coastal environment is constantly submitted to many sources of releases from human origins, resulting in low concentrations of contaminants and most coastal marine species are exposed to these low doses during their entire life. Because biological mechanisms to respond to acute and chronic exposure may be different, the concentration sensitivity is also different. It is thus a challenge that stake holders in charge with the environment protection regulation can lean on robust scientific basis addressing the consequences of chronic exposure of species to low doses of pollutants. For long life-span species, investigating chronic exposure means long term experiments with many potential technical biases. A relevant strategy is to use early stages of development. Even if the exposure time is limited compared to the life-span of the organism, if exposure starts at the very beginning, it covers the whole lifetime up to the end of the experiment, which is relevant in terms of chronicity. In addition, early stages of development are often more sensitive to pollution than adults, so using them for ecotoxicology obviously makes sense. Zinc is an essential trace metal in living organisms but it becomes toxic at high concentration. In the coastal marine environment, its concentration is influenced by many human activities, among which the use of antifouling paint and sacrificial anodes. Our study addressed the effects of zinc on Pacific oyster (*Crassostrea gigas*) spat. Young oysters were exposed to a range of zinc concentrations, added to seawater as ZnCl₂ up to 2 mg.L⁻¹, as of 1 day post-metamorphosis and for 10 weeks. They were fed with micro algae at the same cells concentrations. Zinc was measured in seawater and in oysters throughout the experiment. Survival and growth were monitored on large sample populations to deal with individual variability. A dose-response curve analysis allowed deriving the EC₅₀ for growth. In parallel, target genes analysis included heat shock proteins, metallothionein (MT), detoxication machinery, regulation of oxidative stress and cell cycle. Gene transcriptional expression was quantified by RT real time PCR. Significant change was only detected for MT after 1 week exposure to the highest zinc concentration. However growth was affected as early as a few days of exposure to relatively low dose and turned out to be an earlier and more sensitive marker of the effect of zinc on oyster spat.

TU 371

Assessment of sacrificial anode impact by zinc accumulation in oyster *Crassostrea gigas*: comparison between a long- and short-term laboratory tests

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In marine environments, sacrificial anodes made of zinc are currently used to mitigate marine corrosion as part of cathodic protection systems of immersed metallic structures. Zinc is an essential metal for all living organisms that can be potentially toxic when present in excess. The aim of this study was to assess the bioaccumulation and the effects of zinc released using an electrochemical device providing controlled zinc concentrations from sacrificial anode degradation to mimic the in situ conditions. The work was carried out on oyster *Crassostrea gigas* by performing two in vivo tests. The first test was conducted over a period of 10 weeks at a concentration of 0.53 mg Zn L⁻¹ to simulate long-term exposure, and a second one lasted for 168-hours at a concentration of 10.2 mg Zn L⁻¹ to reproduce short-term exposure. During exposures, zinc concentrations were measured in oyster gills, digestive gland and soft tissues and, endpoints such as mortality, immune parameters and mRNA expression of genes associated with oxidative stress and cell detoxification were analysed.

At the end of the 168-hour exposure experiment, 81.8% mortality rate was recorded, and the bioconcentration factor (BCF) exhibited values up to 405 in the digestive gland. In contrast, the results obtained though chronic exposure showed a higher BCF (13,397), whereas no mortality was detected. Moreover, zinc was able to modulate immune system activities. Indeed, all of the immune system biomarkers studied, except the number of circulating haemocytes which decreased in both experiments, were stimulated by a moderate level of zinc and inhibited by a high level. Our exposure conditions did not induce SOD or MXR mRNA expression. However, a dose- and time-dependent increase of MT mRNA correlated with Zn accumulation in gills and digestive gland of exposed oysters was observed. These results demonstrate the great capacity of *Crassostrea gigas* to accumulate zinc released from anode, especially when low concentrations are released, as in the case of anode dissolution used as cathodic protection. In addition oyster physiological endpoints (e.g. immune parameters, stress biomarkers) are sensitive to acute Zn toxicity but are only moderately affected by a mild Zn concentration. This study confirmed the necessity to monitor this zinc contamination source in marine environments.

TU 372

Effect of herbicides on embryo-larval development and metamorphosis in the Pacific oyster, *Crassostrea gigas*

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The summer mortality syndrome which sporadically threatens oyster farming appears to be due to a combination of several intrinsic and extrinsic factors including physiological stress related to reproduction and xenobiotic stress. In this context, terrestrial inputs including pesticides could be involved in oyster mortality events, not as a single causative agent but as additional stressors. The study was carried out in the frame of the European Projet Chronexpo. The aim of this project was to evaluate the impact of different contaminants (including pesticides) used in the region surrounding the English Channel on the early stages of *Crassostrea gigas*. Experiments were conducted on glyphosate, its main metabolite (AMPA), two commercial formulations (Roundup Express[®] and Roundup Allées et Terrasses[®]) and also adjuvant (POEA: Polyethoxylated tallowamine) alone. Mecoprop, Mecoprop-P and their main metabolite (2-methyl-4-chloro phenol; 2M4C) were also tested.

The effects of these substances and copper sulphate (used as a positive control) were studied in D-hinged larvae in order to assess their potential embryotoxicity by considering both the rate of abnormal larvae (countings) and the kind of abnormalities (multi-variated analyses). The effects were also researched in pediveliger larvae ready to metamorphosis (calculations of metamorphosis rates). For metamorphosis test, we optimized Coon & Bonar's protocol because of the difficulty to count moving larvae. For each endpoint (embryo-larval development and metamorphosis rates), results of 4 experiments allowed us to determine the EC50 values.

As regards embryotoxicity, the 2 kinds of Roundup appeared about 10-fold more toxic than glyphosate and AMPA and the toxicity of 2M4C reached 3-5-fold those of active substances. In the metamorphosis assay, a similar result was recorded and, glyphosate, AMPA and mecoprop appeared little toxic (EC50 >100 mg/L). Finally, the comparison between the 2 larval stages generally showed a greater sensitivity of D larvae (embryotoxicity) compared to pediveliger larvae. For most of the molecules, results showed that embryotoxicity assay is more sensitive than the classic standardized acute toxicity *Daphnia magna* test. This work is the first part of a study which will be continued by long time exposures and will allow us to provide guidance on pesticide regulation in the frame of the European Directive "Marine Strategy"(2008/56/CE).

TU 373

Ecotoxicity of nitramines, important transformation products of amines used in carbon capture

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Due to the imminent use of amines in the CO₂ capturing process from gas power stations in Norway, it is anticipated that the inputs of amines and transformation products into the environment will increase. The two main groups of transformation products with the most potential to cause environmental harm have been identified as nitrosamines and nitramines, both of which are considered to be carcinogenic. Recent theoretical modelling as well as laboratory experiments have found nitramine compounds, 2-(nitroamine) ethanol (CAS: 74386-82-6) and dimethylnitramine (CAS: 4164-28-7) to be present. However, despite the likelihood of these compounds increasing in the environment, no environmental toxicity data for these compounds currently exists. Therefore, the aim of this work was to provide an environmental risk assessment for the selected nitramine compounds taking into account the key trophic groups within freshwater, marine and terrestrial environments. The toxicity assessment was made using a suite of standardised bioassays for the measure of acute and chronic toxicity, which found the EC/LC50 concentration of the selected nitramine concentrations to be in the low to mid mg/L range for all aquatic toxicity tests. In addition, the sub-lethal effects of these compounds was also investigated, using a 3 tiered approach to assess the potential mutagenic and carcinogenic effects of these compounds. Overall, data to develop the environmental risk assessment for these future environmental pollutants will be presented.

TU 374

Effects of arsenic on physiological parameters of mussels (*Mytilus edulis*) from the Scheldt Estuary (Belgium)

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Mussels are marine organisms widely used as environmental biomonitors, due to the ability to concentrate pollutants in their tissues with minimal metabolic transformations.

Arsenic is widespread in the marine environment and occurs in several chemical forms, principally as arsenate (As V), and to a lesser extent as arsenite (As III) a more toxic form of arsenic. Arsenic introduced into the environment often contaminates aquatic ecosystems, where the residence time has been estimated at about 50 years. This study aimed to determine whether exposure to specific heavy metal arsenic has an effect on the physiology of mussels. The effect of arsenic in mussels were analyzed in an experiment with 150 mussels divided in 7 different groups and exposed to 6 different concentrations of As(III) and one control for 10 days, during this period several physiological parameters were analyzed (Scope for Growth, energy reserves, body condition index, Mts and arsenic accumulated by the mussels). Arsenite does not seem to affect the physiological parameters of *Mytilus edulis*, but the organisms can accumulate As (III) proportionally to the concentrations that it is exposed, especially at high concentrations. Several authors were not able to find this relation, but this is due to the low concentrations used in their experiments. The present study has shown that the arsenic accumulated in the body of mussels did not have adverse effects to the mussels, at least in a short period of time and for those physiological parameters measured, but after 10 days of exposition some parameters (lipids and SFG) have positive correlation with arsenic in sea water, suggesting an effect of arsenite. Long-term experiments should be performed to really understand these effects.

TU 375

Changes in the biomarker responses of the razor clam *Solen marginatus* as an indicator of varying contamination exposure in two Portuguese coastal lagoons: a case study

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Coastal lagoons are highly productive marine environments that provide a wide range of ecosystem services. Nevertheless, these habitats are particularly vulnerable to direct and indirect sources of pollution, which leads to environmental degradation and ecosystem impairment.

The aim of this study was to monitor the health status of two natural populations of *Solen marginatus* from Ria de Aveiro and Ria Formosa, two Portuguese coastal lagoons located in Aveiro and Algarve, respectively. In order to achieve this, individuals from both places were sampled, in two successive years, and an additional group of individuals was sampled from a reference site (São Jacinto, Aveiro). For each sampled population and sampling time, lipid peroxidation and the activity of glutathione-S-transferase, catalase and cholinesterase were determined. In addition, total mercury burden in whole organisms was also monitored as mercury is one of the major contaminants that can be found in these coastal lagoons. Significant differences were observed between the two lagoons regarding the mercury burdens accumulated and the enzymatic activities measured, suggesting that the two populations are exposed to different environmental quality and contamination levels.

TU 376

Marine ecosystem health assessment through the Integrative Biological Index (IBI) in mussels from Portugal NW and Basque coasts

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In order to assess the biological effects of pollution, mussels *Mytilus galloprovincialis*, were seasonally sampled in seven localities subjected to different levels of pollution along the North Iberian Peninsula Coast (NIPC) over one year (April, June, October 2010 and in February 2011); 4 in the NW Coast of Portugal (Vila Chã, São Bartolomeu do Mar, Viana do Castelo and Carreço) and 3 in the Basque Coast (Arriluze, Gortiz, Mundaka). In each locality, a battery of biochemical and cell and tissue-level biomarkers were applied: acetylcholinesterase (AChE) and glutathione S-transferases (GST) enzymatic activity, levels of lipid peroxidation (LPO), lysosomal enlargement (lysosomal volume density, Vv), lysosomal membrane stability (labilisation period, LP), cell type replacement (volume density of basophilic cells, VvBAS) in digestive gland epithelium, and changes in the morphology of digestive alveoli (mean luminal radius to mean epithelial thickness MLR/MET). These biomarkers were integrated in the *Integrative Biomarker Index* (IBI), which was developed within the framework of the Prestige oil spill (POS). The IBI index revealed that mussels' health was affected in chronically polluted localities. Star plots accompanying IBI provided complementary information concerning mechanisms of biological response to environmental insult. The NIPC is a high risk area for marine spills due to intense marine traffic. This is an important integrative field study to understand the present environment health status and to determine the levels of general stress on core localities which provide reliable assessment of future impacts of pollutant. This study was supported by the Government of the Basque Country through a post-doc grant to L. Garmendia (ref. BFI09.244) and K-EGOKITZEN project and by the Portuguese Foundation for the Science and Technology and FEDER funds through the project RAMOCS (ERA-AMPERA/0001/2007; EU AMPERA ERA-NET, ERAC-CT2005-016165).

TU 377

Metal exposure and associated effects in mussels: an integrated approach - hyphenated analytical techniques and biomarkers

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Aim of the study was the assessment of metal exposure in coastal areas under indirect or direct influence of maritime activities and nautical tourism (port and marinas) using integrated biological-chemical analytical approach and mussels (*Mytilus galloprovincialis*) as bioindicator species.

Next to the natural sources, a large number of metals are present in the environment as a result of human activities. Since the ban of organo-tin compounds, Cu is the main component of anti-fouling paints, with the result that boat traffic is a major anthropogenic source of Cu in the coastal aquatic environment. Besides, zinc oxide is generally used in combination with Cu(I) as a booster. As a result of the leaching of chemicals from the painted surface, concentrations of metals are significantly elevated in semi-enclosed marine systems, such as harbours, marinas and estuaries, where the transport, berthing or docking of vessels is extensive.

In this study we applied new methodological approach - investigation of metal distribution in the cytosolic fractions of proteins separated by size exclusion chromatography (SEC-HPLC) and detected by inductively coupled plasma mass spectrometry (ICP-MS). Additionally, the cellular energy allocation (CEA) methodology was applied as a general biomarker of physiological status of mussels. Supplementary, a non-target bioanalytical approach utilizing 2D gel-electrophoresis will give hints for further potential protein biomarker. Thus, this study represents a contemporary and new approach in quantification and assessment of the environmental risk caused by metal exposure, as well as the potential toxic effect of metals on mussels.

The study was performed on indigenous mussel populations sampled in the middle part of the eastern coast of the Adriatic Sea at the locations of two ports, two marinas and a reference (unpolluted) site. The results obtained from the sampling in March 2011 will be presented.

This study was performed within the scope of the DAAD project (Project-ID 50752021 Deutscher Akademischer Austausch Dienst) during which scientific collaboration between the research groups from Croatia and Germany was established.

TU 378

An improved on-line clam-based behavioral monitoring system allows an approach to estimate dose-response profiles of clams exposed to waterborne copper

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The purpose of this study was to develop an improved on-line clam-based behavioral monitoring system. The system included a valvometry apparatus and three kinds of valve closure analytic programs that can offer a real-time and cost-effective method to construct an approach for estimating dose-response profiles of clams (*Corbicula fluminea*) exposed to waterborne copper (Cu). The valvometric technique can reduce the environmental stress of observed bivalves to promote a measuring precision in the spontaneous status of recording daily valve movements. The clam-based on-line behavioral response monitoring system was used to respectively monitor the valve closure responses of 16 *C. fluminea* exposed to unpolluted environment and various Cu concentrations. In this study, a probabilistic-based approach describing the valve behavioral response of *C. fluminea* exposed to unpolluted environment and Cu was developed. The magnitudes of shell gape of 20 and 50% were respectively adopted as the determining thresholds of the valve closing (VC) and siphon extension (SE) status to digitalize the valve movements in bivalves. The observed data of valve closure response was analyzed to construct the time-varying dose-response profiles (RVC and RSW) based on an empirical three-parameter Hill model. It also allowed the estimation of the integration time-specific EC50^{VC} and EC50^{SW} values as a bioassay approach. The daily valve opening and closing rhythm were characterized by a three-parameter lognormal function. The time-specific EC50^{VC}s and EC50^{SW}s were accordingly estimated to be 70.56, 48.78, 35.85, 19.8, 6.54, 4.97 and 73.2, 43.69, 19.52, 8.77, 6.58, 5.61 µg L⁻¹, respectively, at integration times of 10, 15, 30, 60, 120 and 300 minutes. The results revealed that the RSW-based bivalve behavioral observation has a better sensitive response for detecting a lower waterborne Cu concentration than that of RVC within one hour. The results also demonstrated that the response sensitivity of clams exposed to Cu depended on the initial valve opening and closing state of *C. fluminea* after Cu occasional addition, indicating that clams in response to different Cu exposure concentrations resulted in various valve closing rates. In the future, an estimation of dose-response profiles of clams exposed to waterborne contaminant should take into account the related response characteristics of valve activities to promote the predictive capabilities of this bioassay approach.

TU 379

Glutathione S-Transferase, glutathione peroxidase and acetylcholinesterase activities in mussels transplanted to harbour areas

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As part of an integrative monitoring campaign involving water and sediment chemistry, in situ bioassays, and mussel bioaccumulation and biomarkers, *Mytilus galloprovincialis* mussels of standard size were transplanted from a clean location to five sites in two important harbours from the Atlantic coast of Spain (Vigo and Pasaia) at the 2008 year. After a 30-day field exposure, the enzymatic activity levels of glutathione S-transferase (GST), glutathione peroxidase (GPx) and acetylcholinesterase (AChE) were quantified in the gills of transplanted mussels (n=12). GST activity is induced by organic pollutants, GPx activity is induced as a response to oxidative stress, and AChE activity is specifically inhibited by organic pesticides such as organophosphates and carbamates. The concentrations of major contaminants (trace metals, polychlorinated biphenyls and polycyclic aromatic hydrocarbons) accumulated in the mussels tissues were also measured. Mussels from the most polluted sites consistently exhibited a significantly higher GST activity compared to the control site (p<0.05); whereas AChE activity was significantly inhibited. Both biomarkers showed the same pattern of GST induction and AChE inhibition during the two sampling years. The GPx activity was occasionally induced, but the pattern of response was less consistent than for the other two biomarkers. The most intense biological responses were recorded in the inner parts of the estuaries, where the most polluted areas were located. The above results suggest that GST and AChE activities can be used as potential biomarkers of toxicity for active monitoring in marine coastal ecosystems.

TU 381

Impact of suspended particles on bioavailability of petrogenic PAH in cod (*Gadus morhua*), mussels (*Mytilus edulis*) and passive samplers exposed to produced water

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Caged organisms and passive samplers are routinely used to monitor environmental impact of operational discharges from offshore oil and gas production. Comparison of exposure situations over time may be required. Sorption of Produced Water related hydrocarbons to particles in the sea water may influence the bioavailability of these compounds. Temporal variations of microalgae density may therefore affect exposure measurements in caged organisms and passive samplers. In order to investigate the consequence of differences in biological particle density on bioavailability of petrogenic polycyclic aromatic hydrocarbons (PAHs), a laboratory study was conducted in blue mussel (*Mytilus edulis*), Atlantic cod (*Gadus morhua*) and passive samplers (SPMDs) exposed to PW. Organisms and SPMDs were exposed for 17 days to diluted PW in a continuous flow-through system. The dilution was set at 0.1%, from day 6 until day 10 the flow was set to 0.2% to mimic real field conditions with varying PW concentration over time. The exposure set up included: a negative control with sea water only, a positive control with diluted PW only and three tanks containing diluted PW and algae mix at low, medium and high concentrations. The following parameters were analysed: PAHs in water, PAH concentrations in SPMD and mussel soft tissues, and PAH metabolites in fish. Due to the important role of gills for absorption of waterborne PAHs, a histopathological investigation of fish gills was conducted as a support parameter. The presence of realistic densities of organic particles had only minor impact on the bioavailability of low molecular weight PAHs in fish, mussels and passive samplers. Bioavailability of 3-ring PAHs was only significantly reduced in mussels when algae particle density was high (about 50000 cells/ml).

TU 382

Estimation of experimental conditions to maximize mussels shell capability in trace metals accumulation

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The evaluation of mussels shell capability of concentrate heavy metals was performed by means of a set of laboratory experiments conducted with mussels collected in unpolluted sites exposed to sub-lethal concentrations of a known toxic metal. Multiple cycles of accumulation and depuration were carried out all along the experimental period; the constant monitoring of experimental conditions and periodical metal determination in shell and tissue samples allowed the individuation of the accumulation and depuration patterns in both Bivalves materials in the selected species. Alternative exposure to contaminated and clean seawater respectively was performed in order to record the different metal concentration trends in shell and soft tissues. In fact it is demonstrated that once the metal is incorporated in the crystalline calcium carbonate lattice, his loss from the shell during mussels lifetime can be considered of no concern; in the other hand it is known that soft tissues are able to release contaminants in response to changed environmental conditions. The aim was to establish a set of experimental conditions able to optimize metal accumulation in mussel hard parts and to calculate the specific MATC (Maximum Acceptable Toxic Concentration) value. So it was possible to settle physical, chemical and physiological parameters in order to maximize the shell metal content and set the basis for implementation of bioinertization strategies exploiting mussels metabolic activity and detoxification mechanisms.

TU 383

The effect of sublethal nickel concentrations on *Mytilus galloprovincialis*: a multiple biomarker study

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Nickel is a known mammalian carcinogen and mutagen, typically found at low concentrations in the marine environment (<1 µg l⁻¹), but enriched at coastal locations near mining operations. This study used a multiple biomarker approach to investigate the toxicity of sublethal concentrations of nickel to *Mytilus galloprovincialis*, a key bioindicator organism. Mussels were exposed to sublethal concentrations of nickel (0-3600 µg l⁻¹) for 5 days. In addition to determining tissue-specific nickel accumulation, the biomarkers analysed were DNA strand breakage (comet assay; a biomarker of exposure), induction of micronuclei (a biomarker of effect), histopathology and expression of several key genes known to respond to oxidative or toxicological stress. Nickel accumulated in mussels at all concentrations, in the tissue-specific order byssus > digestive gland > gills > foot > adductor muscle > mantle. Significant genotoxic damage was observed by both the comet assay and micronucleus test, but only at 3600 µg l⁻¹ nickel. Correspondingly, semi-quantitative analysis of histopathology in the gills indicated an increase in structural damage at concentrations ≥ 1800 µg l⁻¹ Ni. Quantitative RT-PCR revealed overexpression of the glutathione-S-transferase (gst) and metallothionein (mt20) genes in gill tissue after exposure to 3600 µg l⁻¹ nickel, compared to the controls. Expression of these genes is known to be induced following xenobiotic exposure, especially to metals. The p-glycoprotein (pgp) gene, which has been implicated in multi-xenobiotic resistance (MXR), was markedly overexpressed in all nickel exposed mussel gills, with the highest rate of expression at 18 µg l⁻¹ Ni. These results suggest that nickel is genotoxic to mussels at 3600 µg l⁻¹ and that this genotoxicity correlates with responses at the molecular (i.e. biomarker of exposure) and tissue (i.e. biomarker of effect) levels. As a result, we recommend that effort is made to accurately quantify nickel concentrations in highly polluted waters near nickel mines (e.g. the New Caledonian lagoon), and that remedial action may be needed if values exceed 3600 µg l⁻¹.

TU 384

Biomarkers responses of the native clam *Scrobicularia plana* and changes of macrobenthic functional composition in a shallow tidal creek affected by fish aquaculture effluents

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The effects of solid organic wastes from a marine fish farm on sediments were tested using biomarkers in native clam (*Scrobicularia plana*) as biochemical indicators and macrobenthic community as ecological indicators. The clams and macrobenthic samples were collected in the intertidal sediment in October 2010 from five sites of the Rio San Pedro creek, following a gradient of contamination from the aquaculture effluent to the control site. Phase I and Phase II detoxification enzymatic activities (ethoxyresorufin O-deethylase (EROD), glutathione S-transferase (GST)), antioxidant enzymatic activities (glutathione peroxidase (GPX), glutathione reductase (GR)) and oxidative stress parameters (Lipid Peroxidation (LPO) and DNA strand breaks) were measured in clams' digestive gland tissues. Numbers of species, abundance, richness and Shannon diversity indexes were the biodiversity indicators measured in macrofauna. In parallel, redox potential, pH and organic matter in sediment, and dissolved oxygen in the interstitial water were measured in situ. Significant (p < 0.05) increases of GPX enzymatic activity, DNA damage and LPO, together with significant (p < 0.05) decrease of biodiversity indicators were observed in the areas close to the aquaculture effluent. Biomarkers (DNA, LPO and GPX) were significantly (p < 0.01) negative correlated with pH, redox potential and dissolved oxygen and positively correlated with organic matter. On the contrary, macrobenthic biodiversity were significantly (p < 0.01) positively correlated with pH, redox potential and dissolved oxygen and negatively correlated with organic matter. It has been demonstrated that effluents from fish aquaculture activities in Rio San Pedro creek may induce oxidative stress in soft-sediment species which may lead to alteration of the biodiversity and health status of the exposed organisms.

TU 385

Mechanisms of acute toxicity of metals in estuarine and marine invertebrates

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In freshwater invertebrates, acute metal toxicity is generally associated with the disruption of ionic and osmotic regulations. In contrast, the mechanisms involved in acute metal toxicity in estuarine and marine invertebrates are not well characterized. Therefore, in the present study the most recent information generated by our research group on the possible mechanisms involved in the acute toxicity of metals in these animals was reviewed. Data on the acute effects of metals (copper, silver, zinc, and nickel) on biochemical and physiological biomarkers in estuarine and marine invertebrates (foraminifers, sea urchins, sea anemones, sea cucumbers, copepods, isopods, shrimps, crabs, and clams) were considered. Biomarkers evaluated included those associated with ionic and osmotic regulation of body fluids (ion and osmotic concentration and activity of associated enzymes), respiration (oxygen consumption), cellular oxidative status (enzymatic and non-enzymatic agents from antioxidant defense system), and energy metabolism (activity of enzymes from glycolysis and Krebs' cycle). As observed in freshwater invertebrates, data available for the different groups of estuarine and marine invertebrates investigated indicate that ionic and osmotic imbalances can be observed when euryhaline animals are acutely exposed to metals in brackish waters, especially in very diluted salt water. However, no alterations have been observed when these animals are exposed to the metals in marine waters, suggesting that another mechanism might be involved in the acute toxicity of dissolved metals other than the imbalance of ionic and osmotic regulations, except for nickel. In this case, oxidative stress, as well as respiratory and energy metabolism impairments should be considered as potential mechanisms of acute metal toxicity in estuarine and marine invertebrates.

TU 386

Will biotic and abiotic factors increase the toxicity of environmental contaminants in exposed sea snails?

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Historically, mercury contamination has received an extensive attention due to its impact on human health and wildlife. Currently, concerns about climate changes and the way they might affect the environment are being more and more studied. Moreover predator-prey relations have been progressively more pointed as stress sources. Environmental contamination and biotic and abiotic stress co-occur, thus it is important to assess and understand the synergistic or antagonistic effects of these changes (e.g. salinity) and/or predation combined with common environmental contaminants.

Kairomones are signs produced by the predator and that can be detected by the prey, causing stress and consequent fitness costs. In order to measure those costs and evaluate the effects of simultaneous exposure to multiple stressors, the sea snail *Gibulla umbilicalis* was exposed to increasing concentrations of mercury and salinity and/or predatory risk, simulated by a concentration gradient of kairomones produced by a sea snail predator - sea star. Mortality, feeding rates (indirect measure by post-feeding exposure egestion) and avoidance behaviour were assessed. In the end of the experiments, animals were sacrificed and cholinesterases' activity and energy reserves were measured. Our results have shown that although responses could be triggered by single stressors, the combination of contaminants with abiotic or predator-specific compounds induces

stronger responses.

Studying both types of stressors and its interaction with contaminants can be crucial to provide improved interpretation of ecological effects of relevant scenarios for environmental risk assessment.

TU 387

Effects of mercury contamination on acute and chronic parameters of exposed sea snail *Gibbula umbilicalis*

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The Minamata (Japan) incident in the 1950's triggered the global concern regarding the mercury contamination hazard. Mercury is a very common contaminant in the environment, having natural sources, such as volcanism, and anthropogenic origins, such as mine tailings or industrial effluents.

Exposure to some environmental contaminants, even for short periods of time, may cause serious damage to exposed individuals. Thus, to evaluate the possible effects of mercury in the sea snail *Gibbula umbilicalis*, acute and chronic assays were performed by exposing the animals to increasing doses of the contaminant for 96 and 168 hours. Mortality, feeding rates (indirect measure by post-feeding exposure egestion) and avoidance behaviour were assessed. In the end of the experiments, animals were sacrificed and cholinesterases' activity and energy reserves were measured.

Although evaluated at individual level, the effects observed here might be translated to the population and community level, since feeding impairment and change in energy allocation may have effects on the organisms' fitness and reproductive performance. Moreover, modification in cholinesterases' activity and its common link to behaviour shifts might lead to severe implications in population dynamics.

Results have shown that most of the parameters evaluated tend to be impaired by this common contaminant, showing the deleterious effects that this compound may have at the population level and on marine life.

TU 388

Investigation on differential sensitivity of marine mollusk to various doses of detergent

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The problem of anthropogenic impact on marine aquatoria is one of the most actual nowadays. Synthetic detergents has been detected in municipal wastewater effluent and surface waters at different concentrations ranging from mg/kg to low mg/l. Detergents occur in the environment as complex compounds and could cause potential risk for humans and aquatic organisms. Few chronic ecotoxicological data are available with respect to detergents' effects in mussels. The critical issue is the choice of methods to determine concentration response threshold and the level at which detergents are essential for organism health status.

Objective of the present study was to develop improved method for assessing potential effect of detergent agent (e.g., SDS - dodecylsulfate of sodium) on marine mussels' cardiac activity and behavior. The study was conducted on adult 3-years mussels *Mytilus galloprovincialis* Lam. Continuous recordings of the heart rate and valve movements in the mussels were carried out in the laboratory under nearly natural conditions with running ambient seawater and addition of detergent. Previously, there had been reported that concentration of 1,7 mg/l detergent is the threshold for mussels to change their filtration activity. Our results have indicated that concentration of 0.5mg/l is the detection threshold limit of SDS in mussels, which could affect on heart rate and behavior (valve gape). In high concentrations detergent caused acute toxic action, which lead to rapid valve closure, isolation and subsequent bradycardia. Results have proved that implementation of behavioral and physiological biomarkers simultaneously could be effective tool to early detection stress exposure effects in marine organisms.

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TU 389

Biomonitoring trace metals in *Mytilus galloprovincialis* off the west coast of the Cape Peninsula, Cape Town

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The concentration of trace metals (Fe, Mn, Cu, Zn, Cd and Pb) in the mussel (*Mytilus galloprovincialis*) were measured along the west coast of the Cape Peninsula, Cape Town, from autumn 2010 to autumn 2011. Sampling took place at Scarborough (considered an unpolluted site), Hout Bay, Green Point, Milnerton and Bloubergstrand. The samples of whole animal soft tissue were analysed using an Atomic Absorption Spectrophotometer (AAS). Metals in *M. galloprovincialis* were significantly lowest at Scarborough and at the other sites differed significantly between seasons, with winter having the highest significant difference. The efficiency of metal accumulation was measured using the Biosediment Accumulation Factor (BSAF). The results showed that the BSAF was highest in Cd, Pb, Zn and Cu, with the lowest BSAF reported in Fe and Mn.

TU 390

PAM chlorophyll a fluorometry, field and laboratory research for corals and seagrass along the coast of Qatar

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A two year-long field program was conducted to evaluate the effectiveness of PAM (Pulse Amplitude Modulation) chlorophyll a fluorometry in monitoring the health of coral reefs and seagrass along the coast of Qatar. The technique was calibrated and validated as a means of assessing sub-lethal stress for coral in the region. The reduced photosynthetic efficiency indicated by the PAM, without clear visual signs of impairment in coral structure, was generally followed by necrosis of parts of the colonies; this was visually verified in subsequent campaigns. Two PAM derived measurements; photosynthetic efficiency and Rapid Light Curves (RLC) were well correlated with visual observations on the health conditions of the organisms. Furthermore, this work has suggested a critical threshold value applicable to photosynthetic efficiency for corals in this environment. For seagrass, there was correlation between RLC data and visual observations, such as high epiphytic coverage; however, PAM measurements did not appear to provide as strong an indication of sub-lethal stress before deterioration as obviously as it does for corals. This research has now been integrated with laboratory studies utilizing both the diving PAM used in field research and the imaging PAM, which can measure photosynthetic processes and obtain detailed visual images of photosynthesis. Gulf coral collection and cultivation and its detailed monitoring were the first steps of corals (and seagrass) laboratory experimentation. PAM measurements were performed on the *Acropora* sp. colonies prior to and after collection. "Mother" colonies were tagged and photographed to document their recovery. PAM measurements were continued in the laboratory in order to monitor the recovery of the nubbins after collection and transfer. Protocols for future coral experiments are being tested and adapted. Laboratory experimentation is also underway on seagrasses to better understand the driving factors that influence sustained photosynthetic performance for this important ecological resource.

TU 391

Toxicity tests using bioluminescent Phytoplankton

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In the field of risk assessment of chemicals entering the marine environment, tools are needed as the pressure of several pollutants impacts marine and estuarine ecosystems. Relevant sources may be the chemicals from gas/oil production platforms and sea-side refineries. The discharge of compounds to the marine environment will be receiving additional attention in the EU Marine Strategy and rapid assessment tools are needed to identify and prioritize sources. Most recently, the disinfection of ballast water will be significant interest as the International Convention for the Control and Management of Ships' Ballast Water and Sediments will introduce worldwide requirements for treatment standards. Most of these treatment systems use chemicals; and therefore risks need to be identified before market (certification) and after (monitoring and control). Therefore, a rapid toxicity method to determine the effects of contaminants in marine waters has been developed and used in such tests. This innovative test method uses bioluminescence from marine dinoflagellates, and can serve as a quick and easy to use test to screen for effects in marine waters. The test is ASTM Standard E1924 "Standard Guide for Conducting Toxicity Tests with Bioluminescent Dinoflagellates". The QwikLite(R) toxicity system is a rapid, cost effective, portable bioassay based on inhibition of light produced by marine bioluminescent dinoflagellates. The bioluminescent species *Pyrocystis lunula* are used according to their robustness and sensitivity for various toxic substances in the environment. Currently, the method using the dinoflagellates is pilot tested in the marine laboratory. The first results on reference compounds and environmental samples will be presented.

TU 392

Development and validation of a colorimetric methodology to assess macroalgae chronic toxicity - a case study with *Ulva lactuca*

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Xenobiotics are increasingly present in everyday life and are easily integrated into marine ecosystems. In aquatic systems macroalgae are continuously exposed to

contaminants. Since they represent a very large biomass, they can act as an important sink for contaminants becoming a gateway for higher trophic levels. Moreover, it is known that macroalgae, especially estuarine, are very important in the aquatic system due to several aspects: easy to find and to identify, vast biomass percentage and great bioindicators, making them potential sentinels of to monitor xenobiotics in aquatic environments. Macroalgae presence or absence, as well as their look-like may reflect the current state of the marine ecosystem, thus it is mandatory to develop new tools and methodologies to quickly and cost-effectively assess their status. The green macroalgae *Ulva lactuca* was used as a model species. The macroalgae, when exposed to xenobiotics, can show signs of decay by losing their colour and their texture. But these signs only are assessed by visual inspection. To be sure the algae are in decay and correctly quantify it, a new method was developed by measuring the colour across a colour scale. For this a colorimeter (Chroma Meter CR 400/410- Konica Minolta) was used and color variations calculated when exposed to contaminants. Also its validation by measuring photosystem electron transfer in exposed macroalgae was performed. The methodology revealed to be a sensitive and effective measure of xenobiotic toxicity and a prospective tool for environmental risk assessment.

TU 393

Analysis and environmental distribution of organic micropollutants in urban protected salt marsh areas

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Coastal ecosystems concentrate most of the marine organisms and species compared to open ocean areas. However, they are often heavily affected by human activities that can jeopardize their populations. Therefore, contamination by organic compounds discharged by nearby industries and cities has attracted considerable attention from multiple perspectives. Our first objective in this work was to develop a fast, easy and low-solvent consumption multiresidue method for the simultaneous identification and quantification of a wide variety of classic and emerging pollutants that often impact coastal marine systems. Stir bar sorptive extraction (SBSE) and liquid desorption (LD) coupled to gas chromatography tandem mass spectrometry (GC-MS/MS) were used for determining polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), organochlorine and organophosphorus pesticides, triazines, plasticizers and some personal care products (triclosan, UV filters and fragrances). Briefly, 10 mm stir bars coated with polydimethylsiloxane (PDMS) were placed in a flask containing water samples (100 mL) and stirred for 24 hours at room temperature. Then, bars were sonicated during 30 min using a reduced amount of ethyl acetate (0.2 mL) to release the analytes. This extract was analyzed by GC-MS/MS, so sub-ppb levels could be detected. Several parameters were optimized: extraction and desorption time, ionic strength, presence of organic modifiers, pH and MS/MS transitions. The second objective was applying this method to characterize the presence and distribution of micropollutants in two protected salt marsh areas from SW Spain that are adjacent to cities (Cadiz and Huelva, respectively). 20 water samples were collected at each area during summer 2011. Concentrations were often below 1 µg/L for most analytes; nevertheless some of them such as triclosan (up to 0.3 µg/L) can act as endocrine disruptors and affect marine organism populations after chronic exposure. Overall, results from this study provide an overview on the state of the two sampled marine environments with respect to chemical pollution, being useful for carrying out future toxicological assays with native species.

TU 394

Mixture toxicity of anthropogenic and natural compounds to marine micro-algae

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Primary production by micro-algae embodies the carrying capacity of marine ecosystems and is primarily linked to nutrient availability and light. However, recent studies indicate that certain industrial chemicals may have a direct impact on coastal plankton communities and hence on the carrying capacity of estuarine and marine ecosystems. At the same time the frequency and intensity of toxic algal blooms in the coastal zone are increasing globally, resulting in increased levels of natural toxins expecting to affect coastal ecosystems. These different chemical stressors are hypothesized to disturb regulatory mechanisms within algal communities, modifying the competitive abilities of individual species and resulting in shifts from highly nutritious to unfavourable algal species that destabilize the food chain. However, insight in the potential antagonistic or synergistic action of toxicants and toxins is lacking, which may mask or enhance the toxic effects. Therefore this study aims to provide information on the combined toxicity of both type of compounds to a range of marine micro-algae. Three algal species (*Dunaliella tertiolecta*, *Phaeodactylum tricornutum* and *Thalassiosira pseudonana*) were exposed to two anthropogenic compounds (Irgarol and Tributyltin) and two natural toxins (Decadienal and microcystin). For *D. tertiolecta* binary equitoxic mixtures of the anthropogenic compounds and the natural toxin Decadienal were tested according to the Toxic Unit (TU) concept. Reduction in effective photosystem II (PSII) efficiency was determined by Pulse Amplitude Modulation (PAM) fluorometry after 4.5 hours as a measure for toxicity. A difference in species sensitivity was observed with *T. pseudonana* being the most sensitive species. Irgarol is the most toxic compound for all tested species and the natural toxin Microcystin is the least toxic. Both natural toxins are clearly less toxic to the tested algae than the two anthropogenic compounds. A small, less than additive mixture effect was observed for Decadienal and TBT, while a large more than additive mixture effect was found for Decadienal and Irgarol. These results suggest that single compound toxicity data can not be used to predict the mixture toxicity of these compounds. A species, compound and mixture specific response was observed which underlines the complexity of determining the toxic pressure of coastal waters on the primary producers.

TU 396

The use of protozoa in ecotoxicology: from biological model to environmental biomonitoring application

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Nowadays, anthropogenic activities have resulted in accumulations of inorganic and organic pollutants in the environment as well as in the food chain, hence leading to serious ecological and human health problems. This may pose a risk to benthic and epibenthic organisms and it is crucial to discover tools that will identify adverse effects of sediment-associated chemicals on benthic organisms as well as link toxicity mechanisms to biological effects. Due to their nature as a eukaryotic cell/organism and their position in the food web, ciliated protozoa are suitable models for evaluating the biological effects of chemicals in living organisms as well as in estimating pollution levels in aquatic environments. Lethal and sub-lethal effects of exposure to inorganic and organic pollutants were tested on the cell mortality, replication rate, lysosomal membrane stability and endocytosis rate of the marine ciliate *E. crassus*. Increasing concentrations of mercury, copper, and benzo(a)pyrene, individually and as mixtures, were investigated in our study as they might be bioavailable in naturally occurring polluted sites. Exposures to binary mixtures of all studied pollutants were performed showing both inorganic-organic and inorganic-inorganic additive and/or antagonist effects. Moreover, medium salinity was also varied to mimic estuarine-like environmental conditions linking biological response to ionic strengths. Results of laboratory experiments underlined that, due to the short time and simplicity of the test procedures, the use of protozoa such as *E. crassus* is a promising and convenient bioindicator for evaluating the toxicity of different environmental matrices like pore water, sediments and wastewaters - polluted by heavy metals and organic pollutants. To validate the laboratory experiments a pilot biomonitoring program was performed in the Venice Lagoon, a peculiar, fragile ecosystem characterized by high economic and ecologic values. The responses of the battery of biological indexes in *E. crassus* were combined with sediment and elutriate chemical analyses. Overall outcomes were able to discriminate a gradient of stress in the four investigated sites. Furthermore, biological responses were linked to the specific occurrence of a certain class of pollutants in both sediments and elutriates.

TU 397

Can foraminifera be reliably used as environmental indicators?

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Sediment samples from around the Robben Island sewage pipeline and a fish factory pipeline in St Helena Bay were examined for foraminifera as well as for a suite of environmental factors. X-ray analysis was conducted on “live” foraminiferal tests to determine correlations with trace metals within the sediments. The species diversity, richness and abundance were negatively correlated with trace metals. The percentage nitrogen was negatively correlated with diversity while the percentage carbon was positively correlated with abundance and richness. The mean grain size was negatively correlated with richness, diversity and abundance, although these correlations were low. The percentage carbon, percentage nitrogen, and the mean grain size most influenced community structure. The dominant genera in St Helena Bay were negatively correlated with trace metals and percentage nitrogen. The dominant genera from around Robben Island were most strongly correlated with the mean grain size. *Bolivina*, *Elphidium* and *Quinqueloculina* appeared to be most strongly affected by environmental factors than the other genera and may be regarded as good bio-indicators. *Ammonia*, although dominant was less correlated with environmental factors and because it is considered an opportunist. No significant correlations could be found between the trace metal concentrations of the tests and the sediments. It appears that foraminifera can regulate the concentration of trace metals within their tests, evident in the low concentrations of trace metals in tests despite high concentrations in the sediments. St Helena Bay samples also displayed a higher concentration of trace metals but a lower Ca:Mg ratio than those of Robben Island. Trace metal concentrations appear to interfere with the laying down of calcium carbonate within foraminiferal tests.

Despite variability and patchiness normally found in benthic meiofauna, trends found in foraminiferal assemblages could be related to environmental conditions, particularly the trace metal concentrations within the sediments. Despite the trace metal tests not being significantly correlated with the trace metals in the sediments, trace metals do appear to have an effect on Ca:Mg ratios within the tests.

TU 398

The effect of a Copper and Zinc mixture on *Dunaliella tertiolecta*

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Toxicity experiments are usually performed with one compound, but contamination in the field generally consists of mixtures of toxicants. The aim of this study was therefore to determine the effect of a Cu+Zn mixture on the marine alga *Dunaliella tertiolecta*. Growth rate (cell count) and photosystem II efficiency (Pulse Amplitude Modulation fluorometry) of *D. tertiolecta* were determined after three days of exposure to Cu, Zn and equitoxic mixtures of Cu+Zn, composed according to the Toxic Unit concept. Our results showed that growth of *D. tertiolecta* was more sensitive to Zn than to Cu. Photosystem II efficiency was less sensitive to the metal(mixtures) than growth. The relative

insensitivity of photosystem II efficiency to these two metals requires determination of growth inhibition in order to prevent underestimation of metal(mixture) toxicity. The toxicity of the mixture of Cu+Zn was more than concentration additive and could thus not be predicted based on single metal toxicity. Hence, a reliable estimation of metal toxicity requires experimental verification of predicted effects.

TU 398

Toxicological effects of three polybromodiphenyl Ethers (BDE-47, 99 and 154) on growth of marine algae *Isochrysis galbana*

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Polybrominated diphenyl ethers (PBDEs) are highly persistent anthropogenic contaminants found in trace amounts in many environmental compartments far from their source areas, posing a risk to aquatic ecosystems. Our objective was to determine the relative toxicities of three BDE congeners such as BDE-47, 99 and 154 on marine phytoplankton algae *Isochrysis galbana*. For a highly sensitive endpoint: a 72h-inhibition of autotrophic growth rate was calculated according to standards methods. No observable effect concentration (NOEC) values were 2.53, 3.48 and 12.3 µg L⁻¹ for BDE-47, 99 and 154, respectively and LOEC values were 5.06, 6.96 and 24.60 µg L⁻¹ for BDE-47, 99 and 154, respectively. The calculated IC10 (the concentration inhibiting growth rate by 10%) corresponded to 9.3, 12.78 and 54.6 µg L⁻¹ for BDE-47, 99 and 154, respectively. The 50% inhibitions of growth rate (IC50) values were: 25.7 µg L⁻¹ BDE-47, 30.0 µg L⁻¹ BDE-99 and 243.7 µg L⁻¹ BDE-154. Therefore, the acute toxicity of PBDEs decreases as the degree of bromination increases, the order of toxicity being BDE-47> BDE-99> BDE-154. Significant (p < 0.05) adverse effects were observed for all compounds at concentrations >15 µg L⁻¹. Our results indicated that under laboratory conditions PBDEs were acutely toxic to seawater algae at concentrations near 10 µg L⁻¹. However, further work is required to investigate long-term effects in these and other aquatic organisms.

TU 400

Tissue accumulation of fluoranthene and its metabolites in the shore crab *Carcinus maenas* in relation to oxidative stress

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Concern around contamination of the aquatic environment by polycyclic aromatic hydrocarbons (PAHs) is high due to their toxic, carcinogenic, mutagenic and/or teratogenic effects. Fluoranthene (FLU) is a priority PAH that is commonly detected in sediments, water and biota of European estuaries. It is persistent and an important lipophilic constituent of crude oil. Early detection of environmental exposure and effects of FLU on marine organisms is thus an issue of utmost relevance. *Carcinus maenas* is a natural inhabitant of European coasts and estuaries. It is a suitable sentinel species widely used to assess exposure to contaminants, including PAHs. This work investigated the accumulation of FLU and its metabolites in the tissues of *C. maenas* following a 7-day laboratory exposure. Effects of FLU on oxidative stress biomarkers were also investigated. Crabs were collected from a low impacted estuary. In the lab they were acclimated (7d) in filtered seawater (15 psu; 16°C) and exposed individually (9 per treatment) to 5 FLU concentrations between 1.90-75 µg/l. At the end of the bioassay, crabs were ice-anaesthetized and samples of digestive gland were collected for biomarker determinations: glutathione-S-transferases (GST) and glutathione reductase (GR) activities, levels of total glutathione (GT) and lipid peroxidation (LPO), and FLU-type metabolites as measured by fixed-wavelength fluorescence (FF). The remaining whole soft tissues were used for chemical analysis of FLU by GC-MS. The results indicate a strong concentration-dependent accumulation of FLU (measured by GC-MS, $\beta = 5.21$, $P < 0.001$, $R^2 = 0.993$) in the soft tissues and FLU-type metabolites (measured by FF, $\beta = 6.09$, $P = 0.002$, $R^2 = 0.931$) in the digestive gland. A strong positive linear relationship ($\beta = 1.15$, $P = 0.003$, $R^2 = 0.911$) between accumulation of FLU (GC-MS) and FLU-type metabolites (FF) was also found. GST activity and TG levels were significantly increased in crabs exposed to concentrations ≥ 12 µg/l, compared to controls. GR activity was significantly increased, and LPO was decreased, in crabs exposed to concentrations ≥ 30 µg/l, relatively to controls. In conclusion, accumulation of FLU and its metabolites appears to be a useful biomarker of exposure and bioavailability of this PAH to *C. maenas*.

Work supported by national (FCT, MCTES) and European funds (FEDER) through CARBATHMES Project (FCOMP-01-0124-FEDER-007383, COMPETE Programme) and an FCT PhD grant to APR (SFRH/BD/65456/2009).

TU 400

Comparison of proteomic profiles in gills of shore crabs (*Carcinus maenas*, L.) from urban and reference sites and variation of polyubiquitinated proteins

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Coastal waters adjacent to urban and industrial zones can be deeply impacted by chemical pollution that represents a threat for the health of resident organisms. Pollution-induced alteration of cellular processes notably includes the modulation of protein metabolism and homeostasis, so that proteomics can help to understand the sub-lethal effects of chemical stress. Especially, the study of protein ubiquitination provides a particular insight into the effects of environmental stress in terms of damages to biomolecules and regulation of degradation systems as well.

We investigated the response to environmental stress in *Carcinus maenas* (L.) by comparing 2-DE proteomic profiles and protein ubiquitination in gills of crabs sampled in a polluted site and a reference site at low tide. Proteomic profiles showed significant differences according to animal origin despite high individual variability. A small set of regulated spots (Student's t-test, $n=7$, $p<0.05$, 20% FDR) successfully classified the two sites by hierarchical clustering, confirming the discriminatory power of gill proteomic pattern. Immunoblotting of polyubiquitinated proteins with polyclonal antibody and ECL detection enabled to visualize 20 spots in average. In spite of the high individual variability of the pattern, two spots exhibited significantly different ubiquitination levels between conditions (Mann-Whitney U test, $n=3$, $p<0.05$).

These results highlight that protein ubiquitination assessment represents a promising approach to evaluate and gain insight into stress response in marine ecotoxicology. Proteins whose expression and/or ubiquitination level are affected by crab location will be subjected to identification by mass spectrometry, in order to tentatively elucidate the mechanisms involved in pollutant response.

TU 401

Does contamination impact the shore crab (*Carcinus maenas* L.) resistance to an additional stress? An insight through a multiparametric approach

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The shore crab *Carcinus maenas* (L.) represents the most common crab species and a key component of littoral ecosystem in European shores. The wide geographical distribution of populations, their presence in pristine as much as heavily polluted areas and their intertidal location reflects a remarkable ability to cope with different stresses, due to a great physiological plasticity notably. However, the compensation capacities that underlie tolerance to stress may be impacted by the life history of crabs in terms of exposure to pollutants notably. In addition to the chronic exposure to contaminants, a novel insult is indeed likely to generate a too high degree of stress that would overwhelm resistance capacities of organisms.

To test whether and understand how chronic exposure to pollutants affects the resistance capacities of *Carcinus maenas* to stress, we imposed a 4-days air exposure to crabs sampled in a polluted area, Le Havre harbour, and in a clean area, Yport rocky shore (France) and analysed stress markers as well as proteome variations in gills. For each site, antioxidant activities (catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, glutathione S-transferase), protein expression (2D-electrophoresis, SDS 12% polyacrylamide gels, pH 3-10) and polyubiquitination (immunoblotting of 2D-E gels, ECL detection) profiles of air-exposed crabs were compared to those of control individuals that were continuously kept in water. Crabs from Le Havre showed a higher mortality rate (5%) than crabs from Yport (0%) in the course of the experiment, but no difference was found between air-exposed and control groups whatever the sampling site. Results indicate an altered resistance capacity in crabs living in Le Havre harbour in comparison with those from the clean site Yport.

Proteins showing different expression or polyubiquitination levels will be identified by mass spectrometry in order to elucidate the mechanisms underlying stress response. We intend to further investigate the response to prolonged air exposure in a less resistant crab species, the flying velvet crab *Necora puber* (L.), sampled in the same sites as *C. maenas* in order to compare the physiological and cellular responses to stress between the two models.

TU 402

Effects of mercury in natural populations of the sea anemone *Actinia equina*

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Mercury (Hg) is considered to be one of the most toxic metals to biota, being capable of biomagnification and bioaccumulation in food chains. High concentrations of Hg were associated with developmental and behavioral abnormalities, impairment in growth and reproduction, and reduced survival. Though this metal has been extensively studied, several knowledge gaps still exist, mainly regarding its effects in different types of organisms. Accordingly, this study aimed at assessing the effects of a short-term exposure to Hg in natural populations of the sea anemone *Actinia equina*. To attain this objective, four natural populations of *A. equina* were sampled in the NW Atlantic coast. From each population, forty individuals were collected and acclimated in laboratory, under optimal conditions, for one month. Afterwards, organisms were weighed (7.09 ± 0.18 g mean $\pm$ SE), and using a flow-through system were exposed to 100 µg L⁻¹ of Hg and to artificial sea water solely, during 96h. At the end of exposure, organisms were immediately deep-frozen (-80°C) until further analysis. The tissue of pedal disc was analyzed for bioaccumulation and biomarkers (biochemical: glutathione S-transferase-GST, catalase-CAT and lipid peroxidation-LPO and metabolic: lactate and alanine) to compare the uptake, oxidative damage and metabolic patterns among populations after being exposed to Hg. All population exhibited significant bioaccumulation of Hg and a significant change in the activity of LPO when exposed to Hg. On the contrary, exposure to Hg caused no significant changes in the activities of GST, CAT and in the levels of alanine and lactate. Furthermore, significant differences in the response patterns to Hg among the four populations were observed. With the two populations originated from sites with a higher Hg contamination showing a lower bioaccumulation of Hg and a higher oxidative stress. Also, the ratio of lactate/alanine (an index of redox state of the cell) accomplished with the biochemical biomarkers responses revealed that those two populations exhibited a lower stress redox. This findings suggest that the use of several endpoints at different levels of biological organization is the important for the evaluation of Hg effects in the biota.

TU 403

A short-term in situ sediment assay based on the postexposure feeding of the estuarine isopod *Cyathura carinata*

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In situ assays based on feeding depression have been proposed as sublethal assays which allow to assess direct and immediate contaminant effects on key ecosystem functions (e.g. organic matter decomposition, grazing), long before direct effects on individuals (e.g. growth) are extrapolated to effects at higher levels of biological organization - the traditional approach when selecting sublethal test endpoints. In situ assays provide a more realistic exposure scenario than assays conducted under laboratory controlled conditions, which is particularly relevant for estuarine ecosystems where environmental conditions are highly variable. In this context, the objective of the present study was to develop and evaluate a short-term cost-effective in situ sediment assay based on the postexposure feeding of an estuarine species widely distributed and with an important ecological role in estuarine foodwebs. The selected species was the benthic isopod *Cyathura carinata*, a secondary producer, prey to fish and birds, occurring along the North Atlantic coast and in the Baltic and Mediterranean seas. The methodology for feeding quantification was first developed and optimized under laboratory conditions with *Artemia franciscana* (Crustacea) nauplii. Then, the sensitivity of the postexposure feeding response to a reference chemical - cadmium - was compared to that of lethality, by conducting tests during a 48-h exposure period. Finally the in situ postexposure feeding assay was evaluated by deploying organisms in chambers at reference and contaminated Portuguese estuaries (Mira and Sado Rivers, respectively). The influence of different exposure conditions (sediment particle grain size, temperature, salinity, and food availability) was also taken into account through a multiple regression model, with the ultimate goal of discriminating contaminant effects from those due to environmental factors. The in situ postexposure feeding assay with *C. carinata* was found to be a potential useful cost-effective tool for estuarine sediment toxicity assessments.

TU 404

Toxicity of the biotransformation products of four methylated PAHs and one PAH produced by *Nereis diversicolor* and *Nereis virens*

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Risk assessments of spilled oil generally focus on the *content* of specific PAHs in a contaminated site. When the content of these compounds are below the set limit, elimination of the contamination has succeeded. However, when spilled oil is biotransformed, a wide range of water soluble products are created. These compounds can reenter the sediment by deconjugation reactions, but can also act as toxicants in the water column. If these transformation products are toxic, they do not only expose organisms in the water, they are also subjected to spreading in the environment in a much larger scale.

The objective of this study is therefore to: 1) assess of the toxicity of the biotransformation products of 1-methylphenanthrene, 3,6-dimethylphenanthrene, 1-methylpyrene, 5-methylchrysene and phenanthrene produced by the two benthic invertebrates *Nereis diversicolor* and *Nereis Virens*, and 2) identify the most important water soluble transformation products.

The biotransformation products from PAH exposed *N. diversicolor* and *N. Virens* were collected by extracting the test water of laboratory mesocosms exposures at different times. The water soluble biotransformation products were extracted and concentrated by SPE, and their toxicity (EC50) were determined by exposing *Daphnia magna* to dilutions of these extracts. The water soluble biotransformation products were identified and quantified by UHPLC connected in series to a fluorescence detector and a Quadropole Time of Flight Mass Spectrometer. To aid the identification enzymatic deconjugation of the phase II products was performed, and identified by use of Gas Chromatography Mass Spectrometry (GC-MS).

This work presents a novel approach to test the toxicity of transformation products of a contaminant mixture, where organisms are exposed to the entire range of biotransformation products. Also, this work shows the changes in toxicities of the transformation products as transformation of the PAHs progresses, and gives insight to the mechanisms of biotransformation of alkylated PAHs.

TU 405

Vitellogenin and Zona Radiata gene expressions in three different species of catfish from Terminos Lagoon

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In an effort to assess the differences in the Vitellogenin and Zona Radiata gene expressions of three different species of catfish (*Ariopsis felis*, *Bagre marinus* and *Cathorops melanopus*) collected in the Laguna de Terminos, 70 fish were collected at 5 different sites of the lagoon and were dissected to obtain liver. Total RNA was extracted using the Trizol method along with purification with the DNase enzyme. Specific primers of Vitellogenin and Zona Radiata genes were custom designed to amplify and isolate two partial fragments from *Ariopsis felis*, *Bagre marinus* y *Cathorops melanopus* liver tissues. Gene expressions were analyzed using amplification with Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) and results showed statistical differences between Vitellogenin and Zona Radiata values for fish collected at Laguna de Terminos. Data from this experiment show that Vitellogenin gene expressions were more sensitive than the ZR, under normal conditions in the environment. Future studies will be focused on understanding the reproductive cycle and the genetic variability of these fish.

TU 406

Polybrominated diphenyl ethers and their methoxylated analogs in sea bass (*Dicentrarchus labrax*) from Bizerte Lagoon, Tunisia

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Concentrations of polybrominated diphenyl ethers (PBDEs) and methoxylated polybrominated diphenyl ethers (MeO-PBDEs) in sea bass (*Dicentrarchus labrax*) collected from the Bizerte Lagoon and the Mediterranean Sea (reference site) were investigated. To the best of our knowledge, this is the first report of these compounds in marine fish from Tunisia. Concentrations of ΣPBDEs ranged from 37.3 to 218 ng g⁻¹ lipid weight (lw) for sea bass in Bizerte Lagoon, whereas in the Mediterranean Sea, concentrations ranged from 20.8 to 36.6 ng g⁻¹ lw. BDE-47 was the major congener in samples from the two areas. Mean levels of MeO-PBDEs ranged from 49.4 to 798 ng g⁻¹ lw in samples from Bizerte Lagoon. In the Mediterranean Sea, the mean concentration of these naturally produced organobromines was 482 ng g⁻¹ lw. The total PBDEs and total MeO-PBDEs concentration in sea bass from Bizerte Lagoon was similar or slightly lower than those reported for other species from other locations around the world. No significant correlations were found between the organohalogen compounds levels and the fish characteristics.

TU 408

Seasonal pollutant monitoring in discarded fish species: is valorisation appropriate?

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Increased by-catch utilisation interest may come from a greater demand for fish products: the development of new markets for previously discarded species, use of low-value by-catch specimens for aquaculture and animal feed or the creation of value-added products from by-catch or discarded fish for food, pharmaceutical or cosmetic industries. However, the contribution to a sustainable management of this biomass through their optimal valorisation highly depends on the quality of the products to be obtained from them.

Many studies in the scientific literature, like surveys of fish and fish products in markets of different countries [1] or monitoring reports of Public Administrations and the EU [2] reported significant levels of this kind of pollutants (especially of dioxins, polychlorinated biphenyls-PCBs, organochlorinated pesticides-OCs and heavy metals) in several cases for commercial species of different fisheries. Hence, it is logical to assume the presence of contaminants in other non-commercial species, although contamination levels in these non-targeted and/or discarded species are not usually assessed. However, a sustainable management of discards passes through the evaluation of their pollutant content, especially if it is considered that the products of discard valorisation are mainly concentrates (as fish oil and meal). Pollutants contained in the raw material are usually present at higher concentrations in the valorised product, especially if the product has a high fat content. In fact, some marine valorised by-products present pollutant levels of concern [3].

In this sustainability framework, FAROS project, co-funded under the LIFE+ Environmental Program of EU (LIFE08 ENV/E/000119), aims as one of its main objectives to analyze the valorisation potential of fish discards in order to contribute to their sustainable management by minimizing discards/by-catch through their optimal valorisation to recover and to produce valuable chemicals of interest in the food and pharmaceutical industry [4]. To properly define these adding-value processes, monitoring of metals, PCBs and dioxins are being performed on the most discarded species identified in FAROS métiers.

TU 409

First evidences of suboptimal health status of red mullets from the priority polluted Mediterranean area of Portman (SE Spain)

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The Portman area (Cartagena, SE Spain) is one of the areas most heavily polluted by metals in the western Mediterranean Sea and highly impacted by emissions from oil-related and chemical industrial activities in the adjacent Escombreras Valley. An integrated assessment was conducted of the health status of red mullet (*Mullus barbatus*) from three priority pollution areas of the Spanish Mediterranean coast, including Portman. A suite of general physiological indicators (lipid content in muscle tissue, condition factor, hepatosomatic and gonadosomatic indexes) and hepatic biomarker responses (ethoxyresorufin-O-deethylase (EROD) activity, metallothionein concentration and DNA damage) were determined. In addition, fish muscle tissue and sediment samples were taken and analysed for concentrations of arsenic, trace metals, Polychlorinated Biphenyls (PCBs), chlorinated pesticides (dichloro-diphenyl-trichloroethane (DDTs), α-hexachlorocyclohexane, lindane, hexachlorobenzene, cyclodien insecticides and trans-nonachlor) and polycyclic aromatic hydrocarbons (PAHs). Our results showed that fish from Portman accumulated the highest concentrations of mercury, lead and arsenic and also of some PAHs and PCBs congeners. In addition, they had significantly lower lipid content, condition factor and gonadosomatic index, as well as the highest the highest levels of EROD activity and DNA damage of the areas investigated. Contaminant body burden in red mullet only partly corresponded to chemical characteristics of the sediments in the areas in which they were collected. Despite current concentrations of contaminants in sediment and fish muscle from the three priority pollution areas of the Spanish Mediterranean shelf investigated herein are at levels that do not give rise to severe health effects in red mullet, our findings indicate that fish from Portman exhibited a suboptimal health status that warrants further study. The integrative assessment strategies, including chemical, (eco)toxicological parameters used in this study can contribute

TU 410

Characterization of selected Metals in United Arab Emirates coastal fish and locally produced vegetables

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Heavy metal pollution of the marine environment has long been recognized as a serious environmental concern. A lack of published literature on heavy metals contamination of local fish and a proper fish advisory in the United Arab Emirates (UAE) is in current need. Moreover, seafood and locally produced vegetables comprises a major portion of local daily consumption. An assessment of the contamination of selected metals including arsenic, cadmium, lead and mercury was made in several UAE food products such as fish, seafood and vegetables (sardine, shakelie, trevaly, mushrooms, cucumbers, peppers, among others) using Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES). The concentration levels were dependent on the food type and metal tested. For example, arsenic was found in sardine and shakelie at concentrations of 0.0241 mg/kg and 0.0298 mg/kg, respectively. The lowest arsenic concentration was obtained in peppers at 0.089 mg/kg. This data provides preliminary information for the further formulation of health risk assessments for the community of UAE.

TU 411

Sediment-contact exposure of medaka eggs to a PAH model: combined chemical, toxicological and proteomic approaches

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Estuarine ecosystems represent nursery areas for many fish species. They are nevertheless submitted to a high anthropic pressure, which is manifested by pollution. Among the most important pollutants are polycyclic aromatic hydrocarbons. The main objectives of this study were to understand transfers between the different compartments during exposure and to see if the macroscopic effects observed were related to proteomic modulations. We spiked reference sediment with 3 concentrations of fluoranthene, then exposed Japanese medaka via direct contact with sediment during their entire embryonic development. Water, sediment and embryos were collected during the exposure and parent fluoranthene was analyzed by GC-MS. Time to hatch was also determined and photography was performed to analyze morphologic abnormalities and total body length. They were then sacrificed in liquid nitrogen to perform proteomic analysis by 2-dimensional electrophoresis. In sediment, the concentration of fluoranthene decreased linearly with time. In water, concentrations were globally constant, with the sediments acting as a reservoir of pollutant. In embryos, the concentrations were more erratic, and were dependent on stage of development more than concentrations in sediment or water with a fast clearance after hatching. At the same time, the highest tested concentration led to hatching failure associated with one day delayed hatching. The rate of several abnormalities increased such as heart edema, skeletal deformities, heart defects and abnormally reducing of yolk sac. These pathologies, related to blue sac disease syndrome, explained the dose-dependent increase of teratogenicity. The proteomic map exhibited 600 proteins. Among these, the expression level was modulated for 77 proteins. Cytoskeleton appeared to be regulated during exposure, as well as proteins related to cell physiology, fatty acid or protein metabolism and transport. Macroscopic and microscopic effects and/or regulations were dose-dependent, which suggest that fluoranthene is involved in a metabolic pathway that we could not determine with our proteomic study. Exposure to environmental concentrations of a single PAH in a realistic way of exposure could lead to proteomic modulations, with macroscopic teratogenic effects. This study was supported by region Haute Normandie and Aquitaine, GIP Seine Aval and University of le Havre.

TU 412

Long-term incubation of adult *Nereis virens* (Annelida: Polychaeta) in copper-spiked sediment: the effects on adult mortality, gametogenesis, spawning and embryo development

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The impacts of copper on polychaetes have received considerable attention with many sub-lethal endpoints developed including effects on reproduction. However, no reproductive experiments with large polychaetes lasting longer than a few days have been attempted. *N. virens* is an ideal candidate for long-term parental exposure investigations as gametogenesis and spawning of a population are highly synchronized. Heavy metals interact with sediment in many ways and spiked sediment has been used frequently to aid in the development of concentration-response relationships. In this study we investigated the effects of copper on the later stages of the gametogenic cycle of *N. virens* through spiking sediment to a range of nominal concentrations (50, 500 and 1000 mg kg⁻¹ dry weight of sediment) chosen to reflect those present in sediments experienced by macrofaunal polychaetes. Subsequent adult mortality and the numbers and timing of spawning events were quantified as well as the effects on oocyte development. Finally, sperm and oocytes were extracted from the adults and using in vitro fertilizations we assessed the developmental success. BCR 3-step standard sequential extraction confirmed that much more labile copper (in actual and percentage terms) was present as spiked concentrations increased, although the residual fractions had similar amounts and this is comparable to those levels found in the tissues of the exposed worms. Adult mortality was not dependent on the exposure time, but higher concentrations induced greater mortality for both sexes. Oocytes were significantly smaller at higher concentrations although pairwise comparisons did not show specific differences. Spawning of males was earlier and more frequent in the higher concentrations. Differences in the number of embryos developing normally after in vitro fertilizations of oocytes fertilized with sperm from exposed males and not exposed males showed that sperm were more susceptible, but oocytes were also affected at the highest concentration. Exposure of adults to copper may have significant implications for adult survival, embryo development and larval recruitment and subsequent colonization of polluted sediments.

TU 413

Oxidative stress, genotoxicity and histopathology biomarker responses in sea bass (*Dicentrarchus labrax*) liver from Bizerte Lagoon (Tunisia)

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The aim of the study was to evaluate the impact of environmental contaminants on oxidative stress, genotoxic and histopathologic biomarkers in liver of sea bass (*Dicentrarchus labrax*) collected from a polluted coastal lagoon (Bizerte Lagoon) in comparison to a reference site (the Mediterranean Sea). To our knowledge this is the first study of enzymatic, histopathologic and genotoxic biomarkers using biomarkers from fish liver for assessment in Tunisia coastal waters.

Antioxidant enzyme activities were lower in fish from the polluted site compared with fish from the reference site, suggesting deficiency of the antioxidant system to compensate for oxidative stress. DNA damage was higher in fish from the contaminated site indicating genotoxic effects. The liver histopathological analysis revealed alterations in fish from Bizerte Lagoon. Hepatocytes featured lipid-type vacuolation and membrane disruption. Results suggest that the selected biomarkers in the studied specie are useful for the assessment of pollution impacts in coastal environments influenced by multiple pollution sources.

TU 414

Effects of hypoxia on spermatogenesis and steroidogenesis of marine medaka (*Oryzias melastigma*) testis: an in vitro study

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Aquatic hypoxia (dissolved oxygen levels less than 2.8 mg O₂/L) has become a major concern and more than 200 "Dead Zones" have been identified by the United Nations Environment Program worldwide. Hypoxia has been shown to be an endocrine disruptor, and impairs developmental and reproductive functions in fish. However, the detailed mechanisms of reproductive impairment of hypoxia remain unclear. Using primary cell culture of marine medaka (*Oryzias melastigma*) testis as an in vitro model, experiments were conducted to decipher the effects of acute hypoxia (2%) on spermatogenesis and steroidogenesis. Hypoxia significantly increased cell death at 24h as measured by Trypan Blue Exclusion (p<0.001, n=6) and flow cytometric analysis using propidium iodide (PI) (p<0.05, n=5). Flow cytometry showed that hypoxia significantly reduced testicular cell differentiation (spermatocytes and spermatogonia) at 48h (p<0.05, n=4), and this was associated with significant down-regulation of expression of steroidogenic genes including CYP11A (p<0.0005), 3β-HSD (p<0.05) and stAR (p<0.001). However, no significant changes in apoptotic cell death were observed following 2% hypoxic treatment at 24 and 48h as analyzed by flow cytometry after labeling with Annexin V and PI. Furthermore, there were no changes in expression of the pro-apoptotic gene BAX and the anti-apoptotic gene BCL2 at these time points. Our results suggest that spermatogenesis and steroidogenesis in fish can be impaired under acute hypoxic conditions, and alterations in germ-line development and steroid hormone regulation are a likely cause for some of the male reproductive abnormalities observed following hypoxic exposure. These molecular changes may potentially reduce reproductive success of males and lead to a decline in natural fish populations.

TU 414

Toxic effects of ZnO nanoparticles towards marine algae *Dunaliella tertiolecta*

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Dose response curve and population growth rate alterations deriving from the exposure to ZnO nanoparticles of the marine alga *D. tertiolecta* were evaluated. Bulk ZnO and free zinc ions were also investigated for comparison. Aggregation state and particle size distribution were monitored during the experimental testing time. The results show that nZnO is more toxic (EC50: 2.42 (0.97-5.36) mg L⁻¹, NOEC: 0.01 mg L⁻¹) than its bulk counterpart (EC50: 4.45 (3.45-5.98) mg L⁻¹, NOEC: 1 mg L⁻¹). Cross-referencing the toxicity parameters calculated for ionic zinc (EC50: 0.65 (0.36-0.70) mg L⁻¹, NOEC: 0.01 mg L⁻¹) and the dissolution properties of the ZnO it can be gathered that the higher toxicity of nZnO cannot be ascribed exclusively to free zinc ions. Nonetheless growth rates of *D. tertiolecta* were not significantly affected by nZnO exposure. Our findings suggest that the pristine size of the dispersed particles affect the bioavailability and the overall toxicity

TU 415

PBDEs accumulation in muscle of Whitemouth Croaker (*Micropogonias furnieri*) exposed to BDE 99 and BDE 153

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Polibrominated Biphenyl Ethers (PBDEs) are flame retardants compounds widely used in plastics, textiles, furniture, electronics, automobiles, and many other materials. Although PBDE are persistent and resistant to degradation, earlier studies have established that some fish can debrominate certain PBDEs congeners. Whitemouth croaker (*Micropogonias furnieri*) is an important species in the Brazilian fishery industry. Therefore, it is important to understand the accumulation pattern of PBDEs in its muscle. During 30 days, 60 Whitemouth croakers were exposed to BDE 99 and BDE 153 through diet (100 ng fish⁻¹ day⁻¹), followed by a 30 days period of depuration. At days 0, 1, 5, 15, 30, 45 and 60 muscle sample were collected from at least 3 fishes. Overall, the Whitemouth croaker exposure to BDE 99 ended up accumulating BDE 47 in their muscle, indicating their capacity for processing BDE 99. On the other hand, BDE 153 accumulated without any transformation.

TU 416

Inhibition of GST, GPx and AChE activities in marine mussels by exposure to the dissolved polybrominated diphenyl ethers BDE-47

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The environmental presence of polybrominated diphenyl ethers (PBDEs), among which BDE-47 is the most abundant, make toxicity data necessary to assess the hazard risk posed by PBDEs to aquatic organisms. In this study, mussels, *Mytilus galloprovincialis*, were continuously exposed to 8 µg/L BDE-47 for 30 days. After the exposure phase, the mussels were allowed to depurate in clean sea water for 10 days. Samples were collected at time 2, 5, 9, 15, 20 and 30 days of exposure to toxic. In a second experiment mussels were exposed for 30 days to three BDE-47 concentrations. In both experiments samples were used to measure glutathione S-transferase (GST), glutathione peroxidase (GPx), and acetylcholinesterase (AChE) activities. GST and GPx were inhibited after 20 and 30 days exposure, while AChE was inhibited already from the second day of exposure. After the 10 days recovery period in clean water the three enzymes recovered the initial levels of activity.

TU 417

Differential gene transcription in *Poecilia vivipara* exposed to copper

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The increasing industrial activities and the use of CuSO₄ as a fungicide in agricultural practices, as well as in the control of algae and pathogens in fish culture ponds have raised the copper concentration in aquatic systems. Furthermore, occasional accidents have aggravated this situation introducing substantial amounts of copper into aquatic environments. Copper is accumulated mainly in the liver and gills and excreted via bile and kidneys. The aim of this study was to identify genes differentially expressed in liver of *Poecilia vivipara* (Guppy) exposed to waterborne copper (20 µg Cu/L) for 24 h, employing the subtractive suppressive hybridization (SSH) method. Results showed that 65 gene fragments were differentially expressed, 37 were up-regulated and 28 were down-regulated. The up-regulated genes were bile salt export, Complement Component 3c, CYP450, enolase, Apolipoprotein B, C1 and E, microsomal glutathione-S-transferase, NAD dehydrogenase, ubiquitin a-52, plasminogen and novel protein. The down-regulated genes were Apolipoprotein A1, Coagulation factor 2, HSP70, Liver-type fatty acid Binding Protein, serine--pyruvate mitochondrial-like. The identified genes are associated with different metabolic functions like biotransformation, protein, lipids and energetic metabolism, indicating the susceptibility and/or molecular responses of this organism to the toxic effects elicited following the trace metal exposure.

TU 418

Spatial distribution and accumulation patterns of cyclic methyl siloxanes (cVMS) in fish from Northern Norway

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Cyclic volatile methyl siloxanes (cVMS) have come under environmental scrutiny in recent years in regards to their potential persistence and bioaccumulative nature. As polymeric ingredients in the synthesis of silicone products, cVMS are categorized as high production chemicals where they are used heavily within the personal care products and cosmetics, as well as other facets of industry (surface treatment agents, plasticizers, construction materials, mechanical fluids). Although the majority of cVMS emissions are to the atmosphere (90%), the remaining emissions are discharged via wastewater effluent into local watersheds and can accumulate in aquatic dwelling organisms. However, little data exists on the spatial distribution of cVMS in the aquatic environment. This study will investigate the spatial distribution of cVMS within Northern Norway and if physiological factors (e.g., age, body length, weight) have an effect on cVMS accumulation within fish. Atlantic cod was collected in 2010 and 2011 near the city of Tromsø (site A) and 30 km north of the city (site B) in Northern Norway. All cVMS were detected in cod livers sampled with highest median concentrations for D5, followed by D6 and D4. Significantly higher median concentrations for octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) were observed in fish collected at site A (D4: 60 ng/g lw; D5: 1380 ng/g lw) compared to site B (D4: 10 ng/g lw; D5: 139 ng/g lw). However, no significant differences were observed in the spatial distribution for dodecamethylcyclohexasiloxane (D6) between sampling sites. Concentration of D5 within cod liver was found to be negatively correlated with age. No correlation was observed between D4 and D6 concentrations and age; however, negative correlations were observed for body length and weight. Concentrations of D4 and D5 significantly decreased away from point sources indicating rapid elimination of these chemicals within fish. No significant differences in concentration for D6 between sampling sites may indicate greater persistence of this chemical to elimination processes. Decreasing cVMS concentrations with age (D5), body length and weight (D4 and D6) may be an effect of enhanced metabolic activity and/or growth dilution within older fish.

TU 420

Organochlorine pollutants in oceanic migratory birds: influence of body condition in PCBs and DDTs concentrations among different tissues

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Albatrosses and petrels (Procellariiformes) are migratory oceanic birds of considerable conservation interest. Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) were assessed in the subcutaneous fat, liver and muscle of one hundred birds belonging to eight species of Procellariiformes collected during their migration period in southern Brazil, one of the most important feeding areas for these species. The influence of body condition during the migration period in the distribution of organochlorine contaminants was evaluated, showing that it is a significant factor in the variation and redistribution of these compounds in the tissues of these birds. Birds with good body condition presented PCBs and DDTs concentrations in the fat tissue significantly higher than those found in the liver and muscle tissue. On the other hand, 60% to 70% of the specimens with signs of starvation such as the depletion of fat reserves had higher DDTs and PCBs concentrations in the liver than in the fat tissue, although the difference in the concentrations of OCs between these two tissues did not achieve statistical significance. These findings suggest a tendency toward an increase in the concentration of these compounds in the liver and muscle of birds with poor body condition. The concentration of these compounds is influenced by the body condition of the bird at the time of collection and the analysis of different tissues may generate different findings regarding contamination in birds. Lipid reserves are extremely important to the achievement of high energy cost activities to seabirds, mainly those with extended reproductive periods and that spend most of their lives at sea migrating long distances, such as Procellariiformes. The process of maintenance and utilization of lipid reserves during the life cycle of the bird imply in a frequent redistribution of OCs in the organism, when the negative effects of these contaminants may be manifested.

TU 420

Development of appropriate bioassay and statistical methods for determining survival sensitivities of Antarctic marine biota to metal exposure

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Developing water quality guidelines for Antarctic marine environments requires understanding sensitivity of biota to contaminant exposure. Toxicity tests with Antarctic invertebrates have shown response times to metal exposure takes far longer than the durations of standard bioassay methods. Test methods which address the biological characteristics of these cold climate species and the environment need to be developed. This study investigated the effects of five metals on behaviour and survival of an Antarctic amphipod, *Orchomenella pinguinis*, over 30 day exposures. Multiple observations were made assessing mortality rate response of this species to metal exposure. As traditional bioassay methods use probe analysis for individual period endpoints treated independently, a new statistical model that combines bioassay model with a survival time model was developed. Interval-censored survival times were modelled using a generalised additive model (GAM) with multiplicative effects for concentration level used to incorporate the bioassay component. The time period by concentration level interaction was included as a random effect term with this mixed model version of the GAM (called a GAMM) allowing model adequacy to be investigated. The advantages of this approach are that (i) it smooths through noisy periodic mortality data "gaining strength" by estimation of the trend across multiple observation periods exploiting all data simultaneously, (ii) the simple multiplicative model, if adequate, does not allow cross-overs of time trends across concentrations that make interpretation difficult, and (iii) it allows the uncertainty in estimates of lethal concentrations to be more adequately modelled.

Response times vary between metals with amphipods exposed to copper producing behavioural changes and mortality at a faster rate than exposure to cadmium, lead or zinc. Nickel did not affect the behaviour or survival of the amphipod during the 30 day exposures despite using concentrations > 1mg/L. Results from comparisons of both approaches indicate that standard bioassay methods have serious limitations when applied to Antarctic species which are generally long lived, have longer developmental times, and slower metabolic processes and potentially metal uptake than species from other climatic regions. Recommendations are made for further toxicological assessments for the future development of Water Quality Guidelines to protect Antarctic marine environments.

TU 421

PAH in fish: biological tool for Guanabara Bay environmental evaluation

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The Guanabara Bay is an estuarine ecosystem with high anthropogenic influence, receiving chronic urban and industrialized pollution from Greater Rio de Janeiro, Brazil. In this scenario it was carried out a study with sampling collection from August to October 2008 to investigate PAHs and their metabolites in *Mugil liza* fishes captured in the Guanabara Bay.

It was analyzed 41 muscular tissue samples for 38 compounds, including 16 PAHs EPA + alkylated ones. In the bile samples, 13 metabolites of PAHs were analyzed in 30 *Mugil liza* fish samples. The metabolites analyzed were OH-Naphthalenes, OH-Biphenyl, OH-Fluorene, OH-Phenanthrenes, OH-Pyrene, OH-Chrysene, OH-Benz(a)pyrene. Statistical analyzes showed that the data concentration has a normal distribution and drawn from the same population in both determinations, PAHs and their metabolites. It was verified a medium concentration of 274.54 ng.g⁻¹ for PAHs in muscular tissue and 1,038.95 ng.g⁻¹ for PAH metabolites in the bile. On the other hand, it was not observed correlation between PAH values in fish tissue and in the bile.

The data from this study was compared to other estuarine and coastal regions in the world reported in the literature. The PAH metabolite concentrations in Guanabara Bay were in the same range of various regions that receives chronic anthropogenic pollution as expected. However, PAH results in muscular tissue, when compared with three international concentration-based and risk-based criteria revealed that PAHs in *Mugil liza* fishes from Guanabara Bay was not a significant threat to public health.

To sum up, the data of this study can be used as background for Guanabara Bay. The bioindicator in this study, *Mugil liza* fish, has shown to be effective to evaluate the contamination and exposition of Guanabara Bay ecosystem to PAHs.

TU 422

Polycyclic aromatic hydrocarbons inputs in an Amazon estuary (Belém/Pará -Brazil)

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitously distributed in the aquatic environments, coming from natural and anthropogenic sources. PAHs can be produced due to anthropogenic activities, such as fossil fuel combustion, boating activities and forest fires. Natural low molecular weight (LMW) PAHs are also known to be produced inside termite nests and by woody plants. Few studies were developed in the Amazon region concerning PAHs sources and distribution. The study area, Guajará Bay in northern Brazil (48°30'W and 1°30'S), is part of the largest watershed in the world and has great ecological importance due to its vast biodiversity. One of Amazon's biggest cities, Belém, capitol of the state of Pará, is located in its margins. Most of the sewage produced in the city is released directly into Guajará Bay without any treatment. For this preliminary work, estuarine surface sediment samples were collected around Belém, near the main input channels. Sediments were Soxhlet extracted and PAH concentrations were determined by gas chromatography coupled to mass spectrometer detector (GC/MS). Total PAH concentration ranged from 8.41 to 90.0 ng g⁻¹ (d.w), indicating a low contaminated environment. PAHs are probably diluted and degraded during transport as a result of the area's strong hydrodynamics. Parental high molecular weight PAHs show higher levels than LMW and alkylated PAHs in all samples. LMW PAHs, which could be correlated to biogenic or petrogenic origins (naphthalene and phenanthrene), were not found in significant concentrations. In most samples, perylene had the highest concentration among individual compounds, suggesting diagenetic sources. These results, combined with specific compound ratios, suggest that diagenetic and pyrolytic inputs (from fossil fuel burning and specially forest fires), are the main sources of PAHs to Guajará Bay.

TU 423

Ecotoxicological and chemical parameters as tools to develop an integrated approach in a coastal ecosystem

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As most coastal zones, Venice Lagoon is subject to the deposit of sedimentological materials, in form of inorganic and organic detritus, nutrients and heavy metals, and there is also a very strong anthropic pressure; consequently, heavy metal amount is much bigger in lagoon sediment than into overlying water. In fact, remobilisation processes are able to bring to diffusion of contaminants into water body trough bioturbation, concentration gradient or resuspension caused by flooding, tide or boat traffic, all processes that could easily change their geochemical parameters (e.g. redox potential) and make them more bioavailable. On the long term, this complex net of events can cause a loss of integrity of the ecosystem, and as a consequence, a loss of biodiversity and a potential risk for human health. In the specific case of organisms that have a great socio-economical importance due to human food consumption, analysis and monitoring of production sites are a priority, and the "standard" approach to the concept of environmental quality is no longer sufficient, because it does not consider long-term ecotoxicological responses and what a systemic alteration of the entire ecosystem could cause to the populations living along the coast.

This study starts from the concept of "biomarker" in a multidisciplinary approach aimed to evaluate the "state of health" of some aquaculture sites and of the Mollusc Bivalves growing up into. Model organism for the research is *Tapes philippinarum* a species of Mollusc Bivalve of the Veneridae family that in recent years has shown an increased economic value and is in need of support in an optic of social sustainability.

In light of all this, ecotoxicological markers have been used (e.g. heavy metal bioaccumulation and tissue partitioning, metallothionein induction, condition index, influence of geochemical texture) to evaluate the environmental quality of productive sites and also analysis of geochemical speciation have been carried out to assess the actual bioavailability and mobility of abiotic matrix's pollutants present inside the trophic chain.

TU 424

Effects of Pollutants in aquatic ecosystems of the cross-border region of the southwest of Iberian Peninsula

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The southwest of the Iberian Peninsula is characterized by the presence of diverse aquatic environments, estuaries and marshes which environmental situation depends critically on the impact of pollutant substances as consequence of mining activities, increase of population densities, agricultural and industrial activities. A great number of contaminants are treated and eliminated in the waste water treatment plants, but recently the presence of new emergent pollutants has been detected which chemical nature limits the action of the treatment systems.

In order to assess environmental risk and deterioration in these ecosystems analytical tools were developed under the framework of Interreg IIIA project Rise sub projects Endocrinobio tox and Farmacotox to characterize and diagnostic the presence of emergent pollutants. Samplings were carried in marshes and aquatic ecosystems of the cross-border region of Spain and Portugal, namely Bay of Cádiz, River Guadiana, Ria Formosa Lagoon and Arade River. Core sediments and benthic organisms (clam *Scrobicularia plana* and polychaete *Nereis diversicolor*) were sampled. Accordingly, molecular biomarkers based on the characterization of antioxidant enzymes and oxidative damages in biomolecules were analyzed, as well as neurotoxicity and endocrine disruption. Biological answers were integrated with the determination of contaminants in organisms' tissues and sediments. Additionally, a proteomic approach was applied to gather comprehensive information about protein changes in organisms' tissues induced by distinct contaminants to identify new biomarkers to be used in environmental quality assessment. The integration of biomarker responses and sediment characterization can be a useful tool for marine pollution monitoring in southwest of the Iberian Peninsula.

TU 425

Water quality assessment in Portimão Harbor

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According to the Water Framework Directive ports carry out specific activities of recognized high economic and social value that in some cases imply heavy hydromorphological changes on the water quality (heavily modified water bodies). The explicit recognition of the importance of port activities justifies the definition of a specific approach to fulfil a good ecological potential. In this context, the European project "PORTONOVO" was developed to standardize methodologies to evaluate and monitor environmental quality in harbor areas all along the Atlantic Area.

In Portimão harbor (estuary of Arade river, south of Portugal) a biomonitoring survey was carried out in seven sites to assess the quality of water, sediment and biota (mussels *Mytilus galloprovincialis*). This involved analyses of: a) water: abiotic parameters (temperature, salinity, pH, and dissolved oxygen), nutrients (nitrites, nitrates and ammonium, silicates and phosphates), chlorophyll a and phaeopigments, and turbidity; b) sediments: organic content, metals (Cd, Cu, Ni, Zn, Cr and Pb), Polycyclic Aromatic Hydrocarbons and Polychlorinated biphenyls; c) biota: condition index, metals (Cd, Cu, Ni, Zn, Cr and Pb), and a battery of biomarkers (lipid peroxidation, antioxidant enzymes (CAT, SOD and GPX), metallothionein, δ -aminolevulinic acid dehydratase, acetylcholinesterase and alkali-labile phosphates). All data was integrated using a Principal Component Analyses and the IBR (Integrated Biomarker Response).

Sediments in the commercial harbor (site 4), presented higher Cd and Cu concentrations associated to organic contaminants. The IBR pointed this site as the second most stressed location and antioxidant enzymes had a great contribution. On the other hand, site 2 (outer basin of the harbor area) was considered the most stressed location, where sediments had higher Cr, Ni and Pb and organisms associated to higher concentrations of several biomarkers. As for upstream of the harbor area (site 6), there was significant impact of nutrients, phaeopigments, turbidity and some abiotic parameters which reflects the impact of a sewage treatment effluent discharge point. Concordantly in this location oxidative stress biomarkers are the main contributors in the IBR outcome.

The biomonitoring survey provided an integrated response to assess the environmental quality of the port area, proving the need to monitor all these descriptors to assess the good ecological status in Port areas.

TU 426

Persistent organic pollutants in *Stenella clymene* from Northeastern Brazil: a preliminary survey

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Persistent organic pollutants (POPs) have been widely reported in different cetaceans species around the world. However, data in dolphins from northeastern (NE) coast of

Brazil are still scarce, and none could be found regarding *S. clymene*. The *S. clymene* dolphin is a deep water species that has only been observed in waters with depths of 250m-5000m or deeper. Thus, it is supposed to be less exposed to coastal contamination. In this study, 3 blubber samples of *S. clymene* (2 males pups and 1 adult female) from NE Coast (Sergipe State) were analyzed to evaluate the occurrence of PCBs, chlorinated pesticides and PBDEs. Although the industrial activities have been increasing in the NE coast of Brazil, tourism and agriculture are still the predominant activities. Concentrations, in wet weight, of DDTs (1692 ng g⁻¹) were the highest, followed by PCBs (580 ng g⁻¹) > Mirex (190 ng g⁻¹) > HCB (104 ng g⁻¹) > HCHs (20 ng g⁻¹) ~ CHLs (20 ng g⁻¹ lw). PBDEs were detected in no sample. Overall, organochlorine levels are lower than those found in dolphins from other regions of Brazil known to be contaminated. The higher levels of DDTs over PCBs indicate the predominance of agricultural sources and/or use of DDT in pest control of tropical epidemic disease, such as malaria. However, DDEs/DDTs ratio higher than 0.65 indicates that DDTs inputs were not recent. Along the Brazilian coast, similar pattern, regarding DDTs/PCBs, was only detected in samples from an estuary in Southeastern Brazil (Cananeia region) that presents very intense agricultural activities. This study used data generated by the Regional Monitoring Program of Stranding and Abnormalities in the Sergipe/Alagoas Basin - PRMEA, from Petrobras, performed by the Aquatic Mammals Foundation -FMA, in partnership with Tamar/ICMBio, as a mitigation measure requested by the Federal Environmental Licensing, conducted by CGPEG/IBAMA.

TU 427

Measurement of naphthenic acids in the receiving waters around offshore oil and gas platforms in the North Sea by passive sampling

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Produced water (PW) is the largest discharge to the aquatic environment by the offshore oil and gas industry. Despite PW being a highly complex and variable mixture, most studies have largely focussed on the biological effects of only two groups of organic compounds; polycyclic aromatic hydrocarbons (PAH) and alkylated phenols (AP). More recently naphthenic acids (NA) have been suggested as representing a large fraction of the oestrogen receptor agonist activity found in PW. However, determining concentrations of NA in the receiving waters around platforms represents a significant analytical challenge not least due to the rapid dilution of the discharges. Passive sampling devices, which offer unattended time integrative sampling with low detection limits, have successfully been shown to be able to accumulate PW originating PAH and AP in discharge receiving waters. Thus in the present work we report from several field and laboratory studies testing the suitability of the polar organic chemical integrative sampler (POCIS) to accumulate NA. POCIS were deployed in a gradient away from the discharge points at several offshore platforms in the North Sea. After a period of six weeks POCIS were collected and extracted before analysis by GC-ToF-MS. The current form of POCIS was shown to be able to accumulate a range of mono to tetracyclic NA, with different degrees of alkylation. Additionally there was good agreement in the pattern of exposure shown by the passive samplers and also in biomarkers in co-deployed blue mussels (data not shown). Results are further discussed in terms of identifying which compounds are responsible for the biological effects shown and on determining the uptake rates of these compounds in POCIS, in order to allow quantitative determinations in the environment.

TU 428

Detection of thyroid disruption potential in sediments and water of Loire river estuary (France) using in vitro PC-DR-LUC assay

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Located in the west of France, the Loire estuary is an area of major ecological interest, subject to strong anthropogenic pressures. Indeed, this area focuses on urbanization, intensive agriculture and industrial-harbor that involves many releases into the environment. Chemical contaminants released have a strong impact on estuarine ecosystems showing particularly endocrine disruption activity. These impacts are widely studied since the 90s mainly the disruption of reproductive function. Currently, other types of disruption are studied in the aim to studied globally reproduction and development of exposed species. This is specially the case of thyroid function disruption as thyroid hormones play a major role on growth, reproduction and development in particular neurotoxicity development. The aim of this study was to evaluate the presence of disruptors of thyroid hormone receptor transcription in the different estuarine compartments (water and sediments). The detection of such compounds was performed using an in vitro test, the PC-DR-LUC assay [1]. This assay consists of PC12 cells expressing the $\alpha 1$ -isoform of TR of avian origin and stably transfected with a luciferase gene controlled by the SV40 promoter, and enhanced by a four-spaced direct repeat thyroid hormone response element. This test allows showing agonist effect when the cells are brooded in presence of an environmental sample extract and antagonistic effect when the test is carried out with addition of thyroid hormone T3 in the sample. Thyroid activity of an extract is calculated from a reference curve using Hill regression and expressed in ng/L equivalent T3. Water and sediment samples were collected during spring and summer 2010 at different sites in the intertidal zone. A 10.5 factor concentration of analytes from water was done using SPE on Waters Oasis HLB phase. Sediments were extracted by ASE using acetone/dichloromethane (50:50). Results obtained for water extracts showed half of the time very low agonist activities, included between detection and quantification test limits. The sediment extracts showed greater agonist activity leading to thyroid activity quantification, especially those collected in spring (75 % of positive responses against 25% in August). Some antagonist effect has also been observed in few sediment samples. Chemical analyses will be performed on the sediment extracts showing thyroid activity in order to identify contaminants responsible for these disruptions.

TU 429

Herbicide trends following the Brisbane River 2011 flood

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The Queensland flood event of January 2011 presented a key opportunity to evaluate the risks presented by herbicides residues in floodwaters to aquatic ecosystems following a major flow event. We commenced sampling 2 weeks after the peak of the flood using a combination of passive and grab sampling techniques covering a range of sites from a urban tributary to the receiving environment. We found unusually high levels of metalochlor in the urban tributary in the initial period after the flood which indicated a prominent source of metalochlor in the catchment of this tributary. In contrast levels of herbicides such as diuron and atrazine which are commonly found in the Brisbane River in the range of 100 - 200 ng/L appeared to be diluted by the floodwaters with relatively low levels following the flood. Subsequent sampling showed decreasing concentrations of metalochlor and increasing concentrations of diuron and atrazine. In Moreton Bay the receiving environment we found consistently elevated concentrations of a broad range of the herbicides. Work is continuing to assess the long term trend of herbicides at sites in the tributary and the receiving environment.

TU 430

Development of integrative sampling strategies for the determination and effect-based evaluation of contaminants in marine and coastal environments

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The determination of persistent toxic contaminants represents an important issue related to the assessment of the environmental quality status of marine water bodies. Due to their tendency to bio-accumulate and thus their potential toxic effects on marine organisms it is essential to analyse their concentration and bioavailability in different environmental compartments but also their potential hazardous effects, especially when they occur in sublethal concentrations, which may cause cumulative and long term effects.

Spot sampling is still an important component of different monitoring tasks, however such techniques are limited since they represent only a minute view of the current status in particular when focussing on the analysis of a dynamic environment such as the German North Sea.

This limitation can be overcome by the parallel application of techniques based on passive and active sampler as integrating tools, which reflect variations of the environmental contamination level more consistently. In addition combined approaches, which utilise biota such as transplanted blue mussels allow also the detection of hazardous effects or new potential biochemical indicators related to the targeted pollutants.

Here first results related to the development and implementation of an integrative sampling approach are described. Therefore stations have been installed on the Island of Helgoland in the Germany Bight acting as a reference sampling site as well as at the estuary of the river Elbe, which represents a heavily impacted station, respectively. The instrumentation, have been placed in May 2010 and since then regular sampling campaigns, which include transplanted mussels as well as water samples have been performed every six weeks. Corresponding long term hydrological data were acquired 24 h per day during the whole year at both sites utilizing the COSYNA data, which allow a precise observation of the physical environmental conditions. First results of the sampling campaigns on contaminant level and physiological status of the active sampler will be presented.

TU 431

Environmental impact of dredged sediment: assessment of metals bioavailability and contamination levels

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The SETARMS (Sustainable Environmental Treatment and Reuse of Marine Sediments) project aims at finding sustainable, economical and environmental solutions to the dredged sediments management. The present work focused on the assessment of the actual contamination levels and potential availability of metallic elements (As, Cd, Cr, Cu, Ni, Pb, and Zn) present in sediments dredged from 13 different harbours along the English Channel.

The total concentrations of the studied elements in the sediments are determined by ICP-AES after acid digestion. The values are compared to the reference thresholds (GEODE1 and CEFAS2) generally used to determine their fate.

Further investigations are performed to get information on the actual contamination level, the natural/anthropogenic origin or the availability of the elements of interest.

Enrichment factors³ are calculated. Single, sequential and kinetic chemical extractions are used to estimate the potential availability of each element.

All the studied sediments present significant enrichment of Cd, Pb, and Zn (EF values > 2).

These results confirm the need of lability/availability studies to perform valuable environmental risk assessment of dredged sediments.

Generally, important environmental risks are suspected when high enrichment are detected. However, we noticed that in some cases, element with high EF values (i.e, for all sample, EF>4 for Cd and Cu) are poorly available, according the various chemical extractions performed (i.e, available/mobile Cu and Cd<20 % of their total concentration).

According to the reference thresholds GEODE and CEFAS, it appears that among the 13 analyzed sediment, nine sediments cannot be submersible without further analysis (>AL1) and four sediments are considered as not submersible (>N2).

TU 433

Occurrence of pharmaceuticals in marine sediments from Masan Bay, Korea

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Concerns have emerged regarding the presence of human-use pharmaceuticals in marine sediments. In this study, the purpose is to determine concentrations of pharmaceuticals and antidepressants in marine sediments from Masan Bay, urbanized and semi-enclosed bay, Korea. We analyzed 19 pharmaceuticals and 10 antidepressants using accelerated solvent extraction and LC/MS/MS. Among target pharmaceuticals, 11 compounds were detected in 10 sediment samples. Cimetidine, acetaminophen, dimethylxanthine, and codeine were highly detected whereas the average concentration of dimethylxanthine was the highest. The highest total concentration of pharmaceuticals was found at the station close to Masan City, and the next highest concentration was found at the station nearby the outfall of wastewater treatment plant. This result indicates that occurrence of the pharmaceuticals in marine environments is associated with human activities. Interestingly, antidepressants showed no detection in sediment samples. In Korea, prescription and application of antidepressants is rare because Korean people doesn't seem to regard depressant as a disease. At present, there is little information on antidepressants in Korea and then we need to more investigate on pharmaceuticals including antidepressants in Korea.

TU 434

Sediment toxicity and contamination in artificial Lake Shihwa of Korea based on three bioassays

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The artificial Lake Shihwa sediments were examined for contamination using three bioassays: survival of amphipod (*Monocorophium aehrusicum*) for bulk sediments, fertilization of sea urchin (*Strongylocentrotus nudus*) for porewater, and bioluminescence of marine bacteria (*Vibrio fischeri*) for solvent extracts. The Lake Shihwa is an artificial Lake which was constructed by a 12.7 km long dyke for water supply to industrial complex and agriculture area nearby. In the Lake Shihwa region, the cities of Shiheung and Ansan (total population exceeds 1 million) and large industrial complexes (total industrial area = 31 km²) are located. The survival of amphipod for 30 sediment samples ranged from 75 to 100% (median: 95%). The sea urchin fertilization ranged from 1 to 100% (median: 42%) and the bacterial bioluminescence ranged from 12 to 631 mg/L (effective concentration 50%; EC50). The lake sediment contamination was low level. In particular, the survival of amphipod was higher than 80% at all stations, indicating no acute toxicity. High toxicity of sea urchin fertilization, less than 10% of the control sample, was found at four stations in inshore and three stations in offshore. High toxicity of bioluminescence test, higher than 60 mg/mL suggested by NOAA, was found at nine stations in inshore and two stations in offshore. This result suggests that the sediment toxicity was more contaminated in inshore of Lake Shihwa impacted by industrial complexes, compared with offshore containing an outfall of wastewater treatment plants. Benthic macrofaunal assemblages and sediment characteristics (total organic carbon and nitrogen, ignition loss, and acid volatile sulfide) measured in this study also supported to this result.

TU 435

A study of PCBs qualitative profile as biomagnification indicator in an Antarctic foodweb: proposition of a new index

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Concentrations of several organic pollutants groups (PCBs, organochlorine pesticides and PBDEs) were obtained for abiotic and biotic compartments from an Antarctic ecosystem. Considering this large database and also the indication from literature that PCBs heavier congeners are environmentally more persistent, which leads to heavier profiles in higher trophic levels, a parallel between PCBs profile and the biomagnification process itself might come up. In this context, a new index purely based on the chlorination level of PCBs is hereby proposed. This index is the weighed mean of the PCBs, normalized by individual total and weighed by the chlorination level, considering congeners from 2 up to 9 chlorines due to the analytical range. The aim of the present work is to investigate quantitatively and qualitatively the adequation of the proposed index. Samples were collected in King George Island (62°05'S, 058°23'W), Antarctica in the austral summers from 2004/05 up to 2008/09: snow and seasonal melting water, Krill (*Euphausia superba*), limpets (*Nacella concinna*), fishes (*Notothernia rossii* and *coriiceps*), birds (egg and liver from *Pygoscelis antarctica*, *papua* and *adeliae*, *Catharacta sp* and *Larus dominicanus*; only liver from *Daption capense* and *Macronectes giganteus* and only egg from *Sterna vittata*) and pinnipeds (fat from *Lobodon carcinophagus*, *Leptonychotes weddellii* and *Arctocephalus gazella* and liver from *Mirounga leonina*). The index presented significant correlation within the invertebrate group. For limpets, PCBs profiles are probably affected by the lighter profile of the seasonal melting, to the point of presenting negative correlation with $\delta^{15}\text{N}$. For birds, compounds showing negative correlation in eggs, and therefore a lesser trend to be excreted, are the ones presenting positive correlation in liver from the same species *grosso modo*. For liver, there is significant correlation with ΣPCBs , corroborating the index. Pinnipeds presented large fluctuation due to nutritional stress made evident by the $\delta^{15}\text{N}$. For the more comprehensive groups, results were significant in almost all of the cases for more volatile contaminants (HCHs, HCB and Chlordanes) and in some other historically related, such as DDTs, PCBs and Mirex, and also with $\delta^{15}\text{N}$ for poikilotherms. Statistically, the proposed index presented significant correlation with contaminants more times than $\delta^{15}\text{N}$ when considered the invertebrate, poikilotherms and homeotherms groups and all dataset as well.

TU 436

DDT: bacterial structure in marine sediment from the NW coast of Baja California, Mexico

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The introduction of persistent organic pollutants in sediments can cause changes in the structure of microbial communities, resulting in a predominance of organisms capable of surviving exposure to synthetic chemical compounds and of degrading pollutants such as DDT. This study aimed to relate particle size, DDT concentration, organic carbon and sulfate-reducing bacteria (SRB) in sediments to their bacterial structure. In 2006, sediment samples were taken at 17 stations along the northwestern coast of Baja California (Mexico). Analyses of DDT (gas chromatography), particle size, organic carbon and SRB were performed. To determine the bacterial structure, total DNA was extracted from sediments using the FastDNA Spin Kit for Soil and amplified by polymerase chain reaction (PCR), and then purified and analyzed by single-strand conformation polymorphism (SSCP) using the DCode System (Bio-Rad). The bands were extracted, purified and amplified by PCR, and the product obtained was sequenced, purified and assembled. The sequences were aligned and compared with sequences deposited in GenBank. A matrix was generated using the Tamura-Nei model; the MODELTEST program was used to construct a genetic distance tree with the neighbor-joining method. The highest DDT concentrations, particles <0.063 μm and highest organic carbon content were recorded at the stations located in the southern part of the study area. Sediment size and organic carbon content were propitious for the development of SRB such as those belonging to the genera *Desulfobulbus* (band 35A) and *Sulfurovum* (band 59A). Band 35A showed similarity to the GenBank sequences DQ351788 and DQ351792 isolated from North Sea sediments polluted by heavy metals. This bacterium likely plays an important role in the biodegradation of DDT in the study area since SRB are known to carry out reductive dechlorination of DDT and DDD. Band 59A, corresponding to *Sulfurovum*, was isolated from sediments from the station with the highest organic matter content (1.51%) and highest number of SRB (140 NMP g⁻¹), and showed similarity to GenBank sequence EF460072 isolated from contaminated Baltic Sea sediments. The SSCP analysis allowed isolation of seven bands belonging to the domain Bacteria and one to the domain Archaea. The deeper stations in the southern part of the study area showed a larger number of bands (greater diversity) than those in the northern part.

TU 436

Using a novel sediment exposure to determine the effects of Triclosan on marine benthic communities

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Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol), is an emerging contaminant commonly used as an antimicrobial compound in many personal care products such as softsoap, detergent, toothpaste, mouthwash, and is infused in many consumer products, such as kitchen utensils, toys, bedding, socks, and trash bags. Triclosan enters estuarine environments primarily via wastewater treatment plant effluent and has been found in sediment cores dating back to the mid 1960s. The objective of our research was to determine the effect of triclosan on intact estuarine benthic communities. We adapted a novel exposure method which brings intact sediment cores into the laboratory and then exposes the benthic community by addition of toxicant spiked sediments to the core surface. Treatments included: 1) field control - no added sediment, 2) laboratory control - clean sediment added, 3) low triclosan concentration (~20 mg/kg dry), and 4) high (~200 mg/kg dry) triclosan concentration. Macro- and meiobenthic communities were analyzed for differences after a two week exposure to either high or low concentrations of triclosan spiked sediments. Results from the triclosan sediment additions indicated a loss in total abundance and number of meiofaunal and macrofaunal taxa communities at the high triclosan concentration compared to the control. This high exposure concentration is similar to LC50s for amphipods and mysids exposed in the laboratory. We also noted a difference in the field control relative to the laboratory control, most likely due to a smothering effect of the added sediment. Differences in benthic communities were not noted between the low triclosan and laboratory control treatments. These data suggest that at elevated concentrations, triclosan can disrupt benthic communities while also indicating the exposure system does not completely emulate exposures in the field.

TU 438

Environmental variables, pesticide pollution and meiofaunal community structure in two contrasting temporarily open/ closed False Bay estuaries

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Environmental variables (including natural and anthropogenic stressors) and meiobenthic communities were sampled in a 'natural' (Rooiels) and a 'disturbed' (Lourens) estuary in the Western Cape, South Africa, bimonthly for 20 months. A primary aim of the study was to assess if the meiobenthic community structure is driven by different variables when comparing 'natural' versus 'disturbed' system. Due to the much smaller catchment of the Rooiels Estuary, many environmental variables were significantly different ($p < 0.001$) from the variables in the Lourens Estuary, e.g. salinity, temperature, pH, total suspended solids, nitrate and depth. No pesticide concentrations were expected in the Rooiels Estuary due to the absence of agricultural development in the catchment. However, chlorpyrifos (8.9 $\mu\text{g/kg}$), prothiofos (22.0 $\mu\text{g/kg}$) and cypermethrin concentrations (0.42 $\mu\text{g/kg}$) were detected frequently, with the highest concentrations recorded during the summer months. Principal response curve analysis showed that temporal variability between sampling dates explained 42% of the variance in environmental variables and pesticide concentrations and spatial variability between the 2 estuaries explained 58%. Variables contributing most to the differences were higher concentrations of endosulfan, p,p-DDE and nitrate concentrations in the Lourens Estuary and larger grain size and higher salinity at the bottom in the Rooiels Estuary. In general the meiofaunal community in the Rooiels Estuary showed a significantly higher number of taxa ($p < 0.001$), a significantly higher Shannon Wiener Diversity Index ($p < 0.001$) and a generally lower meiofaunal abundance with less variability than in the Lourens Estuary. The variables explaining a significant part (14%) of the variance in meiofaunal abundance in the Rooiels Estuary were salinity and temperature, with the Redundancy Analysis indicating that the abundance of most of the taxa increased with higher salinity and temperature, e.g. Upogebia, Nereis, Urota and nematodes were clearly positively correlated to salinity and temperature. The variables explaining a significant part of the variance in the dataset (43%) within the Lourens Estuary were also salinity and temperature but included chlorpyrifos, nitrate and flow (including river and tidal flow).

TU 439

Biomarker applications in different marine ecosystem for estimation of pollution level

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In this study, the health status of the mussels sampled from Saros Bay (Northern Aegean Sea) Çanakkale (Dardanelles) and Istanbul (Bosphorus) Straits was determined by using biomarker methods (filtration rate and neutral red retention time-NRR). For that aim, the Mediterranean mussel species of *Mytilus galloprovincialis* were transplanted to three sites in Saros Bay and one location in Çanakkale Strait (due to insufficient local population). Local individuals were collected from the Istanbul Strait. Filtration rate experiments indicated that Saros Bay is the least polluted among the selected sites with an average filtration rate 4.20 ± 0.44 L/h. Measured average filtration rate for transplanted mussels in Çanakkale Strait was 3.20 ± 0.36 L/h whereas the filtration rate of the local mussels of Istanbul Strait was found as 2.11 ± 0.67 L/h. The results obtained by the application of NRR biomarker were different than that of the filtration rate. From the experiments of the Istanbul Strait's mussels, NRR time was obtained as 167 minutes. Mussels from the Çanakkale Strait and Saros Bay showed lower values of 116 and 105 minutes respectively. The results showed that the health status of the mussels is moderate and the effect mechanisms of the two biomarker methods are different.

TU 440

First investigation of polycyclic aromatic hydrocarbon (PAH) concentrations and patterns in free-ranging cetaceans from Sea of Cortez, Mexico

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The Sea of Cortez (Gulf of California, Mexico) is one of the seas with the highest biodiversity in all the world. This area is where about the 40% of the known marine mammals of the planet live and a third of the world's marine cetacean species. In fact, this ecosystem is known worldwide for its biological richness and, although it is considered one of the most pristine environments, the increase of human activities is producing noticeable perturbations, like pollution from organic compounds such as organochlorines (OCs), flame retardants (FRs) and polycyclic aromatic hydrocarbons (PAHs). The aim of this study was to evaluate the PAH levels of seven species of free-ranging cetaceans sampled in the Sea of Cortez between 2008-2011, before the anthropogenic exploitation, especially related to tourists activities, will have a major impact than today in the Sea of Cortez. PAHs are one of the most widespread organic pollutants found in fossil fuels and also formed by incomplete combustion of wood, coal, diesel, etc. Therefore, a different type of combustion lead to a different production of isomers and relative amounts of individual PAHs, making them potentially useful as indicators of the burning history and the temporary contamination. As a pollutant, PAHs are of concern due to their carcinogenic, mutagenic and teratogenic properties. The species investigated are three Mysticeti - blue whale (*Balaenoptera musculus*), fin whale (*Balaenoptera physalus*) and Bryde's whale (*Balaenoptera edeni*) - and four Odontoceti - sperm whale (*Physeter macrocephalus*), killer whale (*Orcinus orca*), bottlenose dolphin (*Tursiops truncatus*) and long beaked common dolphin (*Delphinus capensis*). All the free-ranging specimens were sampled using the non-destructive technique of the skin biopsy where the subcutaneous blubber was used for the PAH analyses. The results showed that there is a statistically significant difference in the accumulation of these compounds in the blubber of these animals. Overall, the Odontoceti show higher PAH levels than the Mysticeti and, in both suborders, the accumulation seems to be correlated with the size of the species. Furthermore, comparing the PAH fingerprints of the different species, it is possible to note that Odontoceti has a predominance of low molecular congeners (~ 85%) compared to high molecular ones (~ 15%), while in Mysticeti the comparison is almost the same: ~ 55% of low molecular and ~ 45% of high molecular PAHs.

TU 441

Occurrence and potential ecotoxicity of polycyclic aromatic hydrocarbons in surface sediments from the Changjiang Estuary, China

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A new reservoir that sources water from the Changjiang Estuary will supply half population of Shanghai named as one of mega cities in the world facing server water shortage since 2012. Before the construction of the new reservoir, fifteen surface sediment samples were collected in April 2006 for investigating the occurrence and potential ecotoxicity of persistent organic pollutants in the Changjiang estuary. The concentrations of 20 polycyclic aromatic hydrocarbons (PAHs) compounds in the surface sediments were determined by gas chromatography-mass spectrometry (GC-MS). It was found that the sum of 20 PAHs concentrations ranged from 60 to 4176 ng/g with the mean value of 895 ng/g. The highest level of PAHs in the sediments occurred near the outlets of municipal wastewater treatment plants and tributary river mouths (e.g. Huangpu River and Liuhe River), while the lowest PAHs concentrations appeared at the mainstream including the strategic water sources. Molecular indices of PAHs indicated that PAHs in the sediments of the Changjiang Estuary mainly originated from petroleum leakage and petroleum combustion process. Urban sewage and wastewater discharges and shipping activities were the main contribution sources of PAHs. In addition, the levels of Ah receptor agonists were analyzed by ethoxyresorufin-O-deethylase (EROD) bioassay. The results of bioassay showed that the toxic equivalent (TEQ_{bio}) of sediment extracts ranged from 0.54 to 23.47 pg/g dry weight. The results from both chemical analysis and bioassay indicate that the ecotoxicity of PAHs presents high potential only in the sediment from the Huangpu River mouth, while it presents low potential in most of surface sediments in the Changjiang estuary.

TU 442

A biological adverse effects index and its relationship with metals enrichment factors in sediments from Todos Santos Bay, northwest coast of Baja California, México

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The concentration of metals found in sediments is the result of natural phenomena, anthropogenic activities or the combination of both. Therefore, different normalizing techniques have been used to tell the difference between the natural occurrences of metals in sediments from those of anthropogenic sources. Iron, aluminum, grain size and total organic carbon are among the most widely used normalizers. However, the information yielded by these normalizers mostly indicates the degree of enrichment that the metals have in relation to the kind of normalization used. Enrichments calculated by the different normalizations aren't necessarily indicators of toxicity levels. Thus, in the absence of toxicity studies along with measurements of heavy metals concentrations and/or enrichments in marine sediments, concentrations by themselves, even enrichments, cannot be used to suggest the probability of adverse effects on benthic biota.

Todos Santos Bay (TSB) is located 100 km south of the Mexico-USA border on the northwest coast of Baja California, Mexico. Most of the TSB 330 km² (90%) is 10-50 m deep. The city of Ensenada is located in the inner part of the bay; it has 400,000 inhabitants and the main economic activities are: manufacturing, tourism, fishing, and agriculture. During many years TSB has been environmentally affected by domestic and industrial effluents, fishing and boat traffic, and agricultural runoff.

In 1992 and 2004, heavy metals concentrations were measured in surficial sediments from TSB. The aim was to search for relationships between metals enrichment factors and a biological adverse effects index. Unlike Ni, the elements Cd, Cu and Zn showed significant correlations ($p < 0.05$) between enrichment factors and the biological adverse effects index. Cu showed a 0.74:1 relationship, which means that any enrichment, even a slight impoverish (enrichment factors from 0.74 to less than 1.0), could represent biological adverse effects. On the other hand, Cd and Zn enrichments must be > 5.5 and > 1.5 , respectively, in order for the sediments to be considered toxic. In general, data showed that most of the metal concentrations in Todos Santos Bay sediments couldn't cause adverse effects to biota. Only Ensenada's harbor and the zone next to a dredging dumping site showed metal enrichments that could be toxic.

TU 443

The exposure-dose-response framework for ecological risk assessment of estuarine contaminated sediments

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The Exposure-Dose-Response framework was identified to conduct and integrate the Lines of Evidence in Sediment Ecological Risk Assessment. Data from estuarine sediment environments contaminated by copper, cadmium, lead and zinc including sediment chemistry, bioaccumulation, toxicity, and biomarkers for molluscs, crustaceans and fish and benthic community assemblages were mined from literature and from available databases. The collected data were compared with sediment quality guidelines, explored for relationships within the Exposure-Dose-Response framework. The molar sum of the four metals was also investigated to possibly use it as a concise indicator of exposure and dose in relation to response. The Exposure-Dose-Response relationships were also investigated for data collected in Lake Macquarie, an estuarine lagoon on the East Coast of Australia, historically contaminated by copper, cadmium, zinc, lead and selenium. The molar sum of the four metals showed to be a good indicator of contamination and bioaccumulation in relation to response. Results from Lake Macquarie's mirrored the results of the data from around the world. Dose-Response relationships were shown

to be stronger than Exposure-Response relationships in the mined datasets and in Lake Macquarie's, by-passing the confounding factors due to bioavailability of metals in sediment. Molluscs and fish showed more consistent Exposure-Dose and Dose-Response relationships than crustaceans.

TU 444

Developing ecotoxicological methods for relevant test species in coral reef environments

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There is a severe paucity of applicable ecotoxicological data for tropical marine ecosystems and applicability of toxicity data from temperate species has been widely questioned and criticised. The urgent need to rectify critical gaps in our understanding of ecologically acceptable concentrations of contaminants for the protection of tropical marine environments has been widely acknowledged. Research studies have shown that coral species are extremely sensitive to toxicants, however there are many challenges to developing standard toxicity tests using corals.

Our group had been working with the sea anemone *Aiptasia pulchella* (Carlgren 1943, Fautin 2006, currently under review) to develop toxicity tests for commercial use. The use of the species in standard ecotoxicological studies will represent the introduction of a new taxonomic phylum (Cnidaria) to the suite of commercial toxicity tests available for marine waters in Australia. We have developed animal husbandry methods, and ecotoxicological methods for testing contaminants. Results to date show that the sensitivity of this test organism is relative to previous work using corals. Research continues on the development of sub-lethal endpoints for assessment.

TU 445

Occurrence of polychlorinated biphenyls in *Chelonia mydas* from northeastern and southeastern coast of Brazil

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Polychlorinated biphenyls (PCBs) are persistent, toxic and widely distributed through the atmospheric transport and ocean currents. PCBs can mimic natural chemicals such as hormones and can disrupt the chemical processes of living organisms, some weaken the immune system. The lack of an efficient pathway for the degradation of these compounds, combined with their hydrophobicity, has led to its accumulation in various marine organisms and may undergo a process of bioaccumulation and biomagnification along the food chain. Among organisms more susceptible to the actions of organochlorines are sea turtles, especially species such as green sea turtle (*Chelonia mydas*), which has its occurrence associated with coastal regions, where the highest incidence and accumulation of contaminants occurs. The *Chelonia mydas* is the largest hard-shelled turtle, which is distributed throughout the oceans, in areas of tropical and subtropical waters. The present work aims to verify the occurrence of PCBs in the species *C. mydas*. Liver samples were collected in juvenile dead turtles on the coast of Ubatuba (n=30), state of São Paulo and on the coast of Aracaju (n=40), state of Sergipe, located respectively in southeastern and northeastern region of Brazil. This study used data generated by the Regional Monitoring Program of Stranding and Abnormalities in the Sergipe/Alagoas Basin - PRMEA, from Petrobras, performed by the Aquatic Mammals Foundation - FMA, in partnership with Tamar/ICMBio, as a mitigation measure requested by the Federal Environmental Licensing, conducted by CGPEG/IBAMA. The PCBs were extracted with organic solvents and the purification was made with concentrated acid. The identification and quantification of PCBs was performed in gas chromatograph with mass spectrometer. PCBs were found in 76% of samples from Ubatuba and 35% of the samples of Aracaju. The concentrations of total PCBs in liver samples of Ubatuba were, in wet weight: <0.38 to 17.4 ng g⁻¹ and in samples of Aracaju ranged from <0.38 to 142.6 ng g⁻¹. Predominance of light PCB congeners (3-5 chlorine) can be observed in the majority of the samples analyzed, showing similar distribution profile in the turtles of both areas that can be related with long range atmospheric transport. The accumulation of PCBs can be linked with omnivorous turtle diet during juvenile stage. More over the low levels are associated with the maturity of those turtles.

TU 446

Investigation and development of a non-invasive LC-MS/MS quantitative method to determine the stress levels of whales

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The aim of this project is to determine if it is possible to detect the presence of hormones in whale blow samples, and see if the hormone levels could be related to stress due to their environmental situation. If successful the project would enable a non-invasive method to be developed to determine the stress levels of species that are difficult to study. Specific hormones, progesterone and cortisol, were monitored in samples of whale blow from a variety of different whale species. The results showed that both cortisol and progesterone were detected easily in the lowest calibration standard (0.5 pg/μL) suggesting lower detection limits are possible with further method development.

Reproducibility was excellent, %RSD for both compounds were less than 2% for 5 replicates of a 5 pg/μL standard. Linearity for both compounds was excellent; r² = 0.999 for cortisol and r² > 0.999 for progesterone.

Cortisol was detected in the majority (22 of 25) of samples at a level higher than the lowest calibration point. Progesterone was detected in 13 samples higher than the lowest calibration point.

For all samples parallel collection of both MRM & full scan data was obtained and is shown for each of the species types. This set of data enabled other compounds to be simultaneously screened, and any matrix background to be monitored.

TU 447

Measurement of trace element and perfluorinated compound (PFC) concentrations and species identification of shark fins from the Hong Kong market

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Shark populations are declining around the world, largely due to overfishing and the global shark fin trade. In contrast to many other marine predators, such as marine mammals, there is a lack of information on the contaminant status of sharks, likely because sharks are often more difficult to obtain. The larger pelagic species, in particular, are often available only for opportunistic sampling as bycatch, but are well represented in the shark fin market. The purpose of this study was to assess the levels of two groups of contaminants, trace elements and perfluorinated compounds (PFCs), in shark fins purchased from dried seafood shops in Hong Kong, one of the largest markets in the world for shark fins, and to identify the sampled species using DNA sequencing. Shark fins, composed primarily of collagen, can be used for monitoring protein-bound contaminants, and also can be used to assess the hazards of human exposure to these chemicals through the consumption of shark fin soup, a traditional Chinese dish. Shark fins (n=30) from several trade categories were purchased and analyzed for trace element (Ag, As, Cd, Co, Cr, Cu, Mn, Mo, Ni, Pb, Sb, Se, Ti, and Zn) levels using microwave digestion and inductively coupled plasma-mass spectrometry (ICP-MS). A new method was developed for the extraction of short- and long-chain PFCs from shark fins and PFC concentrations were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS). DNA was extracted from the purchased fins using a commercial kit and species were identified using validated primer sequences. Trace element concentrations varied among individuals and species, and hazard assessment of the measured levels indicated that the overall risk to human consumers was low. The results of the species identification confirmed the presence of shark species listed as threatened or endangered by the International Union for the Conservation of Nature on the Hong Kong market, and will be discussed in the context of changes in shark populations over the past 10 years, as well as the occurrence and bioaccumulation of trace elements and PFCs in sharks.

TU 447

Persistent organic pollutants and heavy metals in marine species stranded, threatened of extinction

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Sea turtle and cetaceans are two groups of species with most of their members threatened of extinction in some grade and, therefore, protected. The current social and industrial development has produced new and persistent forms of pollution reaching coastal and marine waters and the ecosystems that these support. Currently, in the Member States of the European Union, the chemical quality of surface waters is regulated by the Water Framework Directive (WFD). In this context, Decision 2455/2001/EC established a list of 33 substances or groups of substances of priority concern because of their persistence, toxicity and widespread use and detection in rivers, lakes, transitional and coastal waters. The current list includes certain PAHs (Polycyclic Aromatic Hydrocarbons), pesticides, PCBs (polychlorinated biphenyls), PBDEs (polybrominated diphenyl ethers), etc. Globally, studies of contaminants in cetaceans and sea turtles stranded have been developed especially in the last ten years. However, still there are few studies exist in this field, and even fewer studies with large numbers of samples to obtain a less biased information. Studies have focused primarily on organochlorine and heavy metal, with very few or absent studies with other persistent organic pollutants. Due both to the fact of location into the top links of the food chain (being some of them super-predators) and to the presence of large blubber layer, they tend to biomagnify and to bioaccumulate persistent organic compounds in the case of cetaceans, and heavy metals, in the case of sea turtles (although in both groups are both types of compounds, the concentration ranges are different). In this work, a review of studies is showed, focusing in the chemicals, levels, organs, and species.

TU 448

Organic pollutants in tern eggs from Rodrigues Island - Indian Ocean

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Very little is known about organic pollutants in biota in general, and marine birds in particular, from the Indian Ocean. The Indian Ocean has Africa to the east and Asia to north - areas where active use of POPs such as DDT is used for malaria control and where legacy POPs such as PCBs have only recently begun to be phased out. The Indian Ocean has many small islands where large marine bird colonies can be found; many of these islands are quite isolated.

The aim of this study was to investigate the potential of such isolated islands as global background sites of persistent organic pollutants. We collected 10 eggs each of the Common Noddy (*Anous stolidus*) and Sooty Tern (*Onychoprion fuscatus*) breeding colonies from Cocos Island (19 43S, 63 17E), a marine reserve near Rodrigues Island.

Rodrigues Island is part of the Republic of Mauritius, and has a small human population with no industry other than fishing and an oil-fired power plant. Pooled samples were analysed for a range of pollutants to select target analytes. Pooled analyses revealed no detectable PBT, PBEB, DPTE, HBB, and BTBPE. Of the PBDEs, only PBDE-47 and -100 were detected. No further analyses of brominated compounds were done. All eggs were then analysed for HCB, PCB, DDT, HCH, mirex, toxaphenes, and chlordanes.

Geometric means were (ug/g wet mass, in all cases, Common Noddy is listed first, flowed by Sooty Tern): HCB 0.37 and 0.4; mirex 0.92 and 0.66; total DDT 1.71 and 2.4; total chlordanes 0.092 and 0.14; total PCBs 2.1 and 2.5; total toxaphenes 0.24 and 0.58; total HCH 0.25 and 0.22. Although the levels were very low and close to detection limits for some compounds, t-tests of log-transformed data revealed significant differences ($p < 0.05$) only for mirex, chlordanes, and toxaphenes.

Both species are pelagic terns with similar feeding patterns. The levels found were low as expected, but the continued presence of compounds such as mirex and toxaphenes in tropical bird eggs from the southern Indian Ocean shows the presence and global distribution of even legacy POPs in isolated oceanic islands. The slight differences between the species indicate possible differences in feeding patterns. Since these species are not endangered and breed in large colonies, their eggs are ideal monitoring tools. Bird eggs from islands such as these and elsewhere in the Indian Ocean may be ideal candidates for monitoring the effectiveness of interventions on an oceanic scale.

TU 449

Assessments of biotransformation and ecotoxicological effects of alkylated Polycyclic Aromatic Hydrocarbons

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Even though spilled oil has been a subject of examination for many decades, there still exist major unresolved gaps in the knowledge of the environmental fate and effects of oil compounds. Alkylated PAHs are much more abundant than regular PAHs. In spite of this and their known persistence and human carcinogenic effects they have only been given little attention regarding ecotoxicological impacts. This work focus on the environmental fate and effects of four methylated PAHs: 1-methylpyrene, 1-methylphenanthrene, 3,6-dimethylphenanthrene, 5-methylchrysene and two regular PAHs: pyrene and phenanthrene. The assessment is divided into: a) determination of biotransformation rate, b) identification of biotransformation products and c) toxicity testing of the methylated and regular PAHs. The toxicity was determined using standard *Daphnia magna* immobilization tests. *Nereis diversicolor* was used as the biotransformer because this worm exists in large numbers, and accounts for a significant turnover of carbon in the marine seafloor. Identification and quantification of biotransformation products was performed using UHPLC connected in series to a fluorescence detector and a Quadrupole Time of Flight Mass Spectrometer. Our results show that:

a. Alkylated PAHs are biotransformed at a much higher rate than regular PAHs

b. Carboxylates are the most abundant transformation product of the alkylated PAHs, whereas no phase I products were found for regular PAHs

c. Common phase II products (glucuronide-, glucoside-, sulfate- and glycine conjugates) are found in both types of compounds, however glycine conjugates are only found for alkylated PAHs

d. It is the hypothesis that the toxicity tests show that the methylated PAHs are more toxic than the regular PAHs, however these tests have not been performed yet.

Whereas a fast biotransformation rate may seem as a good feature for the methylated PAHs, as they will be removed fast, there is also evidence that biotransformation of PAHs may produce compounds that are more toxic than the PAH itself. Hence, a high biotransformation rate may increase the environmental concentration and thus the exposure concentration of toxic transformation products. The high production of carboxylates signify the need of further ecotoxicological assessments of these compounds, as they are produced in high quantity and at a relatively high rate. Furthermore, these products are water soluble and will therefore be transferred to the water column.

TU 450

Addressing emerging issues in marine metal ecotoxicology with novel analytical techniques

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Two emerging issues in marine metal ecotoxicology are (1) the relative importance of animal physiology (elemental homeostasis) towards metal toxicity in transitional waters with intermediate salinities and (2) metal mixture toxicity. For both issues a mechanistic understanding of metal and element homeostasis upon mixture exposure is needed to enhance fundamental knowledge and applied risk assessment procedures. This study aimed at assessing the physiological impact of metal (mixture) toxicity on the homeostasis of essential elements. We used micro X-ray fluorescence (XRF) techniques to analyse mussel gills.

Gills of mussels (*Mytilus edulis*) of approximately 1.5 cm, exposed to a control or copper contaminated artificial seawater, were dissected and (a) immediately cryogenically frozen on-site or (b) dehydrated in a graded acetone:water series and fixed with hexamethyldisilazane (HMDS). Elemental distributions were analysed with synchrotron based micro-XRF. In a second series of experiments, mussels were exposed to different salinities and Cu concentrations, upon which HMDS-embedded gills were analysed with laboratory source based micro-XRF. In a proof-of-principle experiment to study metal mixture toxicity, small *M. edulis* were exposed for 48 h to one of the following treatments in artificial seawater (i) control, (ii) 100 µg/L added Cu, (iii) 1 mg/L added Zn or (iv) a mixture of 100 µg/L Cu and 1 mg/L Zn. Again, HMDS-embedded gills were analysed with laboratory source micro-XRF.

Preliminary results indicate that the internal distribution of metals in mussel gills at the micro scale is not significantly affected by the dehydration and HMDS fixation and enhanced tissue preservation, compared to the cryogenically prepared samples. Gills of mussels exposed to 40 µg/L Cu at a salinity of 32 psu exhibited a decrease in K content compared to the control. Analyses of mussel gills exposed to salinity and combined Cu/salinity stress are ongoing. As an example of differences in elemental homeostasis between single metal (Cu or Zn) and mixture exposed mussels, this K depletion upon Cu exposure was present to a lesser extent upon Zn exposure, but was absent upon exposure to the mixture.

Micro-XRF can provide fundamental mechanistic knowledge about physiological effects of metal mixtures or metal toxicity in transitional waters. It revealed different physiological consequences of metal mixture exposures compared to single metal exposures.

TU 452

Salting-out effect in aqueous NaCl solutions- experimental data and modeling

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Increasing NaCl concentrations in aqueous solutions shift the equilibrium partitioning of organic solutes towards non-aqueous phases like organic matter or air. This so called salting-out effect has long been known but there are rather few experimental data and many of these data are contradicting. Here we present experimental data on the salting out effect of NaCl on a large and diverse set of organic chemicals together with an evaluation of various modeling approaches.

TU 453

Photobromination of phenol and salicylic acid in coastal marine waters

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A large number of experimental studies aiming at understanding the photo-fate of organic pollutants in fresh surface water have been published. In contrast, considerable less attention has been paid to the possible environmental significance of photoreactions of anthropogenic compounds in coastal seawater. The formation of halogenated organic compounds occupies a special position since they have complex diversities of biologic effects: Immunotoxicity, endocrine toxicity, reproductive toxicity, carcinogenesis. The biotic processes are the main published and focused natural origins of halogenated organic chemicals while to date relatively little attention has been focused on abiotic chemical processes for organohalogen production. It is thought that abiotic chlorination or bromination is due to the formation of $\text{Cl}_2\cdot^-$ or $\text{Br}_2\cdot^-$ radicals as a consequence of Fe(III) or Fe(II) - dissolved organic matter complex irradiation or as a consequence of the reaction between hydroxyl radicals and Cl^- or Br^- species. However, the quantitative relevance of these processes has not been fully assessed yet and probably do not account for the total abiotic generation of halogenated derivatives in seawater. This work presents results of photohalogenation studies in coastal seawater, using phenol and salicylic acid as model molecules. Natural seawater was sampled in the Gulf of Lyon (southern France), a coastal area with depth around 10-20 m, characterized by a modest hydrological recycle, and subjected to noticeable anthropogenic inputs, from the city of Marseille and the Rhône river. Phenol and SA were added to the seawater at environmental concentration levels ($< 1 \mu\text{g/L}$) and the samples were irradiated under simulated solar light in presence of different radical generator (i.e.; superoxide and nitric oxide). The extension of indirect photolysis and the formed intermediates were evaluated. The degradation mediated by natural photosensitizers formed several secondary pollutants including mainly brominated compounds (i.e., 2,4,6-tribromophenol and 5-bromosalicylic acid) and to a lesser extent hydroxylated and nitrated derivatives. Brominated intermediates might originate from the reactivity of hypobromous acid (HOBr) resulting from the oxidation of bromine ion by peroxyxynitrite (ONOO^-) and should be catalysed by bromamines. A potential photochemical source of tribromophenol in coastal seawater will be discussed.

TU 454

Laboratory scale simulation of CO₂ leakages from marine stable geological storage: metal accumulation and biological effects on the benthic organism *Ruditapes philippinarum*

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Nowadays, the injection and storage of CO₂ in marine geological stable formations is proposed as one of the potential strategies to decrease the atmospheric CO₂ concentrations in order to avoid the abrupt and irreversible repercussions of climate change. Nevertheless, practical implementation of this technical option could produce significant impacts on marine ecosystems due to possible CO₂ leakages that may occur during the injection and sequestration procedure.

This study aims to quantify the possible effects of variation in sediment pH derived from CO₂ leakages on metal bioaccumulation. To this end, a lab scale experiment involving direct release of CO₂ was conducted using the model benthic organism, *Ruditapes philippinarum*. Bivalves were exposed during 10 days to sediment samples under established pH conditions (8.0-6.0). Survival, burrowing activity and metal accumulation (Fe, Co, Pb, Mn, Al, Zn, Cu, Hg and As) on whole body were employed as endpoint. After 10 days of exposure, 100% mortality was observed at pH 6.0. Non significant differences for mortality rate were found in 7.0 and 6.5 treatments with regard to the control (7.9). Burrowing activity was affected by pH, decreasing the activity at low pH levels. In general, metal concentrations in clam tissues were affected by pH. These results

conclude that CO₂ leakages might provoke an increasing of sediment toxicity linked to metal mobilisation.

TU 454

New piling materials for seawater environments: an ecotoxicological assessment

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Since many centuries, estuarine and lagoon environments, and marine coastlines required huge amount of wood for maritime works, above all mooring piles, docks, bulkheads and navigation channel marks. Recently, wooden piles used in Venice lagoon (Italy) showed an increased decay rate mainly due to the action of woodborers. It has been estimated that along the shipping channels there are about 22,000 wooden piles serving as channel marks and around 10,000 are present for boats mooring. Each year, a large number of them must be replaced. Local authorities in order to keep navigation safety and increase environmental protection, reducing wood consumption, identified a set of ready-to-use piling materials that are expected to be long lasting. Particularly, traditional, alternative and improved woods as well as other synthetic materials have been taken into account during our survey. Their ecotoxicological impact has been assessed considering a battery of testing species (*Vibrio fischeri*, *Phaeodactylum tricornutum*, *Crassostrea gigas*, *Amphibalanus amphitrite* and *Tigriopus fulvus*) and various endpoints. Tests were performed on leachates obtained according to standard procedures. Integrated toxicity data evidenced a series of pros and cons for both traditional and synthetic materials. Some of them were deemed as not suitable, whereas for others specific scenarios of use were suggested.

TU 455

Mobility and mineralogical speciation of aluminium in contaminated sediments and clays

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Aluminium sacrificial anodes are commonly used for the protection of metallic structures in marine harbours. The environmental impact of these anodes remains poorly understood. Indeed, the anodes solubilization contributes to the increase of aluminium concentration in water and finally in sediments (1).

The aim of this study is to investigate the mineralogical speciation of the solubilized aluminium in sediments and clays.

Sediments are brought into contact with seawater contaminated with aluminium either by using aluminium sacrificial anodes in a thermostated cell with a classical three electrodes system or by dissolving an aluminium salt: aluminium sulphate (Al₂(SO₄)₃·18H₂O) or aluminium chloride (AlCl₃·6H₂O).

In order to evaluate the potential aluminium mobility and the evolution of its mineralogical speciation, single and sequential extractions (2) were applied to natural sediments and clays before and after aluminium contamination. All the leachates are analysed by ICP-AES (Varian, Vista MPX).

The results show that whatever the mode of contamination was, the additional aluminium is more labile than aluminium present initially in sediments and clays.

TU 456

Identification of contaminants in marine waters using high resolution fractionation combined with mass spectrometry

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Primary production by microalgae embodies the carrying capacity of marine ecosystems and is primarily linked to nutrient availability and light. However, recent studies indicate that certain anthropogenic contaminants and natural toxins may have a direct impact on coastal plankton communities and hence on the carrying capacity of estuarine and marine ecosystems. However, little is known about unidentified contaminants and mixture effects of anthropogenic compounds and natural toxins under field conditions. Linking biological responses and chemical compounds is often problematic due to the large numbers of different compounds present in marine waters. In our study we aim to identify the contaminants that are responsible for toxic effects on photosystem II efficiency in microalgae using GC-MS and LC-MS. We use Effect Directed Analysis (EDA) to identify the compounds that have an effect on microalgae in order to quantify the toxic pressure in Dutch estuaries and coastal waters. In EDA, analytical chemistry is combined with bioassay analysis to isolate and ultimately identify the compounds in a complex sample that are responsible for the observed effects. We use passive samplers to extract contaminants from estuarine/marine waters during a period of 6 weeks. By using ultra performance liquid chromatography with UV detection, extracts are fractionated into 96 wells plates, resulting in far more, smaller fractions per sample than commonly used in EDA. After evaporation of the solvent the well plates can be directly used for microalgae toxicity assessment using Pulse Amplitude Modulated fluorescence (PAM). With this high resolution fractionation technique the complexity of the fractions is reduced to simplify the chemical identification of the contaminants responsible for the biological effect.

TU 458

Human health risk assessment of selected metals in the diet of the UAE community

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The Arabian Gulf is a rich ecosystem with abundant fish supply; hence, fish constitutes a major portion of the daily meal for not only the people of United Arab Emirates (UAE) but also the region in general. The UAE has also recently diversified the production of vegetables in the local agricultural zones. The population of the UAE has a great diversity in its cultural distribution and those different cultures have dietary choices typical for them; therefore the risk of metals in the diet of different cultures may vary depending on those choices. This research was done using the concentration of several metals in locally produced seafood and vegetables to formulate a health risk assessment for the community of the UAE based on their dietary habits and weights. Samples were acid digested and analyzed for metals on an Inductively Coupled Plasma Spectrometer. UAE community dietary choices and body weights were characterized by conducting a survey on 100 students of various cultures from the American University of Sharjah finding their eating habits and average body weights. Results were then formulated to give consumption rates for the average person and the number of times the food type could be safely consumed within a certain time period. The survey suggests that those individuals from the Middle East, Europe and North Africa were at a greater risk than others because of their high intake of seafood. Also, those from the Asian subcontinent were at greater risk due to smaller body sizes than those from other regions. Overall, risk of metals depended on food type and metal considered. For example, with regards to arsenic, risk levels were low, because although the fish Shekalie had the highest arsenic levels, the average person in the UAE could still eat over 5.4 kg/day of the fish without surpassing the maximum intake levels suggested by the World Health Organization.

TU 458

Novel effects of natural and anthropogenic halogenated phenolic compounds on developmental stages of the zebrafish *Danio rerio*

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In recent years halogenated phenolic compounds (HPCs) such as hydroxylated polybrominated diphenyl ethers (OH-PBDEs) have been found in many species from the Baltic Sea, e.g. cyanobacteria, red alga, blue mussels and a variety of fish species (1, 2). Although these substances seem to be primarily natural products (3) they can also be produced by *in vivo* metabolism of PBDEs. It has been shown that many marine algae species (e.g. red algae) are able to produce OH-PBDEs (4). The amount of product formed depends on environmental factors like temperature, light, wind or nutrient supply. Variations, e.g. due to climate changes might increase the levels of OH-PBDEs produced dramatically (5). Recent studies have shown that one of the most ubiquitous OH-PBDE found in marine fish, 6-OH-BDE 47, is toxic to zebrafish *Danio rerio* embryos (6). This developmental toxicity is, at least in part, due to potent inhibition of mitochondrial oxidative phosphorylation (OXPHOS), thereby disrupting the main source of cellular energy. In this study, we hypothesize that other HPCs may also modify energy metabolism and that this effect might explain some of the severe health effects observed in Baltic Sea wildlife, such as wasting syndrome, decrease in fat content and reproductive insufficiency. Several HPCs found in different fish species in the Baltic Sea have been identified and synthesized and are currently being tested for effects on OXPHOS, with both *in vitro* and *in vivo* models. We have developed a novel method for monitoring the disruption of OXPHOS *in vivo* in a small fish species (*Danio rerio*). The effects of HPCs on mitochondrial respiration in the living organism are measured using various techniques, including oxygen consumption, acid production and mitochondrial membrane potential. Preliminary results indicate that a number of HPCs disrupt OXPHOS. Studies are currently ongoing to link these novel effects on energy metabolism in the zebrafish embryo with effects, like a decreased fat content, found in fish sampled from the Baltic Sea.

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TU 460

The challenge of sustainability of marine resources: results of FAROS Initiative

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Discards are described by the FAO as "that portion of the total organic material of animal origin in the catch, which is thrown away, or dumped at sea for whatever reason. It does not include plant materials and post harvest waste such as offal. The discards may be dead, or alive", i.e. both no commercial as well as target species. No commercial species are generally dumped because it is undesirable (i.e. there is no market or other use for it) and therefore uneconomic to keep on board. Target species may be discarded due to poor quality, undersized specimens (below Minimum Landing Size) or quota limitations among others.

Nowadays, there is a common and positive agreement (among citizens, NGOs, the fishing sector, policymakers, scientist, etc.) that perceives discards as very negative and that solutions have to be implemented. In this framework of promoting the responsible and sustainable management of the European fishing activity, the European Commission developed a number of actions directed to the development of policies "to reduce unwanted by-catches and eliminate discards in European fisheries", as well as to make "the best possible use of the captured resources avoiding its waste". In this framework of defining strategies and solutions to accomplish with the EU policy of "no discards", the

Project FAROS - co-funded under the LIFE+ Environment Program of the European Union - takes advantage of the background of experience, good practices and previous technologies to give the guidelines to define a global and real-time information system both on board and in land. This system will be the core of an efficient and optimal discards management network of the actors involved in the fishing activity (fishermen and fleets, ports, industries, etc.) by exploiting of the existing synergies between them in order to attain the minimization of discards/by-catch as well as their optimal valorization to recover and to produce valuable chemicals of interest in the food and pharmaceutical industry.

TU 460

Brominated flame retardants and dechloranes in eels from German rivers

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The European Eel (*Anguilla anguilla*) is a catadromous, carnivorous fish. It is widely distributed over Europe and has a high economic value for the fishing industry. Its overall population has been declining rapidly since the 1980s and has by now dropped to 1% of the average population during the 1970s. In consequence the International Union for Conservation of Nature (IUCN) has rated the eel as "critically endangered".

In order to prevent a further decline it is crucial to identify the causes as well as implement measures to stabilise the population. Currently several possible natural as well as anthropogenic causes are being discussed. Chemical pollution as a possible cause has become one of the main focuses as the eel is predestined to take up large quantities of especially lipophilic organic pollutants such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and brominated flame retardants (BFRs) due to its high lipid content.

The focus of this research project is the analysis of polybrominated diphenyl ethers (PBDEs), alternate brominated flame retardants (alternate BFRs) and Dechloranes (DP, Dec-602) in elvers, yellow- and silver eels from different German rivers with GC-MS in order to compare the quality of these rivers as habitats for eels (in terms of chemical pollution) and to estimate the BFR contamination during the eel's life cycle.

Seven PBDEs could be detected in yellow and silver eels with BDE-47 as main congener.

Silver eels show especially high concentrations of PBDEs in fish fillet with up to 18 ng/g wet weight (equal to 60 ng/g lipid weight) of sum PBDEs. Alternate BFRs such as pentabromoethylbenzene (PBEB) and 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE) as well as Dec-602 and the syn- and anti- isomer of Dechlorane Plus (DP) could be detected in all life cycle stages analysed with concentrations between 0.04 ng/g ww (equal to 0.1 ng/g lw) for Dechloranes and 2.5 ng/g ww (equal to 10 ng/g lw) for DPTE. For PBDEs equally high concentrations have been reported in studies from other countries such as Belgium. The high concentrations are alarming, as many BFRs are toxic for aquatic organisms and are therefore likely to affect the eel's health and ability to reach its spawning ground. There are so far no other data for Dechloranes in aquatic biota in Europe.

ET14P - Bringing ecological processes into ecotoxicological risk assessment

TU 461

Effects of polycyclic aromatic hydrocarbons on growth and grazing rates of Atlantic Ocean Phytoplankton communities

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Pollutants exert a significant toxic effect in organism's worldwide, altering the species' interactions and ecology. Despite their impact on ecosystems, effects of pollutants on species interrelations have received limited attention in the scientific community, being scarce the experiments performed with natural communities. It is therefore important to analyze the role of species interactions in toxicological responses in the field.

Among the broad spectra of pollutants reaching the ecosystems, Polycyclic Aromatic Hydrocarbons (PAHs) are of special interest. Introduced in remote oceanic regions by atmospheric transport and subsequent deposition, and their bioaccumulation on planktonic food webs subsequently stress marine communities. Some studies analyzing the PAHs effects on zooplankton and phytoplankton populations independently showed a variety of responses and sensitivities, being in some cases phytoplankton populations are highly resistant to PAHs. However, their interactions have been scarcely analyzed until now.

It has been observed that zooplankton can be more sensitive to these pollutants than phytoplankton, the balance between growth and losses of phytoplankton could be strongly affected by PAHs.

Here we quantified the growth and grazing rates of natural oceanic phytoplankton communities and the effects of PAHs in the balance between grazing and growth in these communities by dilution experiments, following the modified dilution method of Landry and Hassett (1982). The phytoplankton abundance was analyzed by flow cytometry and Chlorophyll a concentrations by fluorimetry during the Malaspina Expedition 2010 across Atlantic Ocean.

Experiments showed that the presence of PAHs affected the grazing rates and the balance between growth and grazing in phytoplankton, which may impact in the oceanic food web. Grazing on the total phytoplankton community was slightly reduced by PAHs, although the growth rate was less affected, suggesting more effect on grazers than on phytoplankton at the levels tested. The analysis of the grazing and growth rates in the presence of PAH's on the dominant picocyanobacteria populations indicated strong differences. *Prochlorococcus* showed more sensitivity to PAHs than *Synechococcus*, as some previous studies show, and our results suggest that *Prochlorococcus* sp. grazers could be sensitive to PAHs, more than *Synechococcus* sp. grazers.

TU 462

Aquatic ecosystem model framework for use in refined environmental risk assessment

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Recently, new approaches of combining toxicokinetic-toxicodynamic (TKTD) models and individual-based population models (IBM) are being developed to simulate effects of chemical exposure on the population level of sensitive organisms. These individual-based population models provide applicable endpoints for the ecological risk assessment such as time to recovery, extinction probability, or mean population density.

In this presentation, we will introduce a concept to integrate the TKTD and IBM models in an ecosystem model framework, which on the one hand allows to consider new more ecological relevant endpoints on the ecosystem level in the risk assessment (which is also of interest in the context of ecosystem services), and on the other hand enables to evaluate population endpoints derived from model simulations under altered, more realistic environmental conditions.

Therefore we use a complex ecosystem model for lakes (StoLaM), which includes important properties of standing waters such as lake morphometry (including water volume and depth), trophic conditions (dynamic nutrient cycle), as well as food web composition and complexity. These lake properties are adjustable for specific issues and will usually influence the environmental conditions of planktonic populations significantly. Additionally, time variable weather conditions and exposure patterns as important forcing functions for the physical condition of ponds and lakes are also included in this modelling concept.

By using this model concept indirect effects can emerge on both, population and ecosystem level: indirect effects on the biomass of planktonic organisms due to competition and predation dependent on the implemented food web, as well as indirect effects on water quality parameters such as oxygen concentration, algal biomass and water turbidity. These indirect effects have to be addressed in higher tier studies. For example, time to recovery of a treated planktonic population may vary due to indirect effects, e.g. increasing resources, decreasing competition and predation, respectively.

We will show a framework of TKTD models, IBM models and an aquatic ecosystem model for quantifying responses to stress at various levels of ecological complexity for planktonic organisms of standing waters.

TU 463

Understanding the influence of competition on effect and recovery of *Daphnia* spp. from pesticide exposure in outdoor microcosms

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Competition is an ubiquitous factor in natural populations and has been reported to alter the ecological impact of xenobiotics. We exposed zooplankton communities to esfenvalerate (0.03, 0.3, and 3 µg/L) in outdoor pond microcosms and investigated the short-term and long-term effects on populations of *Daphnia* spp. To cover a broad and realistic range of environmental conditions, we established 96 microcosms with different treatments of shading and periodic harvesting.

Results showed that the pesticide effect on *Daphnia* spp., was strongly enhanced by intraspecific competition until 11 days after contamination. We further revealed that recovery of populations depended on the strength of the initial pesticide effect and the related increase of less sensitive, but closely related competing taxa. These observed interactions were independent from the applied treatment of shading and harvesting and observed to be most pronounced at 0.03 and 0.3 µg/L esfenvalerate, concentrations characterised by partial mortality of the daphnids shortly after contamination. We conclude that understanding and quantifying the influence of biotic/abiotic factors are important for a more realistic prediction of toxicant effects in the field.

TU 464

Effects of mercury on biomarkers of *Corbicula fluminea*, a non-native invasive species in Europe

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Mercury is considered one of the most toxic metals and a high concern over its toxic effects on organisms, populations and ecosystems exist. Contamination of aquatic ecosystems by this metal can occur from several sources including after its use as biocide. Here, the effects of this metal on interspecific competition were investigated using the non-native invasive species (NIS) *Corbicula fluminea* and other bivalves which are native competitors of this NIS in ecosystems of the Iberian Peninsula. *C. fluminea* was selected as a NIS model since biological invasions by this species have been identified as one of the major causes for the decline of native molluscs' populations in local rivers (e.g. Minho River). Animals from different species were exposed in laboratory conditions to several concentrations of mercury. Several biomarkers of neuromuscular function, energy production, anti-oxidant defences and feeding rates were used as effect parameters. Results indicate that mercury caused toxic effects in all the studied species but *C.*

fluminea was less sensitive to mercury than its native competitors. Furthermore, the findings indicate that contamination of aquatic ecosystems by mercury may act in favor of non-native invasive species in situations of competition with native ones.

This study was carried out in the scope of the project "NISTRACKS - Processes influencing the invasive behaviour of the non indigenous species *Corbicula fluminea* (Mollusca: Bivalvia) in estuaries - identification of genetic and environmental key factors" funded by the Portuguese Foundation for the Science and the Technology (FCT) (PTDC/AAC-AMB/102121/2008) and FEDER COMPETE funds (FCOMP-01-0124-FEDER-008556). P. Oliveira had a PhD grant from FCT (SFRH/BD/82402/2011) funded by national funds of the MCTES and FEDER European funds (POPH-QREN-Tipology 4.2.).

TU 465

Predation: boost or buffer of chemical effects?

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Current environmental risk assessment schemes predominantly rely on standard laboratory test systems and thereby largely ignore natural interactions. However, natural populations regularly undergo shifts in abundance and structure and are subject to diverse interactions such as competition or predation.

Predation mostly appears to be selective, thus has the potential to significantly affect both dynamics and demography of a prey population. The effect on population demography will thereby depend on the predator type. In aquatic systems, gape-limited predators such as fish or large insects tend to prefer the largest prey available, whereas size-selective predators, e.g. small insect larvae, are restricted to smaller prey (direct effect). Moreover, it has been shown that predator kairomones might cause shifts in life history traits, including variations in the size at maturity and the size of newborns (indirect effect).

Chemical stressors were often found to act on certain life history traits or selectively affect only the most sensitive life stage within an exposed population. Therefore, the magnitude of effects caused by chemical exposure might depend on population demography under more natural conditions.

With regard to the combination of both chemical and predation stress, we hypothesize that predation will either increase or buffer the effect of chemical exposure depending on the type of the predator, and that kairomones might enhance the respective effect. We test the hypotheses by using an individual-based model that makes predictions on *Daphnia magna* population dynamics and that allows for the systematic exploration of predator type as well as kairomone effects.

Our study reveals that in case of a chemical that mainly affects small individuals, direct and indirect effects of gape-limited predation favour size classes that are more vulnerable to chemical exposure. In this particular case, the release of a chemical would contradict co-evolutionary processes in the predator-prey relationship. By contrast, chemical effects will be lower in systems that include size-selective predators. As a consequence, extrapolation of effects from those systems will underestimate population vulnerability in systems without any predation or with gape-limited predation.

TU 466

Inferring the effects of chemicals on biodiversity: a case study with the herbicide linuron

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Ecological risk assessment aims at the protection of ecosystem structure and functions. Biodiversity is a widely accepted indicator for ecosystem structure and can be assessed using various indices. Currently, information on the effects of chemicals on these indices is lacking, making it difficult to explicitly include biodiversity as a protection goal in risk assessment.

Here, we analyzed the results of a microcosm study assessing the effects of the herbicide linuron on a ditch ecosystem. Using a novel statistical modeling technique called generalized additive mixed models (GAMM), we studied the effects of initial diversity, linuron concentration and time on biodiversity. Six biodiversity indices of the phytoplankton community were used: species richness, rarefied richness, Margalef index, Shannon evenness, Fisher's alpha and reciprocal Berger-Parker index. Each biodiversity index quantifies different aspects of biodiversity (e.g. richness, evenness). The advantages of GAMMs over classical regression models are that (1) they allow for non-parametric and non-linear relationships between explanatory and response variables and (2) they can incorporate temporal autocorrelation, typical for time series data. The analysis showed a negative effect of linuron on all biodiversity indices except for the reciprocal Berger Parker (dominance) index, which was positively affected.

Differences between biodiversity indices could be observed in (1) the time and (2) linuron concentration at which an index was affected. For four out of six biodiversity indices, a lower initial value resulted in a stronger effect of linuron, indicating the increased susceptibility of less diverse communities.

For a low initial biodiversity index, a significant difference between control and treatment could be observed at 4-10 µg/l for richness-related biodiversity indices. Dominance and evenness were only affected at higher concentrations (>100 µg/l), demonstrating the multidimensional concept of biodiversity.

In conclusion, all aspects of phytoplankton biodiversity (except for dominance) were adversely affected by linuron. This is one of the first studies to explicitly assess the effects of chemical toxicity on biodiversity by applying advanced statistical models. A comparison between our results and those obtained with the principal response curve approach would yield insight into the sensitivity of this new technique to detect changes in community structure following pesticide exposure.

TU 467

Increasing resource availability compensates atrazine effects on the benthic diatom *Seminavis robusta*

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Ecotoxicological experiments are typically performed under standard conditions to reduce uncertainties and to allow comparisons between studies. However, standard conditions can be unrepresentative for the field as natural events (e.g. seasonality) and anthropogenic contributions (e.g. carbon emissions) continuously induce shifts in environmental conditions.

In the current study, we examined to what extent the sensitivity of the benthic diatom *Seminavis robusta* to the herbicide atrazine, a commonly used photosynthetic inhibitor, is changed after addition of a carbon resource. To this end, the benthic diatom *S. robusta* was treated with 0, 20 and 100 µg/l atrazine in 20 ml microcosms with f2 medium (artificial seawater with nutrients) for 5 days at 15 °C. Half of the microcosms received a single addition of sodium bicarbonate (NaHCO₃; 3.36 g/l) at the start of the experiment. Tested endpoints were diatom density measured by inverted microscopy and cell quality measured with Pulse-Amplitude-Modulation (PAM) fluorometry. The density of *S. robusta* increased with time in all treatments. Diatoms without additional carbon supply showed overall a similar biomass yield for the 0 and 20 µg/l atrazine treatments i.e. 1.09[GREEKX]10⁶ and 1.26[GREEKX]10⁶ diatom cells, respectively after 5 days. The diatom density in the 100 µg/l atrazine treatment was however strongly impacted as the density dropped to 50% of the control densities. In addition, atrazine considerably reduced the cell quality of the diatoms in the 100 µg/L treatment (max. quantum yield of photosynthetic activity Fv/Fm = 0.086) where the cells in the 20 µg/l treatment (Fv/Fm = 0.457) did not differ from the ones in the control (Fv/Fm = 0.472).

Addition of the carbon source NaHCO₃ to the experimental units yielded a smaller difference in diatom densities between all herbicide treatments when compared with the treatments without additional carbon. Average cell density at the end of the experiment was 1.21[GREEKX]10⁶, 1.11[GREEKX]10⁶ and 1.02[GREEKX]10⁶ cells in 0, 20 and 100 µg/l atrazine, respectively. Yet, the extra C-supply did not compensate for the differences in cell quality between the high (100 µg/l) and low (0 and 20 µg/l) atrazine concentrations. In conclusion, addition of a carbon source counterbalanced effects of atrazine on the biomass yield but not on the cell quality of *S. robusta*. Research is ongoing to examine the implications of the reduced cell quality for higher trophic levels.

TU 468

Environmental context determines community sensitivity of freshwater zooplankton to a pesticide

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The environment is currently changing worldwide, and ecosystems are being exposed to multiple anthro- pogenic pressures. Understanding and consideration of such environmental conditions is required in ecological risk assessment of toxicants, but it remains basically limited. In the present study, we aimed to determine how and to what extent alterations in the abiotic and biotic environmental conditions can alter the sensitivity of a community to an insecticide, as well as its recovery after contamination. We conducted an outdoor microcosm experiment in which zooplankton communities were exposed to the insecticide esfenvalerate (0.03, 0.3, and 3 µg/L) under different regimes of solar radiation and community density, which represented different levels of food availability and competition. We focused on the sensitivity of the entire community and analysed it using multivariate statistical methods, such as principal response curves and redundancy analysis. The results showed that community sensitivity varied markedly between the treatments. In the experimental series with the lowest availability of food and strongest competition significant effects of the insecticide were found at the concentration of 0.03 µg/L. In contrast, in the series with relatively higher food availability and weak competition such effects were detected at 3 µg/L only. However, we did not find significant differences in the community recovery rates between the experimental treatments. These findings indicate that environmental context is more important for ecotoxicological evaluation than assumed previously.

TU 469

Pesticide stress in mountain streams: manipulative experiments and metrics sensitivity

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Pesticide loads in streams are potentially one of the most relevant stressor for macroinvertebrates communities. Nevertheless real effects provoked at the community level are still largely unknown.

To increase our knowledge of effects at this level of organization, descriptive approaches of natural systems are useful but not sufficient, since huge natural and anthropogenic variability may act as noise, causing the power of the investigation to drop. Biomonitoring should be coupled with manipulative experiments able to establish causal relationships between an induced stressor and the observed effects. Furthermore, to get a deeper knowledge of complex systems, it is necessary to make experiments which reflect this high degree of complexity.

In the present work the experimental system was composed of 5 artificial streams, which reproduced a mountain lotic environment under controlled conditions. The artificial streams flow alongside of the Fersina River (Trentino-Alto Adige, northern Italy), whose upstream basin is characterized by the absence of relevant agricultural activities. The artificial streams are fed by the water of the adjacent river and the sediments are naturally colonized by macroinvertebrates drifting from upstream. The aim of the study was to investigate in detail whether (and how) the biological community was influenced by pesticides pulse exposures, in a semi-natural environment, in which nevertheless any other noise source was minimized. An hydraulic system was built in order to pour into the flumes controlled amounts of plant protection products in known concentrations. The experiments have been carried out to reproduce concentrations and timing of a realistic scenario for the investigated area (intensive apple orchards cultivation). During the productive season (March-July) we carried out 5 experiments: biological community was regularly sampled and nominal concentration of water were tested. Organisms were counted and classified until the lowest practically feasible taxonomic level. The interpretation of the results was performed comparing the output of different metrics. Sensitivity of the different metrics was analyzed considering single exposure events as well as overall temporal trends. Results show how some common taxonomic metrics (e.g. taxa richness, Shannon index, etc) are not always suitable to identify the effects of pesticides at the community level, while ecologically based indexes provide much clearer indications.

TU 470

Effects of pulse salinity releases on stream communities: a mesocosm approach

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Secondary salinisation is a major and growing threat to the world's freshwater ecosystems, yet its effects on aquatic invertebrates have been poorly described at a community level. Here we use a controlled experimental setting to evaluate stream community responses to different salinity treatments (5, 10 and 15 mS cm⁻¹) over a one month period. The stream mesocosm consisted of 12 artificial channels and was operated as a flow-through system, relying on water pumped from a diversion channel of the Llobregat River (Barcelona, Spain). Different concentrations of a salt saturated solution were released in pulses from a 2000 l tank using an irrigation system. River cobbles were collected from a riffle segment of the Llobregat River and transported in individual mesh baskets to the stream mesocosm site. Weekly samples of invertebrates associated to the cobbles, invertebrates' drift and algal biomass were collected. Leaf consumption rates were measured in order to explore possible sub-lethal effects of the salt spikes. The fungal community colonizing the leaves was also characterized. Preliminary results show that the salt treatments caused a decrease in the invertebrate densities, a higher invertebrate mortality and a decay of the leaf consumption rates. The salt spikes prevented algal growth, but no effect was registered for the fungal communities.

TU 471

Life history traits as predictive parameters for recovery

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Using predatory mites as a paradigm we investigate to what extent life history traits may help to explain or predict effects of mortality factors such as insecticides. In particular we try to relate recovery to population age structure. Predatory mites overwinter as adult females and the winter generation dies in spring after oviposition has been resumed. Consequently, population development starts from a cohort of eggs. Eggs of predatory mites are often placed in particular microhabitats (acarodomatia) and may be considered well protected to adverse conditions, including exposure to insecticide spray. In the course of the season population age structure stabilizes, but initially it changes rapidly. Using a modelling approach we showed previously that in combination with the presence of a protected life stage (eggs) population age structure might be paramount to recovery time. Depending on population age structure 100% mortality of mobile stages may be followed by full recovery either within days or within months. We have put this hypothesis to an experimental test by exposing predatory mite populations in an apple orchard and in a vineyard to insecticide sprays at regular intervals (10 days) throughout a growing season and by monitoring population development of each exposed cohort for 20 days. We found that for the same species in the same season, effects of insecticide sprays may indeed range from no effect after 3 days to a strong effect even after 20 days of recovery time. Such contrasting effects are not predictable from life history traits alone. We conclude that life history parameters have no predictive value for recovery time without knowing population age structure in combination with the vulnerability of the various life stages.

TU 472

Avoidance data tells you more than meet the eye! Can it be used to investigate the re-colonization of contaminated sites?

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Ecological recovery is gaining importance in both prospective (e.g., pesticides) and retrospective (site specific) ecological risk assessment. Thus, modelling recovery, and associated recolonization, is of paramount importance to better define levels of protection for the soil system. The recolonization process of contaminated soils is conditioned not only by the existence of suitable habitat conditions (including the degree of contamination) but also by the existence of a soil community acting as an external source of individuals. Moreover, their ability to detect the contaminants and to move towards contaminated patches is a key issue.

Soil avoidance tests are widely used and give us information about the ability of organisms to detect the contaminants and, more important, the percentage of the population that is able to avoid it (not able to colonize the contaminated soil). In this context, avoidance data may also provide information on the potential for recovery of soil fauna at contaminated sites. So, a concentration causing a certain percentage of avoidance (ACx) can be used to derive a concentration originating a certain percentage of colonization (the "colonization concentration" - CCy) by considering that $CCy = AC(100 - x)$.

To validate this "colonization concentration" concept we used the earthworm *E. andrei* as a model organism and copper as a model chemical in a 3 step process: (1) calculate ACx values and confirm that the avoidance test using a dual test chamber and how it is currently performed (placing the animals in the middle line) gives the same results when organisms are put either in the control (avoidance response) or in the contaminated soil (escape response); (2) prove, using a 3 compartment chamber, that the number of individuals expected in the compartment with soil contaminated with the CC50 is not dependent of their previous exposure to the contaminant (i.e., passing through a lower concentration); (3) get empirical data proving the concept over a larger spatial scale by simulating a contamination gradient.

Results obtained bring evidences to prove the concept and showed that avoidance data can be used to tackle recolonization potential. Further experiments at larger spatial and temporal scales and other soil organism groups are planned, but data collected here gives promising perspectives for the use of this endpoint to be included in existing or under development population models tackling recolonization and/or recovery.

TU 473

To move or not to move? Effects of sub-lethal pesticide exposure on movement behaviour of *Asellus aquaticus*

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Different testing strategies are applied for the ecological risk assessment (ERA) for pesticides. This is to gain a comprehensive understanding of potential environmental side effects of pesticide application and, occasionally, also the recovery of affected populations. Virtually none of these strategies, however, account explicitly for the impact of the pesticide on animal movement and the relevance of these effects for population recovery from adverse effects.

For non-flying species like *Asellus aquaticus* and *Gammarus pulex*, recovery by immigration of individuals from uncontaminated sites is an important factor for re-establishing population densities after pesticide induced reductions in densities. However, not much information on movement patterns of aquatic macroinvertebrates is available, nor how exposure to sub-lethal concentrations, habitat quality or population density affect the locomotion behaviour of such individuals.

To investigate the influence of pesticide exposure, habitat quality and density on the movement of *Asellus* and *Gammarus*, we performed video tracking experiments to derive information on movement behaviour of individual *A. aquaticus* under different conditions. The experiments tested for the effect of exposure to two different neuro-toxic pesticides (chlorpyrifos and imidacloprid) at different concentrations with varying population densities in the experimental setup. An additional test studied the impact of environmental factors to derive information on the general movement behaviour of *A. aquaticus* when not exposed. In that respect we studied different population densities and habitat qualities (e.g. presence of food or shelter) since different locomotion activities were assumed under varying conditions.

We found that the different factors influenced the preferred turning angle range, resting times, and movement velocity. Especially, exposure to different concentration levels of the single pesticides showed levelled effects on overall animal activity; resting times increased with increased exposure concentration and directionality of movement paths was found to decrease at the same time. The findings imply that currently applied techniques could lead to unrealistic estimations of recovery timeframes if environmental heterogeneity and/or pesticide residue effects are not accounted for.

TU 474

Efficacy of recent remediation measures in a protected coastal lagoon (Paramos, Portugal): toxicity of surface versus subsurface sediments

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Wetlands, including coastal lagoons, are among the richest ecosystems concerning productivity and are responsible for processes such as biomass production, water replacement, sediment and nutrient retention, and floods control. However, coastal areas are also regions supporting large urban and industrial areas, as well as tourism, all leading to the deterioration of these ecosystems. This is the case of the Paramos lagoon (NE Portugal), which despite its recognized ecological significance has a long contamination history (e.g. domestic sewage, agricultural and industrial effluents) that resulted in high levels of sediment contamination. The present study aimed to assess

the efficacy of the remediation measures already implemented in the Paramos lagoon, by comparing the toxicity of surface versus subsurface sediments, as depth profiles in sediments provide information about the temporal contaminant inputs. Tests with the following battery of species, representative of different taxonomic groups, trophic levels and key ecosystem functions, were conducted: bacteria *Vibrio fischeri* (decomposer), microalgae *Pseudokirchneriella subcapitata* (primary producer), crustacean ostracod *Heterocypris incongruens* (epibenthic omnivorous), and the midge *Chironomus riparius* (benthic insect larvae; deposit feeder). Four sites were selected within the wet area of the lagoon to cover not only the whole lagoon area but also the regions close to the two tributaries (likely to be the most contaminated) and to the non-permanent channel (likely to be the least contaminated). Overall, two sites presented higher organism responses, the one located next to the non-permanent channel, which endures some water renewal, and the one close to the area where remediation measures first took place. The other two sites, one located close to a contaminated tributary and the other close to the centre of the lagoon, were those with lower organism responses and higher uncertainties in what regards risk. Also, for all except one site (one of the most contaminated), organism performances were generally best with surface sediments. Although the selected test battery proved to have potential to map toxicity levels within the Paramos lagoon and areas where remediation measures are being successful, the uncertainties associated to the test results at some sites point to the need for a more detailed sediment toxicity assessment (e.g. additional tests and sites, chemical analysis).

TU 475

A comparison of the chronic toxicity of lead between laboratory and field populations of the great pond snail (*Lymnaea stagnalis*)

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Recent research demonstrated that *Lymnaea stagnalis* is among the most sensitive aquatic organisms for lead. However *L. stagnalis* and other species of the *Lymnaea* genus occur very generally in aquatic systems across Europe, even in polluted water bodies. It was therefore hypothesized that *L. stagnalis* may be more sensitive in the lab than in the field. Indeed the high sensitivity of *L. stagnalis* could be the result of the tendency of laboratory populations to show a higher level of inbreeding and a reduced genetic variety compared to field populations. A reduced genetic variation can lead to a higher sensitivity towards pollutants. We compared the chronic toxicity of lead between three laboratory populations and three field populations of *L. stagnalis*. We exposed juveniles of the different populations for 28 days to lead. We calculated the growth rate based on weekly measurements of the shell length. The preliminary data based on one laboratory and two field populations suggest that the laboratory population has the tendency to be indeed more sensitive to lead than the field populations. To test the underlying hypothesis related to genetic variation, we are genetically characterizing the populations at nine microsatellite loci.

TU 476

Use of indicators of toxicity and biological communities as explanatory variables and responses in ecological risk assessment of an aquatic environment

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This study used toxicity tests with *Hyalella azteca* and *Ceriodaphnia dubia* and biological indicators of zooplankton and benthic communities as part of a model of ecological risk assessment applied to a stretch of the Rio Jacuí-RS. The methodology was considered as an explanatory variable in this study, the evidence for the abiotic compartments, surface water and sediment. The Toxicity Indicator was generated from the use of toxicity tests with *Hyalella azteca* and *Ceriodaphnia dubia* which allowed to assess the ecotoxicological evidence from the results of acute effect, chronic effect or no effect when compared with the proper legal standards of environmental quality in Brazil, so the CONAMA Resolution 357/2005 establishes the criteria for surface water quality class 1 and 2 should not have chronic toxic effects and that water class 3 and 4 should not show acute toxic effect. CONAMA Resolution 344/2004 establishes the criteria for classification of sediments to be dredged two quality levels, a level which provides the low probability of adverse effects to biota and level 2 which provides a likely adverse effect on biota. For the evaluation of biological evidence has been proposed the use of a Biological Indicator based on calculations. For the calculations of Biological Indicator for each sample point data were used in species richness, diversity index of Shannon-Wiener and Pielou's evenness of the number density of the species of zooplankton and benthic communities monitored. The four measures of biological attributes were transformed into proportions of zero to one by dividing their values by the highest value obtained from the sample points. For the expression of the Biological Indicator used the umbrella summation to the ratios obtained for each sample point. As a result the Toxicity Indicator with *Ceriodaphnia dubia* showed no points in most studied effect of toxicity in surface water and sediment with *Hyalella azteca* showed a higher percentage of acute toxicity for all sampled points. Biological Indicator Results were expressed as a numeric attribute ranging from 0 to 4. Within this range the lowest value found for the zooplankton community was the average of the highest P4 and P2 the average. The benthic community presented as the average of the lowest and the highest value P3 average of P1.

TU 477

Perinereis gualpensis (Polychaeta: Nereididae) a suitable specie for biomonitoring south central Chilean estuaries

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Perinereis gualpensis is a key estuarine species from Chilean pacific coast estuaries where sandy mud sediments are predominant. Comparing contaminant concentrations, the responses at different levels of organization in conjunction with field, *in situ* and laboratory approaches indicates the suitability of *P. gualpensis* for future biomonitoring programs. On this basis the obtained results indicate: (1) site-specific responses and fast uptake of Hg from polluted sediments confirms the use of this specie as a bioindicator; (2) the ability to reflect anthropogenic changes through oxidative stress and antioxidant responses and other biochemical responses like Na⁺-K⁺-ATPase activity more consistently during the year; (3) cost-effective reproductive and population responses like oocyte stages proportion, L3 Length frequency histograms and density of individuals for determining long-term pollutant exposure; (4) the ability to assess the behavior responses like burrowing deep profiles to determine effects associated to important ecological functions (ex. bioturbation), and (5) suitability for *in situ* and laboratory experiments for short and medium term exposures to classic and emerging substances. *Perinereis gualpensis* may be a good bioindicator specie for determining the environmental quality of the Chilean estuaries. Supported by CNPq (PROSUL program) and BROMAQUA (BBVA Foundation). JMM is a productivity research fellow from CNPq.

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Application of Methemoglobin formation in *Chironomus* larvae to assess risks to nitrates in a poor urban neighborhood, in Eldoret, Kenya

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The Kenyan poor urban neighborhoods that obtain water from shallow wells which are proximal to pit latrines face risk to nitrates that is hitherto unknown. This study investigated extent of use of water from shallow wells from a poor neighborhoods, in Eldoret, Kenya by interviewing women population. The level of nitrate contamination and potential for use of methemoglobin formation in *Chironomus* to estimate the nitrate contamination was studied. The ubiquitous and hemoglobin - containing chironomid larvae from Lake Victoria basin were identified using morphological characteristics. and *Chironomus*, the most widespread genera was chosen and exposed to different concentrations of nitrate and sampled well water in the laboratory. The *Chironomus* larvae were analyzed for methemoglobin using a spectrophotometer and the levels compared to the exposure to nitrate concentrations of the test solutions. Results established ingestion of substantial quantities of water by the vulnerable members of the society i.e. children, pregnant women, and breast feeding women. The nitrate levels in water ranged from 0.5mg/l to 30.0 mg/l NO₃-N with a mean of 5.7 mg/l NO₃-N. Eleven per cent of the wells had a mean above 10 mg/l NO₃-N, the WHO limit. There was a positive correlation between nitrate concentration and hemoglobin absorbance in the tested cases. The potential for using methemoglobin within *Chironomus* larvae as a biomarker for nitrates in water and hence risks of methemoglobinemia among vulnerable members of the society is underlined.

TUPC1 - Perfluorinated compounds: From emission sources to the place of impact

TUPC1-1

Fate of short chain perfluorinated carboxylic and sulfonic acids in a water-saturated sediment column investigated under near-natural conditions

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Introduction

Perfluorinated carboxylic acids (PFCAs) and sulfonic acids (PFSAs) are mostly present in aqueous media of the environment. As PFCAs and PFSAs are persistent and some are known to bioaccumulate and to have toxic properties their occurrence in surface waters is of concern. Where such resources are used for the production of drinking water via sediment infiltration, an efficient elimination has to be ensured. Up to now, data on the fate of these compounds in sediments is scarce. Hence, the aim of the present study is to gain a better understanding of the fate of shorter chain PFCAs and PFSA during sediment infiltration under near-natural conditions.

Methods and Materials

C₄ - C₁₀ PFCAs, C_{4,6,7,8} PFSAs as well as some precursors were spiked together with a tracer in the supernatant of a water-saturated sediment column (length 1 m). The column was filled with coarse-grained medium sand and was fed with surface water (pH 7-8) from a surrounding pond. Water samples were collected from the supernatant and after 40 and 80 cm of sediment passage in regular intervals.

Samples were extracted using solid phase extraction and LC-ESI-MS/MS was used for instrumental analysis. Concentrations in water samples were corrected with blank concentrations and with recoveries of masslabelled internal standards.

Results

After spiking concentrations of analytes in the supernatant ranged from 1.0 to 15.9 ng L⁻¹. Some precursors were not detected in any samples. Even fewer analytes were detected in 40 cm depth indicating occurrence of loss, presumably due to sorption to sediment. C₄ and C₆ PFCAs and PFSAs showed tracer-like breakthrough in 40 cm and were slightly retarded in 80 cm depth. C₈ and C₉ PFCAs were detected in much lower concentration than shorter chain PFCAs and PFSAs and were retarded by the sediment.

Conclusion and Outlook

Whilst short chain PFCAs and PFSAs were not attenuated by the sediment longer chain ones showed retardation. In the next step the recoveries of the different PFCAs and

PFSAs after sediment passage will be quantified. Furthermore, results will be compared with other studies. The results of the present experiment can help to relate drinking water guidance values to surface water concentrations for cases where sediment infiltration of surface water is used for drinking water production.

TUPC1-2

Chain length and pH effects on uptake and distribution of perfluorinated compounds (PFCs) in maize (*Zea mays*)

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Few systematic studies have been carried out to determine the carry-over of perfluorinated compounds (PFCs) in plants and these have been limited to PFOA and PFOS. Uptake and distribution of PFCs in maize hydroculture were measured dependent upon pH value (pH 5, 6, 7, 8) and chain length (C4 to C10). This allowed potential adsorption effects on substrate particles to be disregarded.

Four time-independent repetitions were carried out for each pH value. The maize was grown in four containers of 40 L nutrient solution under controlled conditions (light intensity, day/night rhythm, temperature, relative humidity) in a climate chamber. The pH value of the root medium was maintained for a week using an automatic pH-stat system. Once the pH was adjusted individual PFC substances were added to obtain a concentration of 100 µg L⁻¹ root medium. The PFC mixture was made up of seven perfluorocarboxylic acids: perfluorobutyric acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA) and perfluorodecanoic acid (PFDA) as well as three perfluorosulfonic acids: perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS) and perfluorooctane sulfonate (PFOS).

To rule out the possibility of cross-contamination and impurities during cultivation an additional container was maintained with control plants (blank value control) which received identical nutrient solution but no PFC mixture. One week after addition of the test substances shoots and roots were harvested and weighed. The roots were washed with 1 mM CaSO₄ solution to remove any PFCs bound in the apoplast. After appropriate sample preparation the PFC concentrations were measured using LC-MS/MS in shoots and roots. Perfluorocarboxylic and perfluorosulfonic acids showed distinct differences in uptake and in distribution in shoots and roots depending on the chain length of PFCs. A possible influence of pH of the root medium on uptake of perfluorinated compounds will be discussed.

TUPC1-3

The importance of dietary intake of perfluoroalkyl carboxylic acids and perfluoroalkane sulfonic acids for the general Swedish population

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Dietary intake has been estimated to be a major pathway of human exposure to perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSA) in several previous studies. However, difficulties associated with the analysis of food samples have hampered the quantification of dietary exposure and determination of which food items contribute most to the total dietary exposure. Furthermore, little is known about how dietary exposure to PFCAs and PFSA has changed over time as a consequence of changing production and use of these chemicals. In this presentation, the dietary exposure of the average Swedish population to PFCAs and PFSA is estimated through analysis of food basket samples collected in 1999, 2005 and 2010. Estimated dietary exposures are compared with previously quantified exposure pathways (i.e. ingestion of house dust and drinking water exposure estimates also made in Sweden) and used to dynamically model expected human serum concentrations of PFOA and PFOS using a recently developed physiologically-based pharmacokinetic (PBPK) model. Exposure to PFOS (796'1424 pg kg⁻¹day⁻¹), PFUnDA (88'212 pg kg⁻¹day⁻¹), PFDA (52'102 pg kg⁻¹day⁻¹) and PFNA (62'83 pg kg⁻¹day⁻¹) was dominated by the consumption of fish and meat. In contrast, exposure to PFOA (322'513 pg kg⁻¹day⁻¹) originated from low levels (8'62 pg g⁻¹) found in several "high consumption" food categories including cereals, dairy products, vegetables and fruit. Although statistically significant temporal trends in the average dietary intake estimates could not be determined, there is preliminary evidence of a downward time trend in the concentrations of PFOS in eggs and meat products and an upward trend of PFDA, PFUnDA, PFDoDA acid and PFTTrDA in fish products, which both warrant further investigation. A comparison of the estimated dietary intake relative to other exposure pathways indicates that approximately 70% of the total exposure to PFOA and PFOS is coming from the diet for the Swedish population. Estimated dietary intakes are used as inputs in the PBPK model and it is shown that model estimated serum concentrations of PFOA and PFOS compare favourably to measured serum levels from the year 2010, suggesting that dietary intakes are now approximately at steady-state with human serum concentrations. It is expected therefore that human serum levels of PFOA and PFOS will stop declining and level off in the near future.

TUPC1-4

Temporal patterns in the dietary exposure to perfluoroalkane sulfonic acids and perfluoroalkane carboxylic acids

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Perfluoroalkane sulfonic acids (PFSA) and the structurally similar perfluoroalkyl carboxylic acids (PFCAs) are today characterized as environmental contaminants of high concern due to their persistence, bioaccumulation potential and wide range of toxicological effects observed in laboratory animals. The hazardous properties of these chemicals have prompted the manufacturing industry in Europe and North America to completely phase out production of PFOS and drastically reduce environmental discharges of long-chain PFCAs. Despite these efforts by industry, wildlife and humans continue to be exposed to these chemicals as a result of legacy sources and an increasing production in Asia. Temporal trend studies of human blood show that concentrations of PFOS and PFOA are decreasing after the year 2000 when the industrial phase out by the largest global manufacturer started. However, levels of long-chain PFCA homologues (with 7 or more carbons in the perfluorinated chain) have increased in human blood until the present day. A primary research interest is to determine if the human body burden of PFCAs and PFSA can be attributed to environmental or to consumer product related sources. In previous work in our group and elsewhere, a hypothesis stating that the ongoing human exposure of PFOS and PFOA is dominated by the intake of environmentally contaminated food has been proposed. However, there is still too few quality food data for PFCAs and PFSA to unequivocally support this hypothesis. This study aims to better test the hypothesis that dietary intake is currently the dominant human exposure pathway for PFSA and PFCAs, by comparing temporal trends in dietary exposure to temporal trends in human blood. In this poster historical dietary exposure data will be presented. These data were obtained by applying novel analytical methods to eggs, milk and fish sampled annually from 1999 to 2010. These food items are expected to make an important contribution to the total dietary intake. The analytical methodology, previously developed and validated, comprised various extraction and clean up procedures, including; liquid-liquid extraction, solid-liquid extraction, ion-pair extraction, solid phase extraction and dispersive clean-up on graphitized carbon. Final separation and quantification of PFSA and PFCAs was performed using liquid chromatography coupled to tandem or high resolution mass spectrometry with instrumental detection limits in the low femtogram range.

TUPC1-5

Evidence of excretion of perfluorinated compounds (PFCs) through eggs in a top predator seabird in the North Atlantic

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Transfer of Persistent Organic Pollutants (POPs) from female birds to eggs has been widely studied in legacy POPs, but less is known about the transfer of Perfluorinated Compounds (PFCs) to eggs. Concentrations of PFCs were measured in eggs and plasma of great skuas (*Stercorarius skua*) collected in Shetland, Scotland, in 2008 and 2009 respectively. Great skuas are predatory seabirds at the top of the marine food chain and as such were expected to have high levels of PFCs, which are known to bioaccumulate. Although not a direct measure of maternal transfer, the comparison between PFC concentrations in eggs and female plasma gives an insight into comparative levels and the validity of using eggs to monitor PFCs in seabirds. In addition, levels of PFCs in female and male plasma were compared to assess if sex has a significant effect on PFC concentrations, potentially through excretion of PFCs into eggs by females. PFCs measured included C4-C10 perfluoroalkyl sulfonates (PFSA), perfluorooctane sulfonamide (PFOSA) and C5-C14 perfluorocarboxylates (PFCAs). Five out of thirteen PFCs, including both PFSA and PFCAs, were at significantly higher concentrations in female plasma than in eggs whilst the concentrations of the remaining eight PFCs detected were not significantly different. Nine out of thirteen PFCs were significantly higher in male than female plasma with the remaining PFCs, PFHpA, PFOA, PFHpS and PFOSA not significantly different. These comparisons, and the fact that female great skuas usually lay two eggs per year, suggest excretion through egg laying is a significant elimination pathway for PFCs. Overall levels of PFCs in great skuas were low compared to other studies of seabirds in similar ecological niches, with mean PFOS concentrations of 24, 23 and 43 ng/g ww. for eggs and female and male plasma respectively. In comparison to chlorinated contaminants measured in the same great skua eggs, PFCs were low with a ratio of ΣOC to ΣPFC of 56 compared to 2.67 for eggs of glaucous gulls *Larus hyperboreus*. The comparatively low levels of PFCs in both eggs and plasma suggest that great skuas may not bioaccumulate PFCs to the same extent as some other seabirds.

TUPC1-6

Mass balance of perfluoroalkyl acids in the Baltic Sea

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Perfluoroalkyl acids (PFAAs) are used in a wide range of consumer products and industrial applications. PFAAs are persistent and some are bioaccumulative and toxic, categorising them as persistent organic pollutants. PFAAs have been ubiquitously found in various environmental compartments such as river water, wet deposition, sediments and biota. The aim of this study was to identify and quantify the major fluxes of PFAAs to and from the Baltic Sea using recent monitoring data from the literature. A mass balance was attempted for perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS).

Riverine discharges, wet deposition, inflow through the Danish Straits and waste water treatment plants (WWTPs) discharging directly into the Baltic Sea were considered as

input pathways. Country specific WWTP emission data on a per capita basis from Sweden, Finland, Estonia, Latvia, Lithuania, Poland and Denmark were used to estimate the total WWTP effluent flux of PFAAs from 55 of the largest cities along the Baltic coastline. PFAA fluxes from 15 of the major rivers discharging into the Baltic were compiled. Inputs through wet deposition directly into the Baltic Sea and inflow through the Danish Straits were calculated. As output pathway the outflow through the Danish Straits was estimated. Sediment burial represented by particulate organic carbon sedimentation was considered as a sink of PFAAs.

The results show that direct wet deposition into the Baltic Sea and inflowing rivers are the two major input pathways of PFHxA, PFOA and PFOS, accounting for the largest part of PFAA inflow. WWTPs along the coastline only contributed with a minor fraction to the total amount of PFAAs discharged into the Sea. The net flow through the Danish Straits was estimated to be the major flux of PFAAs out of the Baltic. Sediment burial was only a non-significant loss mechanism for PFAAs. A sub-study of the river Oder catchment area showed that wet deposition with its runoff into the river was the major input of PFOA and PFOS also to this riverine system, whereas all effluents from WWTPs in the catchment areas contributed much less PFAAs to the Oder. This indicates that wet deposition is the overall most relevant input pathway for PFHxA, PFOA and PFOS to the Baltic environment. Taken together, the sum of all PFAA fluxes to the Baltic Sea exceeded the sum of outflows, suggesting that the PFAA levels in the Baltic Sea are still increasing today.

TUPC1-7

Perfluorinated compounds in effluents from the fifty largest US wastewater treatment plants

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Studies conducted to date suggest that wastewater treatment plants (WWTPs) are one of the primary sources of perfluorinated compounds (PFCs) entering the aquatic environment, and that the concentration of PFCs in wastewater effluents typically exceeds that in surface water, ground water, or drinking water. This has raised concerns about human or environmental impacts among the public, media, wastewater facility operators, regulators, and legislators. However, data on the occurrence of PFCs in wastewater effluents in the US are scattered, due to the logistic challenges and analytical difficulties that conducting a nationally representative survey.

Our current work aimed to: a) assess the presence of PFCs and other chemicals of emerging concern in wastewater effluents in a nation-wide monitoring campaign, as well as evaluate their oestrogenicity, and b) provide an insight on the chemicals potentially reaching the US receiving waters. In this study, the 50 very large municipal WWTPs in the US were identified from the 2004 Clean Watershed Needs Survey. These plants produce about 17% of all the municipal wastewater discharge in the US, serving 20% of the US population. A 24-hour composite sample (total of 8 litres) of finished effluent from each plant were collected by EPA Regional or selected plant personnel, and then shipped to the EPA laboratory. For each effluent sample, 50 high priority pharmaceuticals, steroid hormones, alkylphenolic compounds, Bisphenol A and PFCs were measured along with the oestrogenic potency in a fathead minnow vitellogenin induction assay.

This presentation will focus on the results from the PFCs analysis. PFCs were detected in 100% plants, with C4-10 perfluorocarboxylic acids and C4, C6 and C8 sulfonates being detected in more than 80% of the plants. PFHxA, PFOA and PFOS were the predominant PFCs. Most of the effluents had comparable concentrations of PFCs to those previously reported, with median levels ranging between 10 and 30 ng/L. However, some effluents showed elevated concentrations (e.g. 880 ng/L of PFOS). Some plants sampled multiple times revealed large intra-day variability in the concentration of the selected PFCs. There was no significant correlation found between PFCs concentrations and population/total discharge. Additionally, the effect of treatment characteristics of the selected plants on PFCs concentrations will be discussed in the presentation.

TUPC1-8

Are perfluorinated compounds (PFCs) subject to atmospheric transport and deposition in the high Alpine environment?

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Perfluorinated compounds (PFCs) are of growing concern as global contaminants. They have been detected in biotic and abiotic matrices in both source and remote regions (e.g. Arctic). How they reach such remote environments is still under debate. One of the hypotheses for explaining their environmental fate is that their precursors are subject to long range atmospheric transport (LRAT) from industrialised source regions. For testing this hypothesis, a comparison with well known contaminants, such as POPs was made. POPs are semivolatile substances that can accumulate in cold environments in high latitudes, but also in areas of high altitude like the European Alps. The "cold condensation" effect - the temperature driven shift in equilibrium partitioning towards sorbed phases - results in the accumulation of these chemicals in snow and ice, for example. During periods of thaw, the compounds can be found in the melt water of glacial streams.

This LRAT hypothesis for PFCs was tested in the Alpine environment in Northern Italy, in two altitudinal transects from glacier or mountain snow, to glacial streams and then the lower altitude valley rivers. Snow and water were sampled in summer 2008 from two glacial valleys (Lys and Forni valleys) and then along the catchment to two of the major rivers in Italy: the Po and Adda. Up to 28 perfluorinated compounds were analyzed with HPLC-MS/MS, comprising perfluorosulphonic acids, perfluorocarboxylic acids, perfluoro sulfonamides and perfluoro sulfonamidoethanols. The C4-C8 sulphonic and C4-C12 carboxylic acids, as well as perfluorooctanesulfonamide (FOSA) were detected in the samples.

Generally, higher concentrations of the perfluoroalkyl acids (PFAs) were observed in the valley rivers compared to the higher altitude glacial streams, highlighting the influence of point sources further down the catchment. However, PFAs were detected in the higher altitude streams with concentrations of the perfluorocarboxylic acids (notably PFBA, PFOA, PFNA and PFUnDA) higher in glacier/snow compared to levels in the glacial streams. The causes are still under investigation, but the cold condensation effect cannot be excluded, suggesting regional or long-range transport and followed by efficient deposition of these chemicals to the high alpine environment.

TUPC2 - What is the current state of the science on the fate, exposure and effects of pharmaceuticals in the environment?

TUPC2-1

Towards developing QSPR-MM combined model to predict environmental fate of hormonal drugs

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Natural and synthetic steroids are extensively used in medicine and veterinary, especially in hormonal replacement therapy, oral contraception and athletic performance enhancement. The large, and still growing consumption of these medicines throughout the world placed them among the chemicals considered by environmental scientists. Steroids have been detected in many samples of purified water introduced to natural water bodies. Their presence in drinking water, which has been also reported, poses potential risk not only for ecological system but also for human health. Therefore, a comprehensive environmental risk assessment for hormonal drugs is required.

Factors deciding on environmental occurrence and transport of steroid drugs can be determined from computational modeling, without necessity of performing extensive empirical studies. In our project, Quantitative Structure-Property Relationships (QSPR) technique and Multimedia mass-balance Models (MM) will be applied to evaluate environmental persistence (P_{ov}) and long range transport potential (L RTP) of these compounds. The QSPR modeling will be employed to predicting the lacking phys/chem data for drugs. Phys/chem properties will be, then, utilized as input parameters for Multimedia mass-balance Modeling procedure. Estimated data on P_{ov} and L RTP will allow ranking steroid drugs according to their environmental mobility.

We started from creating and launching a comprehensive database containing all available data related to environment transport and fate of hormonal drugs. The most significant gaps in the phys/chem data were identified. For each medicine a set of molecular descriptors that characterize different aspects of molecular structure were calculated. We obtained a matrix of molecular descriptors reflecting the structural variability in the studied medicines. Data was analyzed by means of chemometrics methods, including principle component analysis and two-ways hierarchic cluster analysis. Medicines were divided into classes containing chemicals with the highest structural similarity. In the same way, we grouped medicines having similar phys/chem properties of the highest meaning for environmental transport and fate processes. Finally, we put together the results of both grouping exercises and defined relationships between both types of the similarity (structural similarity and similarity in properties). This led us to conclusions, useful for defining applicability domains for novel QSPR-MM models

TUPC2-2

Fate of 101 pharmaceuticals in sewage treatment plants, receiving surface waters and exposed biota

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Pharmaceuticals have been found in various environmental matrices worldwide in concentrations that range from low nanogram up to milligram per liter. Pharmaceuticals enter the environment primarily through our sewage systems since many pharmaceuticals are not metabolized completely but excreted unchanged. Pharmaceuticals are potent, biologically active chemicals and there is an increased focus on the potentially negative effects of pharmaceuticals in the environment. The purpose of the present study was to investigate the fate of selected pharmaceuticals in sewage treatment plants (STPs) as well as investigate the levels of these pharmaceuticals in receiving surface waters and exposed biota. Selection of pharmaceuticals was based on ecotoxicological criteria, criteria; primarily potency and potential to bioconcentrate, and a total of 101 pharmaceuticals were included in the study.

92 of the 101 pharmaceuticals were detected in the STP influent of at least one STP, of the four Swedish STPs included in this study and 85 of the 101 pharmaceuticals were detected in the effluents. Removal efficiencies, daily loads and sludge concentrations from all four STPs are also presented.

Fifty-three pharmaceuticals were detected in the surface water samples in the range from low ng/l up to 1.8 µg/L. Measured surface water concentrations were evaluated by comparing them to critical environmental concentrations, i.e. the water concentration that is expected to cause a pharmacological effect in fish. This evaluation showed that three pharmaceuticals in these samples are expected to cause a pharmacological response in fish exposed to these waters.

Twenty pharmaceuticals were detected in the three pooled biota (perch) samples taken in the receiving surface water included in the study. Concentrations were in the low µg/Kg range and highest detected levels were found in the perch caught in close proximity to the discharge point of an STP. Detected pharmaceuticals correlate to the surface water concentrations and previous reports on the occurrence of pharmaceuticals in biota.

TUPC2-3

Evaluation of the mammalian plasma model to predict pharmaceutical responses in fish

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Throughout the drug research and development processes, a considerable amount of data are generated to understand receptor-binding based modes of action and human pharmacokinetics including human therapeutic plasma concentrations (HTPC). Evidence of the conservation of enzyme/receptor activity across species, including mammals and fish, is the basis for the Mammalian Plasma Model proposed by Huggett et al (2003). This theoretical model is intended to facilitate prioritization of the potential impact of human pharmaceuticals on fish by comparing the HTPC to the theoretical Fish steady state Plasma Concentration (FSSPC) to determine an Effect Ratio (ER). The lower the ratio, the more likely the drug will to elicit a pharmacological response in the fish. An evaluation of this model using several pharmaceuticals representing multiple therapeutic areas will be presented. To understand potential pharmacological effects, early life stage (ELS) studies were conducted using the fathead minnow. Measured fish plasma concentrations were obtained following exposure of catfish to the same exposure concentrations used in the ELS studies. These data will be presented and applied to the theoretical Mammalian Plasma Model to demonstrate the value of the model as a prioritization tool for pharmaceutical responses in fish.

TUPC2-4

Metabolism and bioconcentration of the human pharmaceutical amiodarone

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The presence of pharmaceuticals in the environment has become an important topic of discussion with respect to bioconcentration and bioaccumulation of these contaminants. There has been considerable discussion on the use of mammalian data and techniques to better estimate the potential for pharmaceuticals to undergo absorption, metabolism and elimination in fish. However, robust data sets containing fish in vitro metabolism and bioconcentration data are not available for human pharmaceuticals. Using the representative cardiovascular drug amiodarone, in vitro (S9 and hepatocyte) trout liver metabolism studies were conducted to evaluate the loss of amiodarone and metabolite formation over time. In addition, three flow-through fish bioconcentration studies were conducted with exposure periods ranging from 7 to 28d. A comparison of data sets will be presented highlighting the limitations and strengths of S9 and hepatocyte assays within the context of actual bioconcentration data.

TUPC2-5

Influence of pH on abiotic transformation and toxic potency of pharmaceutical NSAIDs and SSRIs

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Environmental pH may influence bioavailability and toxicity of weak acid or base pharmaceuticals. Here we test the influence of pH on abiotic transformation, i.e. hydrolysis and phototransformation, and toxic potency of active ingredients and transformation products of non-steroidal anti-inflammatory drugs NSAIDs (weak acids) and specific serotonin reuptake inhibitors SSRIs (weak bases). Half-lives in water were determined for ibuprofen, diclofenac, naproxen, ketoprofen, sertraline and fluoxetine in a laboratory set-up at three different pHs. Comparison of toxic potency of active ingredients and transformation products was done by performing toxicity tests at the three different pHs. Two toxicity tests were used, *Daphnia* sp 48h immobilization test, and a bacterial growth test using the leucine incorporation technique.

TUPC2-6

Combined effects of acidification and emerging contaminants on immune parameters of the mussel *Mytilus galloprovincialis*

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From the beginning of the Industrial Revolution, human activities have had an increasing influence on terrestrial and aquatic environments. Presently, greenhouse gas emissions and deforestation are indicated as the main cause of global climate changes (GCCs). CO₂ levels in the atmosphere influence both global heat balance and carbonate chemistry in the oceans. In the marine environment, the increment of atmospheric CO₂ levels leads to increasing temperature and seawater acidification. Marine organisms have to face not only reduced pH, but they are also exposed to many anthropogenic substances, whose fate and effects under GCC scenarios are totally unknown. Among emerging environmental contaminants, pharmaceuticals give cause for increasing concern. In this study, the combined effects of sea water acidification, as predicted in climate change scenarios, and Diclofenac, a non-steroidal anti-inflammatory drug, were investigated for the first time in the mussel *Mytilus galloprovincialis*. A flow-through system was used to carry out a three weeks exposure of mussels. In the first week, animals were exposed to three pH values (8.1; 7.7; 7.4) only. Thereafter, mussels were maintained for 7 and 14 days at the three experimental pH values and exposed simultaneously to environmentally relevant concentrations of Diclofenac (0, 0.05 and 0.5 µg/L). Each experimental condition was tested in two replicate tanks. Haemolymph was collected after 7, 14 and 21 days of exposure and 3 pools of haemolymph from 7 mussels each per pH value or pH/Diclofenac combination were prepared. Total haemocyte count (THC), pinocytotic activity and haemocyte proliferation were measured. At reduced pH values, THC significantly decreased after 7 and 21 days of exposure, whereas no significant effects of Diclofenac (alone or in combination with pH) were observed. Exposure of mussels to low pH values generally increased pinocytotic activity of haemocytes. Interestingly, exposure to reduced pH and Diclofenac increased significantly haemocyte proliferation, after 14 and 21 days respectively. Results obtained demonstrated that exposure of mussels to reduced pH is a more stressful condition than exposure to Diclofenac, for the immune parameters measured at least. In any case, further studies will be performed to assess effects of seawater acidification and contaminants on other mussel responses, both physiological and biochemical.

TUPC2-7

Effects of the pharmaceuticals propranolol and fluoxetine evaluated through a battery of biomarkers in the Mediterranean mussel (*Mytilus galloprovincialis*)

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Widespread occurrence of pharmaceuticals is reported in aquatic systems worldwide, posing concerns for the health of aquatic wildlife and a theoretical risk to humans. Pharmaceuticals are designed to have specific (therapeutic) effects at low doses and might have side effects at high doses or after prolonged exposure, which could be induced also in non target organisms. In parallel with studies on specific interactions with molecular targets to understand their mechanism of action (MOA), we investigated the alterations induced by fluoxetine (FX) and propranolol (PROP), alone or in combination (MIX) on Mediterranean mussels, *Mytilus galloprovincialis*, based on unspecific side effects caused in humans. Mussels were exposed for 7 days to the pharmaceuticals, each at 0.3 ng/L concentration selected as environmentally relevant. A battery of 10 biomarkers was then evaluated. Considering the most relevant results, lysosome membrane stability was strongly decreased, while lysosome/cytosol ratio was increased by all treatments. FX and MIX increased both accumulation of lipofuscin and neutral lipids, while PROP significantly increased only neutral lipid accumulation. PROP, but not FX and MIX, increased glutathione-S-transferase and catalase activities. EROD and DBF activities were significantly reduced by all treatments. Mussels exposure to the pharmaceuticals did not induce DNA damage, evaluated by the Hoechst methodology. In conclusion, FX and PROP caused significant stress at environmentally relevant concentrations. Their action is primarily through oxidation pathways with significant destabilization of the lysosomal membrane, which has been shown to be directly correlated with scope for growth reduction in molluscs. In conclusion biomarker expression is a sensitive endpoint, useful to detect the effects of substances, such as pharmaceuticals, present at low concentrations in the environment.

TUPC2-8

In vitro effects of environmental relevant concentration of antibiotics on immune parameters of *Elipio complanata*

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Municipal effluent is the most important contributor of pharmaceuticals to the environment; these pharmaceuticals do not occur as single contaminants, but as complex mixtures. Antibiotics are among the most frequently detected classes of pharmaceuticals in the environment. This study focused on several antibiotics, belonging to different therapeutic class, and environmentally relevant. We chose novobiocin, oxytetracycline, sulfamethazole, trimethoprim, ciprofloxacin and erythromycin. Interaction between antibiotics and immunity in mammals is a well known subject, which has been extensively reviewed, but few to no data are available in molluscs. *Elipio complanata* is likely to be exposed to these compounds. Thus, we assessed the in vitro effects of antibiotics, alone and as a mixture on immunological parameters in hemocytes (viability, phagocytosis, ROS and thiols production, COX activity, lysozyme activity, NO production) in *E. complanata*. Secondly, these effects were compared to the immunotoxicity of Montreal effluent, both filtered (without bacteria) and unfiltered.

The antibiotics alone, as well as the mixture, had effects on the immune response of *E. complanata* at environmentally relevant concentrations. Except novobiocin, each antibiotic and their mixture had globally similar effects on phagocytosis (induction), ROS and thiols production (induction and decrease). Sulfamethoxazole was the most potent antibiotic. The mixture has effects corresponding to the prevailing pattern, except that it sharply decreased COX activity in hemocytes, while no antibiotic did, and to the contrary several increased it.

When compared to the filtered effluent, the mixture had similar effects, but the immunomodulation induced by the xenobiotics in the effluent cannot be only explained by its

antibiotic load. Other xenobiotics are certainly also involved. The whole effluent had similar effects than the mixture of antibiotics and the filtered effluent on phagocytosis, ROS and thiols production. However it increased NO production and lysozyme activity, contrary to the filtered effluent, indicating an important role of bacteria in these responses.

TUPC3 - Occurrence, fate and impact of atmospheric pollutants on environmental and human health

TUPC3-1

Estimating the loss of life expectancy attributable to PM_{2.5} emissions in Europe with the use of high special resolution modelling

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People exposure to PMs can have various health effects which are widely described in scientific publications in the area of observational epidemiology and controlled human exposures [1]. This paper presents the preliminary results of the study on estimation of the Loss of Life Expectancy (LLE) indicator conducted in the framework of the FP7 EnerGEO project. The method used for LLE calculation was based on the approach recommended by the Task Force on Health [2] and described in IIASA's Report [3]. The analysis was carried out for 33 European countries with a spatial resolution of 20x20 km. Only population older than 30 years was taken into account as recommended by Pope et al. [4]. The human health impact was considered over the whole life of the population. LLE are calculated for the modelling time horizon assuming 95 years lifespan of the cohort of age 30, for four starting years i.e.: 1990, 1995, 2000 and 2005. In order to perform calculation different input data were collected. Those includes i.a.: EMEP PM_{2.5} concentration data, SEDAC gridded population taken from SEDAC, UN cohort data from. The results are provided in numerical form as well as in form of LLE maps over Europe. The results show that the estimated health impact due to exposure to PM_{2.5} concentrations is significant with very large variations between countries. Generally, the highest LLE was found for the Netherlands, Belgium, Germany and central Europe and the lowest for Ireland and Scandinavian countries. This simply reflects variation in exposure of people to PM concentration, i.e. low concentrations of PMs in Scandinavian countries. The results show, that in the period from 1990 to 2005 the number of days lost was decreased ca. 40%.

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TUPC3-2

Long-term metolachlor and Atrazine Volatilization monitoring at the catchment scale

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The emission and transport of pesticide vapor from bare soil surfaces constitutes an important pathway that is not well understood. Pre-emergent herbicides typically used for corn production include metolachlor [2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide] and atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] that can be used in spatially large regions land surface such as the Upper Midwest Cornbelt. Offsite movement and transport of pesticides pose a critical concern with respect to humans, animals, plants air and water quality. Factors affecting the volatilization of metolachlor and atrazine include meteorological and soil parameters. The physical and chemical properties of a soil will remain largely stable except for the soil water content which can be highly variable in rain-fed systems. In contrast meteorological parameters often vary from year to year which will affect volatilization losses. Recently long-term studies have shown that volatilization losses for some commonly used herbicides were surprisingly larger than runoff losses. We present the results from a long-term volatilization study spanning over a decade. This 10-year study was conducted to improve our understanding of how local meteorology and antecedent soil conditions influence year to year variability in watershed-scale herbicide volatilization. The study was conducted at a 21-ha watershed site located at the USDA-ARS Beltsville Agricultural Research Center in Beltsville Maryland. Site location, herbicide formulations, and agricultural management practices remained unchanged throughout the duration of the study. A flux gradient technique was used to compute volatilization fluxes using herbicide concentration profiles and turbulent fluxes of heat and water vapor as determined from eddy covariance measurements. Results demonstrate that volatilization losses for these two herbicides were high even though both have relatively low vapor pressures. Herbicide cumulative volatilization losses after 5 days ranged from 5 to 63% of that applied for metolachlor and about 2 to 12% of that applied for atrazine over the 10-year period. The variability in volatilization losses were related to antecedent soil moisture conditions and meteorological input variables.

TUPC3-3

Ecotoxicity and genotoxicity assessment of diesel engine emission

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Air pollution of the world's big cities is a constant problem and source of serious health damages. The concentration of pollution is highly depends on traffic. We examined ecotoxicity and genotoxicity of twelve different diesel vehicles.

Ecotoxicity tests were carried out using the ABOATOX Flash System. This test based on measurement of bioluminescence. The test organism is *Vibrio fischeri*, which is a bioluminescent bacteria. As the bacterial suspension is injected to the sample, the luminous intensity increases, to a peak (maximum) within 30 s (that is why the system is called Flash). Toxic compounds causes decrease of bioluminescence. The results are expressed as the ratio of luminescence. The protocol has been standardised, the ISO standard was issued recently (ISO 21338:2010). Luminescence intensity is evaluated in a kinetic mode. The protocol applicable for testing the toxicity of solid and/or coloured samples. In parallel with the Flash system, genotoxicity was assessed using the SOS-chromotest[TRADEMARK]. This is a colourimetric test, which employs a genetically modified *E. coli* strain (PQ37), and uses the cells own mechanism, (the SOS repair mechanism) to detect genotoxicity.

In case of ecotoxicity analysis, the toxicity of the exhaust gas samples are given as EC50 values. EC50s are expressed as % of the initial concentration. For the tested samples, EC50s showed a considerable variability, fell within the range of 0.25-442.73 of the original suspension. The results of SOS-chromotest[TRADEMARK] analysis showed only a slight genotoxicity for five exhaust gas samples: one sample were equivalent to 0.625 µg/ml 4NQO (positive control), and four samples were equivalent to 0.313 µg/ml 4NQO, all samples in the most quintessential dilutions.

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TUPC3-4

Specific toxic effects of six size-fractions of PM10 from urban locality

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It has been proposed previously that atmospheric pollutants could play a role in causing adverse effects on endocrine regulation and/or reproduction. Their mode of actions could involve modulation of receptors such as estrogenic receptor (ER), androgenic receptor (AR) or aryl hydrocarbon receptor (AhR). Many of the organic pollutants are due to their physical-chemical properties associated with air particulate matter (PM). The smaller size fraction they are associated with the deeper they can penetrate into airways and are thus more likely to induce adverse health effects. In our study, we focused on assessment of specific toxic effects of pollutants associated with six size fractions of inhalable PM. The studied air samples were collected at a traffic-burdened urban site in Brno (Czech Republic) and a village site about 8 km from Brno city to address the influence of city agglomeration as an air pollution source on the surrounding area. The samples were analyzed for aryl hydrocarbon receptor (AhR) mediated effects, anti-/estrogenicity and anti-/androgenicity. These parameters were assessed using in vitro reporter gen assays based on mammalian cell lines. This research was supported by GACR P503/10/P249 and CETOCOEN (CZ.1.05/2.1.00/01.0001).

TUPC3-5

Analysis of brominated and organophosphate flame retardants in indoor environment from Pakistan and Kuwait

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Fire is one of the major source of damage to the properties, loss of life and public expenses. Flame retardants (FRs) are added to the polymeric materials to increase their fire resistance. Polybrominated diphenyl ethers (PBDEs) are one of the most widely used and studied FRs. However, regulation on their use in different parts of the world has lead to the increase in the market share of replacement FRs, such as novel brominated flame retardants (NBFRs), hexabromocyclododecanes (HBCDs) and organophosphate flame retardants (OPFRs). Fewer efforts have been made to measure the human exposure to alternative FRs and that their levels in dust have been scarcely evaluated worldwide. To shorten the knowledge gap regarding these chemicals, alongside PBDEs levels we measured concentrations of major NBFRs, OPFRs and HBCD isomers in indoor dust from Asian countries namely Kuwait and Pakistan.

Dust samples were collected from homes living room floors (n = 15 per country) and from cars (n = 15 per country). We aimed at comparing the two micro indoor environments for the selected regions. Dust samples were extracted by a combination of ultrasonication and vortex, followed by cleanup and fractionation by solid-phase extraction. PBDEs and NBRs were analysed by 6890 Agilent (Palo Alto, CA, USA) gas chromatography (GC) coupled to 5973 mass spectrometer (MS) operated in electron capture negative ionization (ECNI) mode, while OPFRs were measured by GC/MS in electron impact (EI) mode. The separation and determination of α -, β -, and γ -HBCD isomers was achieved using a dual pump Agilent 1100 Series liquid chromatograph coupled to an Agilent 6410 triple quadrupole MS system operated in the electrospray negative ionization mode. Flame retardants (PBDEs, NBRs, OPFRs and HBCDs) were present at various concentrations and with different detection frequencies in the analyzed indoor dust samples. Different exposure scenarios were calculated using the 5th, median and 95th percentile concentrations. Exposure scenarios were calculated assuming 100% absorption of intake dust, 70 kg body weight (bw) for adult and 12 kg bw for toddlers, mean dust ingestion (adults 20 mg/day; for toddlers 50 mg/day) and high dust ingestion (adults 50 mg/day; for toddlers 200 mg/day). Exposure assessment values were lower than RfD values. However, the presence of these chemicals in our indoor environments emphasizes the need to evaluate their health implications for human exposure.

TUPC3-6

Evaluating exposure and risk to non-smokers from tobacco smoke PAH residues on smokers

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Polycyclic aromatic hydrocarbons (PAHs) are a suite of potentially carcinogenic organic compounds generated during the incomplete combustion of carbon-containing material such as tobacco. Though PAH concentrations have been widely studied in first and second hand smoke, there is a paucity of data sets characterizing levels in 'third hand' smoke, the residue adhering to the smoker and smoking environment after combustion. The purpose of this study was to quantify the levels of various PAHs in tobacco smoke residue, or third-hand smoke, and assess whether these concentrations are significant as a vector for exposure to nonsmokers, especially children. To quantify PAH levels within third hand smoke, test subjects' hands used to hold the cigarette were swiped before and after smoking with alcohol-saturated cotton gauze. PAHs were subsequently Soxhlet extracted with hexane. Hand swipes were analyzed for 20 PAHs using gas chromatography mass spectrometry (GC-MS). Smokers' hands have significantly higher PAH levels than non-smokers. For example, after only one cigarette smoked under ambient conditions, total PAHs were as high as 200 ng/hand for smokers compared to <40 ng/hand for nonsmokers. PAH residues on smokers' bodies (and apparel) may represent a potential source of third-hand smoke exposure to nonsmokers, particularly children, and indoor environments.

TUPC3-7

In vitro endocrine-disrupting potential of particulate or gaseous phases of air extracts from indoor or outdoor Paris urban areas on the thyroid or estrogen receptor-mediated transcriptional activity

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Chronic contamination of the atmosphere by a number of endocrine-disrupting compounds (EDC) involves new societal concerns related to the importance of the constant human exposure to trace chemical mixtures and its possible subsequent effects upon health. To date, this question remains poorly documented, especially in France where only the aquatic and the terrestrial compartment were characterized for their endocrine-disrupting activity. Contamination of air has been evaluated primarily by chemical analyses of indicator contaminants, and mainly on compounds associated with particulates. However, gaseous phase of ambient air can also represent a potential source of EDC. In this context and as a first approach, we assessed the in vitro estrogenic and thyroid disruptive potential of both the particulate and gaseous phase fractions of indoor (an office) and outdoor air from Paris urban areas on the two cellular transactivation assays, MELN and PC-DR-LUC respectively, which were previously used in our laboratory to characterise endocrine-disrupting activity of water sources and sediments in the greater Paris area. Four samplings were realized, and selective extractions of EDC potentially present were performed. Among the tested air extracts, the indoor gaseous phase was the more active in both transactivation assays, estrogenicity being quantifiable for all the samplings compared to thyroid activity. Extracts endocrine-disrupting potential was in decreasing order: indoor gaseous phase > outdoor gaseous phase > particulate phase, in a significant manner for the estrogenic effects, and as a tendency for the thyroid effects. Moreover, these differences of activity between extracts were correlated with these of cellular toxicity induced by concentrated extracts, indoor gaseous phase showing then the highest toxic content. Our results are in accordance with literature, i.e. ambient air gaseous phase induces an estrogenic receptor-mediated gene expression more important than the particulate phase. Until now, the thyroid-disrupting potential of ambient air extracts has not been characterized. This is the first report of occurrence of endocrine-disrupting activity of ambient air in France. In perspective of these preliminary results, other and more sampling campaigns could be conducted in different everyday life locations taking into account two different populations, adults and children. Effects of air extracts could be also evaluated on other endocrine endpoints.

TUPC3-8

Air quality evaluation in various indoor environments in the Lancaster University

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The present study aimed at evaluating the air quality in a number of indoor environments in the Lancaster University, in Lancaster, United Kingdom. The study covered a wide range of sites associated to work- (laboratories, offices, library), recreation- (sports centre, bars), private life- (residences, laundry) activities. Target chemicals were volatile organic compounds (VOCs) of concern, including BTEXs (Benzene, Toluene, Ethylbenzene, Xylenes, Styrene), terpenes, siloxanes etc. The daily exposure of students/university staff to VOCs was also estimated.

Hexane and toluene were the most abundant VOCs detected in Environmental Science laboratories, owing to the massive usage of solvents for regular extraction and analysis purposes. Limonene exhibited high concentrations in rooms and public places (cafes and bars), being consistently >100 µg/m³ reflecting the impact of the continuous cleaning that takes place. Benzene, the most hazardous among VOCs, ranged between ND and 46 µg/m³. Some of the highest Benzene concentrations were recorded at the Chaplaincy centre (where religious ceremonies take place), probably attributable to the candles burning and at a bar suddenly after smokers from outdoors were returning indoors. Other solvent-BTEXs occurred regularly in significant concentrations, probably due to the fact that continuous restorations (painting, maintenance, rejuvenations) take place in the Lancaster University.

D3 and D5 cyclic siloxanes (which have been used as additives in personal health care products) were regularly detected at concentrations up to 50 µg/m³. Finally, total VOCs, calculated as the sum of VOCs occurring chromatographically between n-Hexane and n-hexadecane and quantified as toluene equivalents, were compared to existing indoor air regulations or recommendations.

TUPC4 - Sorption and bioavailability in sustainable remediation of organic chemicals

TUPC4-1

Community effect of activated carbon amendment to aquatic sediments

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Many in situ and ex situ studies demonstrated a high potential of activated carbon (AC) to reduce aqueous concentrations of hydrophobic organic contaminants in aquatic sediments. However, the application of AC in sediment remediation also should be ecologically safe. Accordingly, there is a need for information on possible negative effects of AC application on the ecology of aquatic ecosystems, e.g. potential impacts on benthic communities. The aim of the present study was to check colonization rates of AC-treated sediment by estimating species diversity and abundance as a function of time. No statistical difference was found in the number of taxa after 3- and 15-m colonization of AC-sediment. The number of taxa and of individuals was not influenced by AC concentration. Results of the Shannon index analysis did not reveal any difference in colonization of AC-treated sediment both after 3- and 15-m exposure time. However, a higher abundance of sensitive species in 0% AC and higher abundance of tolerant species in AC-sediment observed in the present study might indicate a shift in the benthic community composition.

TUPC4-2

Sequestration of sediment associated PAHs with granulated activated carbon (GAC)

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River and marine sediments are contaminated with a wide variety of hydrophobic organic compounds (HOC), which are recalcitrant for degradation and pose a substantial risk for the aquatic wildlife. Our current work aims at the development and implementation of safe and cost effective remediation technologies based on activated carbon (AC) addition. Whereas AC already has been applied to immobilize HOCs in bed sediments, the intensive removal of HOCs from sediments using granulated coarse AC (GAC) may constitute an important next step in the development of AC related remediation technologies. Therefore, we determined the capacity of GAC to reduce freely dissolved aqueous phase concentrations in sediment natively contaminated with polycyclic aromatic hydrocarbons (PAH). The obtained in situ sorption coefficients for GAC relate to binding of PAH in the presence of sediment, that is, accounting for any effect of organic matter fouling or sediment sorption competition.

TUPC4-3

Biological effects of carbon amendments in PCB contaminated sediments on *Lumbriculus variegatus*

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Carbon amendments are being studied to be used as an *in-situ* remediation method for contaminated sediments. Most studies have focused on the efficiency of the added amendments to reduce the availability of the chemical contaminants. Fewer studies have been directed to follow the potential adverse effects of these amendments. The contaminated sites may have viable benthic community and therefore remediation actions can disturb the ecosystem. On the other hand, bioaccumulation in the food web results in a situation where the organisms in the higher trophic levels are at risk and/or they cannot be used for human consumption, which increases the remediation pressures. In order to find the best method for remediation the potential adverse effects of restoration measures needs to be evaluated. In this study, we assessed the effects of activated carbon and biochar in PCB contaminated sediments on feeding, growth and reproduction of an experimental organism *Lumbriculus variegatus*. We used coal based activated carbon and wood based biochar, which we mixed in separate experiments to the sediments. In addition, we tested if cleaning (hot water, solvent extraction) of activated carbon would affect the measured parameters. Activated carbon had sediment specific adverse effects on feeding and growth. The adverse effects of biochar were minor than those observed with comparable doses of activated carbon. The pre-treatment of the activated carbon by hot water or solvent extraction had only minor effects on the measured biological parameters. The ecological consequences inflicted by carbon amendments should be considered sediment specifically.

TUPC4-4

Bioavailability reduction with activated carbon amendment: comparison of field results and expectations under optimal conditions

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Application of activated carbon (AC) at the pilot-scale in Grasse River, NY USA has been shown to reduce the potential human and ecological health risks posed by polychlorinated biphenyl (PCB)-impacted sediment by reducing bioavailability. For instance, over 3-years of post-application monitoring, bioaccumulation in a freshwater oligochaete worm was reduced by 62 - 99% compared to untreated background sediment, and pore water concentrations measured by an aqueous equilibrium batch test were reduced by >93%. To provide perspective on how the level of reductions achieved in the field relate to the reductions that may be expected under optimal conditions, PCB bioaccumulation in *L. variegatus* from mixed tiller field-amended sediments was compared to exposures where AC was first preloaded with a mixture of PCBs (Aroclor 1260). In addition, aqueous equilibrium measurements were compared to modeled pore water concentrations assuming equilibrium conditions with a range of AC doses according to a dual-sorption model which was parameterized for the organic carbon and activated carbon present in Grasse River sediment. When PCBs were preloaded on AC and amended to clean sediment, the bioaccumulation observed in *L. variegatus* was 93% less for total PCBs compared to spiked unamended sediment, with bioaccumulation of tetra and penta chlorobiphenyls less by 98 and 96%, respectively. When results are compared for a tetra and penta PCB congener which are at similar concentrations in the spiked and field sediments it is evident that bioaccumulation is greatly reduced with field-aged AC amendment but not to the level achieved when the majority of PCBs have been transferred to the AC. Aqueous pore water concentrations modeled at equilibrium were 1-2 orders of magnitude lower than the aqueous equilibrium batch test results. This large apparent difference is partly accentuated by the fact that several measurements were below detection limits and may lie closer to predictions. These results indicate that optimum equilibrium sorption has not been achieved in the field, likely as a result of the combination of relatively slow mass transfer kinetics and sorption attenuation of PCBs in the field by natural organic matter. This field study has shown AC amendment to be effective at reducing bioavailability of PCBs in sediment, but that longer periods of time will be necessary to allow sequestration of PCBs by AC to reach its full potential.

TUPC4-5

Use of bioadsorbents to reduce the leaching in soil of fluometuron and MCPA

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The environmental impact of herbicides, especially their presence in surface and ground waters, can be minimized in a preventive way with the use of controlled release formulations and in a corrective way with the use of barriers or filters that trap and immobilize them. For both applications, materials such as natural clays, anionic or modified, and other bioadsorbents coming from natural wastes have shown their utility. Among the bioadsorbents there is a great interest in biochar (solid residue produced by biomass pyrolysis) as a soil amendment, because it is a stable source of nutrients, and its contribution to carbon sequestration. Due to the important role of the solid natural or added soil components on the processes related to the presence of herbicides in water, it is interesting to compare the effects of biochar to those of organoclays. The aim of this work was to compare the effect of an organoclay saturated with hexadecyltrimethylammonium (SAz-HDTM) cation and three biochars used as a soil amendment (2% w/w) on the adsorption and mobility of the herbicides fluometuron (N,N-dimethyl-N'-[3-(trifluoromethyl)phenyl]urea) and MCPA (4-chloro-2-methyl phenoxyacetic acid). Results showed that all adsorbents increased the adsorption of both herbicides on the soil, decreasing their leaching or percolation losses, except for biochar (BC2). The lower adsorption and higher leaching of both herbicides observed in the soil amended with BC2 is due to the high content on dissolved organic matter (DOM) of the biochar, which probably compete with the herbicides for the adsorption sites and/or the formation of one DOM-herbicide complex with higher water solubility, facilitating its mobility with water. The other biochars are efficient in decreasing the herbicides' percolation by 50-70% for fluometuron and 30% for MCPA. For both herbicides, the organoclay is less efficient as compared to the biochars, decreasing the percolation of fluometuron by 35% and MCPA by 45%. The reduction is directly proportional to the adsorption capacity of this organoclay for both herbicides and is related to the interactions organoclay-herbicides at molecular level, due to the neutral character of fluometuron and acidic character of MCPA. These interactions are also shown by the movement of the peak of maximum concentration of the breakthrough curves in the soils amended as compared to the unamended soil.

TUPC4-6

A method to simultaneously determine reduction in PAH dissolved concentrations and bioaccessibility in carbon amended soils

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Soils polluted with polycyclic aromatic hydrocarbons (PAHs) represent a risk to the environment. Therefore, different soil amendments including activated charcoal (AC), biochar (charcoal) and compost are often added to remediate such soils. On the one hand, this leads to a reduction in the PAH dissolved concentrations, and therefore also bioconcentration and toxicity. On the other hand, strong sorption to the soil amendments can reduce bioaccessibility to degrading microorganisms, decreasing the efficiency of remediation. Therefore, to understand the effects of soil amendment addition, methods are needed that can simultaneously determine both the changes in PAH dissolved concentrations and their bioaccessibility. In this study an approach based on the sequential sorption of phenanthrene (Phe), as a model PAH, into an infinite sink consisting of silicone O-rings was developed. The extent of abiotic desorption ($t = 24$ d) of [9-¹⁴C]Phe from suspensions made up of sandy loam soil (either Outfield, RS, or Olsen) plus amendment (either AC, charcoal, or compost) was investigated. The total amount of Phe desorbed was 6 to 10% for AC, 38 to 44% for charcoal, 87 to 106% for compost, and 95 to 106% for the control without any soil amendment. The temporal development in desorption was accompanied by a parallel decrease in the Phe dissolved concentration. Desorption was then compared to the extent of mineralization ($t = 15$ d) of Phe sorbed to the soil plus amendment suspensions by *Sphingomonas* sp (DSM 12247). Between the different soils and amendments, desorption and mineralization followed the same trends, but mineralization was slightly lower with the peak of Phe mineralization correlated with a peak in bacterial growth. Interestingly, compost induced a higher cell density but inhibited the Phe mineralization. Therefore, desorption of Phe into an infinite silicone sink was a useful tool for predicting the extent of mineralization (bioaccessibility) as well as changes in dissolved concentrations. Furthermore, the silicone O-ring format used in this study was practical to handle and greatly simplified the final Phe analyses.

TUPC4-7

Impact of wood biochar on the biodegradation and bioavailability of naphthalene in soil

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Naphthalene is a major component of naturally occurring coal tar and crude oil. It can however be volatilized or biodegraded efficiently in soil but at high concentrations it could leach to aquatic systems and express toxicity to aquatic life. In order to enhance remediation of naphthalene, biochar, which is a carbon rich product from the pyrolysis of biomass can be utilised to control its fate in the environment. This study investigated the influence of 0%, 0.1%, 0.5% and 1.0% of two different biochar (BC1 and BC2) sourced from wood wastes on the relationship between microbial mineralisation and hydroxypropyl- β -cyclodextrin (HPCD), CaCl₂ and methanol extractions of ¹⁴C-naphthalene (50 mg kg⁻¹) in soil. BC1 was produced at 450°C for 16 hours and BC2 was produced at 1000°C for 1 hour. The amendment conditions were aged for 0, 18, 36 and 72 days. The total extent of ¹⁴C-naphthalene mineralisation was assessed by monitoring ¹⁴C-naphthalene mineralisation over 14 days in respirometric assays using indigenous microflora and compared to HPCD, CaCl₂ and methanol extractions. Results showed that the 0.5 and 1.0% of BC1 and all concentrations of BC2 amendments showed significant reduction ($p < 0.01$) in extent of mineralisation compared to 0% BC and cumulative extractions. Linear correlation between HPCD extractability and total amount mineralised over 14 days revealed very good correlation in all concentrations of biochar amendments BC1 ($r^2 = 0.94$, slope = 0.94, intercept = -0.86) and BC2 ($r^2 = 0.94$, slope = 0.90, intercept = -1.34). However, fastest rate of mineralisation showed poor correlation. Additionally, the CaCl₂ and methanol extractions underestimated and overestimated extent of mineralisation respectively. Bioaccessibility often describes the biodegradation endpoint. This paper thus states that biochar used in this study can reduce the bioaccessibility of naphthalene at high concentrations and that HPCD extraction strongly predicts the bioaccessibility of naphthalene in soils even when amended with biochar. However, the production process, feedstock and soil properties determine the capability of biochar to adsorb organic contaminants.

TUPC4-8

Status on activated carbon amendments to reduce bioaccumulation and possible secondary effects

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Innovative in-situ remediation techniques are being investigated to reduce the release and bio-uptake of contaminants from sediments polluted with hydrophobic organic compounds (HOCs) [1]. Sediment amendments with a strong sorbent material like activated carbon (AC) is one alternative in-situ approach for remediation based on the

theory that HOCs are predominantly bound to charcoal-like particles in natural sediments [2]. Today, these theories are confirmed by multiple studies, which found that water concentrations were reduced by 80% to 99% for PCBs, by 60% to 94% for PAHs, and by 67% to 83% for DDTs. The published data show that by reducing the flux of HOCs to the aqueous phase, sediment AC-amendment also reduces the bioavailability of these pollutants and biodynamic modeling may be used to estimate tissue concentrations. Having established that AC amendments can effectively reduce bioaccumulation, the attention has focused lately on possible secondary effects of AC on the health of benthic organisms. While AC amendment reduces the availability of HOCs, other organic matter, including dissolved organic carbon and nutrients, may become less available as well. However, to the extent that those secondary effects have been reported, they were variable across benthic species and sediments tested and results have to be interpreted carefully regarding the actual implications for field conditions.

For PCB polluted sediments, bioaccumulation decreases with increasing AC dose and with smaller AC particle size. An illustrative application range for in-situ amendments of 3-4% with 70-200 µm AC resulted in a reduction of PCB bioaccumulation by approximately 80-90%. The biodynamic modeling approach predicts PCB bioaccumulation reasonably well and may assist the estimation of the expected remedial success for in-situ amendments when monitoring data are not available. Currently, observed possible secondary effects of AC on benthic organisms are non-toxic, limited to egestion, lipid content and growth, and are not highly significant compared the benefit AC achieves by reducing exposure to persistent and bioaccumulative pollutants.

TUPC5 - Are Environmental Specimen Banks ready to face future challenges of environmental chemistry and regulatory toxicology?

TUPC5-1

Derivation of dynamic reference values for the assessment of contaminant concentrations in fish of German freshwater ecosystems using data of the German ESB

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Accumulation indicators are widely used in biomonitoring studies and programs, respectively. The contaminant concentrations in biological matrices of different sampling sites can show regional differences and time series can show temporal changes. However, in terms of relevance to biological systems, assessing contaminant concentrations is very difficult. Assessment by sublethal effect concentrations or by environmental quality standards is only possible in exceptional cases.

Relative reference standards are an alternative to directly assessing tissue concentrations. However, such reference values did not exist for environment-biomonitoring. The data pool of the German Environmental Specimen Bank (ESB) can be used to derive reference values for Germany. Based on the analytical data of the musculature of bream (*Abramis brama*) the derivation of dynamic reference values was conceptually designed and implemented. The bream (*Abramis brama* L.) is a freshwater species which is annually sampled by the ESB at 17 sites.

A five-classes-system was conceptualised where four reference values define five reference ranges. The reference values correspond to a two-year-period and are constantly updated. The reference values were derived for 21 chlorinated hydrocarbons based on fat and wet weight and for 6 elements based on dry weight and wet weight.

The derived reference values can be used as environmental quality criteria for the assessment of the chemical inland surface water status. Since the sampling areas of the German ESB are representative of Germany, the reference values show the general state of inland surface water pollution in Germany. Because of the short reference periods and the constant updating of the reference values, the temporal development of this state of pollution can be shown. Water and fat content vary considerable between fish species. Using reference values based on fat or dry weight compensates these differences. Thus, the reference values of chlorinated hydrocarbons based on fat and those of elements based on dry weight are an assessment tool for other fish species with comparable metabolism too.

TUPC5-2

Retrospective analysis of perfluorinated alkyl acids in marine mammals from Alaska

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Wildlife from remote locations have been shown to bioaccumulate perfluorinated alkyl acids (PFAAs) in their tissues. There are a number of studies examining the accumulation of PFAAs in Arctic fauna from Canada, Greenland, and northern Europe; however, there are few studies examining the levels of PFAAs in tissues collected from the Alaskan Arctic. In this study, twelve PFAAs, consisting of perfluorinated carboxylic (PFCA) and sulfonic (PFSA) acids, as well as the perfluorooctane sulfonate (PFOS) precursor perfluorooctane sulfonamide (PFOSA), were measured in beluga whale (*Delphinapterus leucas*) and northern fur seal (*Callorhinus ursinus*) livers collected in Alaska between 1987 and 2007 to understand their spatial distribution and temporal trends. The beluga livers were from two isolated populations (Cook Inlet and Eastern Chukchi Sea), whereas the fur seal livers were collected from one location, St. Paul Island. Long-chain PFCA, (9 to 14 carbons), and PFOS were detected in most of the samples (>80%). Cook Inlet belugas had higher concentrations of most PFCA and PFOS ($p < 0.05$), but a lower median concentration of PFOSA when compared to the Eastern Chukchi Sea belugas ($p < 0.05$). Temporal trends examined in both species indicated that most PFCA concentrations increased in belugas from 1986 to 2006 and in northern fur seals from 1987 to 2007 ($p < 0.05$). This work was made possible because of the collection and banking of samples done through the Alaska Marine Mammal Tissue Archival Project and emphasizes the importance of environmental specimen banks as a sample resource for assessing chemical trends.

TUPC5-3

Environmental specimen banking in China: necessity and development

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With a rapid economic development and urbanization of China, the environment has been changed a lot since the year of 1978. Environmental specimen bank (ESB) are very useful to investigate temporal trends in ecosystems, to evaluate the results of governmental environmental policies and regulations, and to provide a resource for evaluating animal health. However, no systematic and comprehensive ESB was built currently. In this study, we summarized the necessity to build the ESB in China, and then discuss our opinion on how to develop the ESB in China based on the ongoing research and global interests.

TUPC5-4

Environmental specimen banking development in Finland

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1. Introduction

Environmental sampling in Finland has traditionally dispersed in several different programs each with its own sample storage strategies and data processing systems. In spite of numerous useful series of samples and data, researchers may often be unaware of the existence of relevant material or specimens obtained. To fulfill these needs the Finnish Forest Research Institute and the Ministry of Education established a research and development project "Environmental specimen banking and co-operation between different institutes in Finland". The project aim was to develop the storage and use of existing specimen material in scientific research and education.

2. Specimen banks in Finland

In Finland there are at this moment two environmental specimen banks, Paljakka and Hakuninmaa, established by Finnish Forest Research Institute (Metla) and Finnish Environment Institute (SYKE). SYKE coordinates and participates in monitoring of harmful substances in aquatic and terrestrial environments.

Metla established a network of 3009 permanent monitoring sites all over the country in 1985-1986 for forest inventory and monitoring environmental changes. During the first two years 6 877 moss, lichen and pine bark samples were collected. The Paljakka ESB was established to store increasing amount of samples in 1994. Stored samples originate from several national and international projects like UNECE ICP Vegetation and ICP Forests. At this moment the amount of samples is estimated to exceed 700 000.

3. Development needs

There is a clear need to develop information availability and co-operation in the utilization of existing environmental data and material. This was also expressed at the International Environmental Specimen Banking and Information Management Seminar. Several topics were emphasized for further discussion:

- Data sharing should be developed and updated continuously.
- Existing sample material is not intensively utilized.
- When environmental monitoring is being harmonized, end-users should be taken account.

TUPC5-5

Long-term monitoring by trees within the German ESB

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Biometrical and analytical characterization of systematically sampled specimens for the German Environmental Specimen Bank (ESB) can be used as a real-time monitoring system as well as a control tool to verify the long-term comparability of the applied sampling system. Samples from trees representing environmental contamination on the ecological level of primary production are sampled within 9 representative areas of different exposure in Germany for up to 24 years. Samples were taken each year; in some cases every second year applying standardized sampling procedures.

A thorough analysis of the data comes to the following results:

- Increased needle coverage over 25 years demonstrate improved vitality of spruce trees near the urban-industrial conurbation of Saarbruecken
- Many of the analyzed substances (PAHs, Pb, As) in conifer shoots from forests near urban-industrial conurbations declined by almost a factor 10 or more from 1990 to

2000.
 - Sulfur in the same samples decreased to about 50% of their former concentrations near Leipzig and about 80% near Saarbrücken.
 - PCB concentrations decreased to about 20% from 1985 to 1997 near Saarbrücken and to about 50% from 1991 to 2000 near Leipzig.
 - From 2000 to 2010 the concentrations of PAHs, Pb, As, S and other substances in conifer shoots from forests near urban-industrial conurbations continued to decrease, but much slower.
 - In remote regions like the National Parks (NP) of Bavarian Forest and Berchtesgaden, PAHs and Pb did not decrease any more after 2000.
 - In samples from the NP Bavarian Forest concentrations of PAHs, Pb, Tl, and As are 1,5 to 5 times higher than in samples from the NP Berchtesgaden.
 - The NP Bavarian Forest is a remote area, but, in opposite to the NP Berchtesgaden, it is exposed to long-range transport of air pollutants from southern Germany and the Czech Republic
 - Since 2000 concentrations of high molecular PAHs, Pb and As in samples of the NP Bavarian Forest tend to exceed more and more those of samples from forests near urban-industrial agglomerations of Saarbrücken and Leipzig.

TUPC5-6

The potential for a UK environmental specimen bank

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There are numerous monitoring and experimental research studies in the UK that involve collection and archiving of environmental specimens. We have estimated the cost of these activities to be approximately £16 million per year. However, there is no current UK-wide strategic coordination of this investment. Although the United Kingdom Environmental Observation Framework (UK-EOF) catalogues environmental observations made for and by the UK, it does not currently catalogue specimen collection. This poster describes a short study to: (i) gather information on current specimen collection and archiving in the UK and (ii) investigate the potential for a UK Environmental Specimen Bank (UK-ESB) that would provide links and strategic coordination between different collections. This study focused on environmental specimens, largely biological and geological samples, but excluding medical specimens.

TUPC5-7

Perfluorinated compounds in liver from wild otters (*Lutra lutra*) from Sweden and Norway, collected between 1972 and 2011 - a new threat to the otter population?

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Liver samples from 122 wild otters from Sweden and seventeen otters from northern Norway were analyzed for the presence of PFCs (PFHxA, PFHpA, PFOA, PFNA, PFDoA, PFUnA, PFDoA, PFTriA, PFTeA, PFBS, PFHxS and PFOS) and 57 of them were also analyzed for PFPeDA, PFDcS and PFOSA.

The otters were killed in traffic accidents, or drowned in fishing-gear, and were subsampled from the Environmental Specimen bank at the Swedish Museum of Natural History in Stockholm and from NIVA Institute, Norway. Ninetyseven of the otters were from southern Sweden and were used for a time trend study from 1970 to 2011.

Seventysix otters were collected between 2006 and 2011 and were included in a geographical study including three areas: southern and northern Sweden and northeastern Norway.

PFOS was by far the most dominant isomer of all analysed. It was found in high concentrations in all samples, ranging from 19-16 000 ng/g w.w.

Two of the isomers, PFHxA and PFHpA, were under detection limit in all samples (<0,2 and <0,3 ng/g respectively, n=56) and PFBS were under detection limit in 40 of 56 samples and therefore these three substances are not used in the analysis.

The Swedish otters had similar or much higher concentrations of PFOS than what was reported from the river otter (*Lutra canadensis*) in North America (25-994 ng/g liver w.w.) and the American mink (*Mustela vison*, up to 5140 ng/g w.w.). The highest concentrations were found in otters from the south-central more urban parts of Sweden.

All isomers but two (PFDcS and PFOSA) showed a significant increase in concentrations since 1970, between 5 and 15 % yearly.

Generally, lower concentrations were found in northern Sweden, and even lower in northwestern Norway. This can be expected since southern Sweden is the most densely populated of the three areas. However, some elevated concentrations of PFOS were seen even in north central Sweden. The range in concentrations was very high. For example, some otters had very high concentrations of PFOS but other otters found nearby had low concentrations. No obvious adverse health effects have been studied within this study. All otters were frozen before sent to the museum or NIVA, so histopathology was not possible to be done.

TUPC5-8

The US Pacific Islands Region biorepository and environmental specimen bank

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The National Institute of Standards and Technology (NIST) maintains analytical chemistry laboratories and a cryogenic specimen bank facility at the Hollings Marine

Laboratory (HML) that is devoted to developing methods, analytical measurements, and quality assurance. In addition, NIST maintains an archive of biological and environmental specimens in support of several research and environmental monitoring programs of other agencies and organizations. Archived specimens include marine mammal tissues, marine sediments, shellfish samples, fish tissues, bird eggs and feathers, human liver tissues and blood samples, and human diet samples. Historically, the biota and environmental samples have originated from the U.S. continental area (including Alaska). In 2010, NIST initiated an expansion of its specimen banking efforts to the U.S. Pacific Islands Region. This region includes various U.S. islands in the Pacific Ocean ranging from the main Hawaiian Islands in the east, to Guam in the west, and from Midway Island in the north to American Samoa south of the equator. NIST is using measurement and specimen banking expertise and capabilities of NIST personnel and facilities at the HML to help address environmental and ecological issues that are unique to the region. This expansion includes establishing a satellite facility on the island of Oahu, expansion of banking for ongoing programs (i.e., Marine Mammal Health and Stranding Response Program, Seabird Tissue Archival and Monitoring Project, and Mussel Watch Program), and the development of new programs for the region (banking for sea turtle health assessment). A biorepository to address coral health issues in the region is also being considered. The expansion of NIST banking into this region presents some challenges due to the region's great geographic expanse, its distance from the continental U.S., local sociological and governmental factors, and some inherent issues concerning environmental degradation and natural resource management.

TUPC6 - Plants and chemicals in the environment: risk assessment, pest management and phytoremediation

TUPC6-1

Insights into the chemical ecology of *Myriophyllum spicatum* adapted for ecotoxicological assays

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Myriophyllum sp. is one of the few dicotylous submerged species in aquatic systems, flowing and stagnant waters as well as brackish systems. Since 1991, we have developed axenic cultures of *M. spicatum* and other milfoil species in our laboratory. Now, *Myriophyllum* has been proposed as future OECD aquatic bioassay system in ecotoxicology. In the past we investigated the allelochemical role of hydrolysable polyphenols, which are present in this species in exceptionally high concentrations. Among them are also so called ellagitannins, well known for their possible antioxidant or pro-oxidant activity. Milfoil polyphenols act as natural herbicides and pesticides against competing algae and aquatic insect herbivores. The expression of these bioactive secondary metabolites is specifically affected by abiotic and biotic stressors. Different environmental conditions such as light and nutrient availability or the presence of bacteria affect not only the concentration and but also the composition of polyphenols in *M. spicatum*. These complex interactions have to be considered in ecotoxicological assays using axenic cultures of *M. spicatum*. Our results indicate that the reaction of the plant towards certain pollutants has to take into account growth conditions and allelochemical interferences with co-occurring species to precisely predict the reaction of *M. spicatum* or other milfoil species to different pollutants.

TUPC6-2

Modified sediment-free *Myriophyllum* biotest

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Some plant protection products and industrial chemicals showed an unavoidable high risk for submersed aquatic plants. Tests with *Myriophyllum* spec. should be performed to reduce uncertainties. *Myriophyllum spicatum* represents such a submerged macrophyte.

Up to know ring-tests with *Myriophyllum spicatum* in an unsterile water-sediment or axenic sediment-free system were performed. Here we present a test design without sediment under unsterile conditions. The pros and cons of this test design will be discussed in comparison to the water-sediment and axenic sediment-free tests.

TUPC6-3

Is toxicological sensitivity of *Myriophyllum aquaticum* compared to *Lemna minor* an argument to refine risk assessment of chemicals?

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Myriophyllum aquaticum is a candidate for an additional macrophyte species in refined risk assessment of chemicals. To contribute to filling in the largely missing data on relative sensitivity of *M. aquaticum* to toxic compounds (compared to *Lemna* sp.), four substances were tested using both species in parallel: atrazine, isoproturon, trifluralin and 3,5 DCP. *Lemna minor* tests were conducted following the standard OECD protocol, while *M. aquaticum* was tested in water-sediment system according to draft protocol suggested for future OECD standard. Growth inhibition in *Lemna* tests was estimated using relative growth rates (RGR) and yield based on total number of fronds, total frond area and total fresh weight biomass. Growth inhibition of *M. aquaticum* was estimated using RGR based on length of the plants above sediment, total length of the plants, number of side shoots, total dry and fresh weight biomass. The only common end point for both tests is the RGR based of total fresh weight biomass at the end of the

test. Therefore, the comparison was based on that particular endpoint only: *M. aquaticum* consistently showed lower sensitivity to all four substances than *L. minor*. However, how relevant those comparisons really are? Or, is it sensible to compare relative sensitivity of two plants using the results of two growth inhibition test? *M. aquaticum* test is conducted in static conditions, at the level of the individual - we measure the growth of individual plants, while *Lemna* sp. tests, run in static - renewal system, provide information on effect of chemicals at the level of population, as we measure the reproduction. Risk assessments based on *Lemna* and its endpoints may not be protective of other macrophyte species not due to potential differences in sensitivity to chemicals with specific toxic modes of action, but primarily due to different life history, growth strategy, exposure route, but also differences in recovery rate after exposure to toxic compound. Therefore, if compared to *L. minor*, the difference in sensitivity is not the critical argument which advocates the use of *M. aquaticum* as an additional macrophyte species in refined risk assessment, but rather differences in growth rate, lag phase and recovery potential from water-column substances as well as its suitability for studies of the effect of sediment-bound pollutants.

TUPC6-4

Use of the aquatic plant *Glyceria maxima* (Hartman) Holmberg (1919) in a growth inhibition test - method development

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According to proposed recommendations for herbicide registration in the EU, evaluation of the effects of some herbicides on the rooted, emergent, monocotyledonous aquatic plants, *Glyceria maxima* (Hartman) Holmberg (1919) may be required. However, a ring-tested method is not available for this species.

Based on the test protocol "Guidelines for assessing the effects of pesticides on the growth of *Glyceria maxima*, University of Bristol, Long Ashton, Bristol, 23.05.2001" the effects of a grass herbicide on *Glyceria maxima* were evaluated in a 28-day experiment under controlled environment conditions.

The test used five concentrations and an untreated control, each treatment with three replicate test vessels containing five potted plants (15 plants in total for each treatment). Six days before the start of the test the plants were divided into sections with two shoots, which were individually planted in artificial sediment. The test substance was applied via the water phase without any renewal of the test media. Assessments of shoot length were made weekly and shoot wet and dry weight was measured at the beginning and end of the test.

The results showed that this test method is appropriate to assess the effects of an herbicide on *Glyceria maxima* although some improvements still have to be considered (e.g. how to inhibit the algae growth in the test vessels completely). The calculated endpoints (growth rate and yield of each parameter) showed acceptable coefficients of variance, which were within 20 to 30 % for the total shoot length, within 10 to 20 % for wet and dry weight of the control plants after 28 days of exposure. The mean shoot length of the control plants nearly doubled within the test period. The main factor which reduced the variance was to start the test with plants having two shoots instead of plants with a different number of shoots.

TUPC6-5

Aquatic macrophytes can decrease the availability of contaminants in mesocosms and experimental ditches

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In aquatic ecosystems submerged aquatic macrophytes influence their environment physically and chemically. Here we focus on the impact of macrophytes on the concentration and speciation of contaminants in experimental systems. Contaminants can adsorb to the biomass of macrophytes, especially when the affinity between the contaminant and organic material is high (i.e. K_{oc} is above 1000 L/kg). We tested the effects of macrophyte biomass and growth form on concentrations and speciation of a highly absorbing pesticide, copper (Cu) and zinc (Zn) in mesocosms (1 m³) and flow-through experimental ditches (length 40 m, depth 0.4 m). The insecticide λ -cyhalothrin served as a model-compound, as its K_{oc} exceeds 150000 L/kg. In the mesocosm study the emergent macrophytes contained high concentrations of λ -cyhalothrin. Concentrations in submerged macrophytes were a factor of 10 lower, while sediment concentrations were 100 times lower than the submerged macrophyte concentrations. The accumulation of λ -cyhalothrin in the emergent macrophytes might be caused by their root uptake which is governed by the acropetal (upward) transpirational stream. In the experimental ditches, adsorption to macrophytes and sediment contributed to a decrease of λ -cyhalothrin concentrations in the outflow water stream when macrophyte biomass is high (378 g.m⁻² high macrophyte biomass vs. 96 g.m⁻² low biomass; water residence time $\pm$ 5 days). In the potted-plants mesocosm study, concentrations of Cu and Zn diminished rapidly, the decrease in the controls including sediment and lacking macrophytes being rapid as well. This implies an important influence of sediment on these metal concentrations. However, the experiments showed that macrophytes also accumulate metals and act as a temporary sink. Mesocosm and laboratory experiments revealed that *Myriophyllum spicatum* and *Lemna* sp. increased the DOC (Dissolved Organic Carbon) levels in the water phase and contributed to the amount of hydrophobic DOC fraction. This fraction is important for binding metals and hydrophobic pesticides. Increased levels of DOC in the water phase result in lower free Cu and Zn concentrations thereby lowering the bioavailability of these metals. In conclusion, macrophytes are an important sink for micropollutants like adsorbing pesticides and metals. Besides direct effects, macrophytes indirectly influence water concentrations by generating DOC, which can bind micropollutants, and by changing the metal speciation.

TUPC6-6

Mitigation of insecticide toxicity to sensitive aquatic taxa by the submersed macrophyte, *Elodea canadensis*

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In ecotoxicology, there is a growing appreciation of the influence that ecological interactions can have on the responses of sensitive taxa to contaminants. However, while most studies have focused on factors that exacerbate the impacts of contaminants, few have examined factors that may mitigate their effects. In aquatic habitats, submersed macrophytes may mitigate the toxicity of contaminants by sorbing them from the water column, potentially decreasing the duration and intensity of exposure for sensitive taxa. However, the extent to which macrophytes can mitigate contaminant effects and the influence that plant density has on this ability have not been examined. In this study, we examined the impact of macrophyte density on the toxicity of the organophosphate insecticide malathion to *Daphnia magna*. To do this, we performed a factorial experiment crossing five densities of the common submersed macrophyte, *Elodea canadensis*, with five malathion concentrations in 1-L outdoor microcosms. We determined the LC₅₀ values for *D. magna* in each macrophyte treatment using Probit analyses and tested for significant differences by comparing the overlap of the confidence intervals. In addition, we examined the rates at which different *E. canadensis* densities detoxified malathion-treated water by removing samples of the water column from each microcosm over a 48-hr period and calculating the LOEC for *D. magna* exposed to each sample using Dunnett's procedure.

Overall, we found that as *E. canadensis* density increased, the toxicity of malathion to *D. magna* declined substantially. For example, the malathion concentration required to kill 50% of *D. magna* was 23 times greater in microcosms containing high, but environmentally relevant, *E. canadensis* densities than in macrophyte-free microcosms. Additionally, we found that the rate at which microcosm water was detoxified increased with increasing macrophyte density. This study is the first to experimentally examine the extent to which macrophytes can mitigate the biological effects of insecticides. The results provide further support that ecological interactions can dramatically influence the effects of contaminants.

TUPC6-7

Rhizofiltration, leaching and electro-recovering: an integrated approach to treat wastewater polluted with lead using water hyacinth (*Eichhornia crassipes*) as bioabsorbent plant

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In this work is presented an integrated study which involves biological, chemical and electrochemical processes in order to treat waters polluted with lead using water hyacinth as bioabsorbent plants. The research was carried out in three stages: i) bio-absorption, ii) leaching, and iii) electro-recovering. In the first stage it was determined that water hyacinth had an maximum Pb bio-absorption efficiency of 77.5%. In the second stage, it was used ammonium oxalate as leachant agent, starting from the hypothesis that besides to extract lead from the biomass this salt will enrich it with nitrogen compounds and therefore it would be used as fertilizer. It was compared two leaching processes: simple (one stage) vs sequential (two stages), determining an Pb extraction efficiency of 52% and 91% respectively. After leaching the amount of nitro-compounds, i. e. N-NH₃, N-Org, N-Total of the leached biomass increased significantly. Finally, in the third stage, the Pb leached and concentrated in the leachate it was recovered by electro-deposit, achieving a maximum recovery efficiency of 93%.

TUPC6-8

Assessing the potential of *Miscanthus x giganteus* for fly ash revegetation and soil restoration

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Fly ash (FA) is the primary byproduct of coal-fired power stations with an annual global production that averages approximately 550 Mega tons. Due to its weight and lack of particle aggregation, FA may spread to long distances and cause serious environmental problems. In order to stabilize this material and reduce detrimental effects, we focused our work on the establishment of a direct vegetation on ash. The objective of this study was to investigate the potential of *Miscanthus x giganteus*, a sterile rhizomatous grass, for fly ash revegetation and soil restoration. To evaluate its growth ability on FA, plant development during pot experiments was compared to that obtain in a reference soil. Several physico-chemical parameters (pH, conductivity, dissolved organic carbon, ammonium, nitrite, nitrate, phosphate and potassium contents) and microbiological parameters (enumeration of total, ammonia oxidising and nitrite oxidising culturable bacteria and community diversity analysis) were investigated after plant growth. Field trials were also conducted in order to test the possibility of cultivation at a global scale. Plants demonstrated an adaptation to the FA substrate both in laboratory and in field scale conditions, with an average stem length that was half of that observed in the reference soil. Besides, significant organic carbon and nitrites inputs could be measured after 17 weeks of laboratory cultivation, indicating an improvement of FA agronomic quality. Finally, we suggest that FA revegetation with *Miscanthus x giganteus* could be a cost effective strategy for long term FA disposal site rehabilitation.

WE 001

Development of an AQUATOX ecosystem model for assessing effects of an herbicide on aquatic food webs

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The use of toxicity experiments in laboratory, mesocosm, and field applications plays an important role in pesticide risk assessment but cannot address all issues related to indirect effects mediated through species interactions within food webs. For example, as part of an application for inclusion of HerbicideX (hypothetical name) in Annex 1 (Annex IIA 1.1), available laboratory toxicity data and aquatic mesocosm results indicated the potential for short-term effects on phytoplankton and periphyton species, but the nature and time-frame of potential indirect effects on aquatic animals, including fish, were unknown. Aquatic ecosystem modeling can be used to evaluate not only direct toxic effects of pesticides but also indirect effects mediated through trophic interactions of species (e.g. competition and predation).

We have developed an aquatic ecosystem model using AQUATOX (version 3.0) to evaluate the potential direct and indirect ecological effects of HerbicideX on freshwater systems. We report here on the design and calibration of AQUATOX to represent mesocosms originally intended for assessing community-level effects of HerbicideX. Physical characteristics, nutrient loading, and major biotic groups in the AQUATOX mesocosm-mimic model corresponded to those of the HerbicideX-study mesocosms. Biological communities included phytoplankton, periphyton, zooplankton, benthic and epiphytic macroinvertebrates, and macrophytes, but not fish as the latter were absent from the mesocosms. To match characteristics of the mesocosms, our mesocosm-mimic model is spatially aggregated (i.e., assumes spatial homogeneity in the water column) and lacks immigration and emigration. The model was parameterized using data from the HerbicideX-study mesocosms, from the AQUATOX internal library, and from the open literature. Information on toxicity and environmental fate of HerbicideX was obtained mainly from the Draft Assessment Report developed by the responsible Rapporteur Member State. Overall, the model is well calibrated (generally within a factor of 2-3) to the mesocosm data, particularly for HerbicideX concentration and biological variables. We conclude that aquatic ecosystem modeling complements empirical approaches to pesticide risk assessment by allowing efficient evaluation of indirect effects, testing of multiple exposure scenarios, and assessment of potential long-term effects and recovery times.

WE 002

Prediction of ecological impact of eutrophication and pesticides in a Luxembourgish stream using AQUATOX: linking ecotoxicological models and biological indices

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One of the main current challenges in the implementation of the WFD is the identification of the pressures that cause the deterioration of ecological status of water bodies and the capability to predict the ecosystem evolution upon mitigating these pressures. Different modelling approaches, linked to monitoring strategies, have been recently proposed to support stakeholders, but these models are often not integrative and/or not predictive and extremely data intensive.

In this study the model AQUATOX, an integrative ecological and ecotoxicological model developed by R. Park and J. Clough and released by EPA, has been tested in one case study catchment in Luxembourg which is subject to strong agricultural pressure. The direct, indirect and synergistic effects of nutrients and pesticide have been analysed to support river basin management and to predict the efficiency of Programs of Measures.

Emission loads are generated by coupling the river basin model SWAT and the models are fed with data on food webs composition provided by public authorities and oxygen, nutrients and contaminants dynamics as well as sediment and periphyton budgets provided by a set of experimental campaigns.

In order to make meaningful prediction with AQUATOX, we related nutrients and pesticides loads to specific community structures, reflecting the ecological state. Simplified food webs corresponding to lead species of the saprobic index classification and matching the corresponding water quality classes have been built within AQUATOX, calibrating nutrients and contaminant tolerance. Based on the expected macroinvertebrate assemblages, the simulations in AQUATOX estimate the presence of indicator species for the different saprobic classes, under the corresponding pollution level.

This approach allows to predict ecological status in water bodies as it is measured in WFD and to distinguish between nutrients/organic and toxicants effects as well as between direct and indirect effects.

WE 003

Species Sensitivity Distribution (SSD): Different data validation approaches and their influence on the final SSD curves for selected herbicides

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Species Sensitivity Distribution (SSD) is becoming a common method for evaluation of ecological risks of chemicals. However, there is still no clear agreement about the standard procedure of SSD curve derivation. Many different statistical and data validation approaches have been suggested. In the present work we study different commonly used data validation approaches, and their influences on the final SSD curves, HC5 and PAF / msPAF values. The study has been prepared using data on seven herbicides relevant in European ecosystem. Ecotoxicity data have been derived from databases (e.g. US EPA ECOTOX) and literature. We have compared acute vs. chronic toxicity data, saltwater vs. freshwater toxicity data, using all endpoints vs. selection of the most relevant endpoints, and finally using data for different taxons. We also compare two approaches of using database records: 'using of all data' and 'detailed validation and searching for duplicates and errors'. These comparisons have been applied to monitoring data from the Scheldt River basin in Belgium, where PAF and msPAF values have been calculated. We discuss statistical evaluation of the differences, as well as influence of different steps in the data validation on the overall outcomes of SSD modelling. [Supported by the EU FP7 project AQUAREHAB and by the project CETOCOEN (no. CZ.1.05/2.1.00/01.0001) from the European Regional Development Fund.]

WE 004

Fuzzy approach for risk assessment of brominated flame retardants in aquatic ecosystems

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Brominated flame retardants (BFR) are pollutants that represent a threat to both human health and environment due to their industrial use, their persistence and their ability to bioaccumulate and biomagnify in food chains, especially in the aquatic one. For the last ten years contamination levels for this type of compounds have been reported for European, North American and Asian human tissue, sediments and biota samples [1-3]. However, monitoring efforts into the assessment of BFRs contamination levels in Latin America are scarce.

In this study, a model for the evaluation of the environmental risk of BFRs in the aquatic ecosystems has been developed. It has been based on a technical application of the Fuzzy Theory [4]. In particular, three interconnected Fuzzy Inference Systems (FIS) have been created through the use of the Fuzzy Toolbox in Matlab. In order to improve and make the model scientifically robust, several international experts have been questioned about different information required to build the fuzzy system. Information from 38 questionnaires have been collected and statistically treated.

The model has been tested in two case studies: Ebro River basin (Spain) and Latin America (Chile and Colombia). The data gathered for each one of the two case studies correspond to two different international projects. The first one is the EU funded AQUATERRA project (2004-2009): Integrated modelling of the river-sediment-soil-groundwater system. It provided to this study data from four samples campaigns in two Ebro tributaries in north east of the Spain, the Cinca River (2002 and 2004) [5] and the Vero River (2004 and 2005) [6].

The second project is the BROMACUA funded by the BBVA Foundation. Contamination levels, bioaccumulation and biomagnification in the aquatic trophic chain are being evaluated in two ecosystems, one from Chile (San Vicente bay and Lenga estuary) and another one from Colombia (delta-estuary of the Magdalena river).

Concentrations in biota and sediments obtained in the sample campaigns of both projects have been used directly as input for the model. In this paper, the characteristics of the developed methodology as well as the main results of the risk of BFRs in these two case studies are presented. One of the main conclusions is that the sites in Spain present higher risk than the ones analyzed in Latin America.

WE 005

A simulation model of mortality, detectability and sampling for anticipating performance of field surveys of plant protection products acute effects on wildlife

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SAGIR is a French national network of environment workers collecting dead or moribund animals to assess the main causes of wildlife mortality. SAGIR's surveys are based on three embedded monitoring approaches:

- a national events survey based on opportunistic collection;
- a focus events survey based on opportunistic collection with a focus to a particular etiology at restricted time and geographic scales;
- an active survey based on more intensive local prospection.

Etiologies considered by SAGIR concerned also Plant Protection Products (PPP) acute effects on wildlife. Although these surveys are used and advised for post-homologation studies, fatalities detected during field surveys are still difficult to relate to actual mortality because of numerous ecological and human factors leading to variation of the detection probability of fatalities.

We built an integrated model to simulate spatial dispersion, persistence time, detection and sampling of carcasses on an area. This model is used to simulate different strategies to estimate the proportion of carcasses contaminated with the PPP and/or its residues in the population of carcasses. The model is composed of three different components.

In the state model, individuals of a generic species are randomly distributed on an area composed of PPP-treated and non-treated plots. Individuals are submitted to mortality rates (natural and related to PPP poisoning). If an animal dies, its carcass has a daily probability to disappear from the area, estimated from field experimental assays. The

output of this state model is therefore a number of carcasses per plot and day. Next, an observation model is considered. Observers have a certain probability to detect a carcass, estimated from distance sampling experimental assays. Finally, a sampling model with SAGIR methodologies simulations is used to assess the proportion of the actual number of carcasses, collected during an events survey or an active survey. This kind of integrated model can be used to simulate different field search strategies and to assess material and human resources necessary to obtain a reliable estimate of PPP or residues prevalence in a population of carcasses. The difficulty to perform such field study is therefore highlighted.

WE 006

DEB model to interpret the combined effects of toxicants and environmental factors: case study on *Folsomia candida*

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Standard ecotoxicological tests are conducted by comparing lethal or sub-lethal effects on exposed and control organisms in constant and favorable conditions. However, organisms typically are found in environments that are neither constant nor optimal. Empirical studies indicate for the presence of direct and/or indirect interactions between chemicals and other external environmental stressors. Unfortunately, it is impossible or economically very expensive to experimentally assess all possible interactions between different environmental conditions and chemicals. We thus need tools that can help to understand combined effects of environmental chemical stressors on individuals and further assist us in assessing effects on populations in their natural environment.

Dynamic Energy Budget (DEB) theory could be such a tool. It predicts the performance of individuals exposed to chemicals and other environmental stressors. DEB theory uses a set of four differential equations to describe the rates at which individual organisms assimilate and utilize energy from food for maintenance, growth, reproduction and development. As these rates depend on the state of the organism (age, size, sex, nutritional status, etc.) and the state of its environment (food density, temperature, etc.), DEB theory allows to mechanistically represent the life history of an individual organisms in a variable environment.

We used an existing software tool, DEB-IBM, to develop an individual-based population model, based on DEB theory, of the collembola *Folsomia candida*, a standard species for risk assessment of soil invertebrates. The model considers the influence of temporal and spatial variation of temperature on *Folsomia candida* populations exposed to cadmium and also includes density-dependent effects. First results are encouraging since they qualitatively reproduce population-level data. More quantitative analyses are required, though. We nevertheless conclude that DEB theory, combined with individual-based population models, is a generic and powerful tool for ecological risk assessment of chemicals.

WE 007

Population-level toxicant sensitivity: stage-specific density dependence and demographic stochasticity in Cu-exposed flour beetles (*Tribolium*)

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Population models are needed for predicted predicting toxicants effects on population-level endpoints such as long-term abundance and extinction risk from measured individual-level endpoints such as survival and fecundity. Demographic processes such as density dependence and demographic stochasticity (uncertainty in vital rates due to random differences among individuals) can complicate prediction of population-level effects. Stage-structured populations are particularly complicated; the different life-history stages may show different sensitivity to toxicant exposure, but the most sensitive stage is not necessarily the most important endpoint for the overall population-level effect. In this study we have investigated the role of stage-specific toxicant effects and stochasticity in populations of the red flour beetle (*Tribolium castaneum*) exposed to a toxic concentration of copper (Cu). We developed a population model based on the so-called LPA model (Larva-Pupa-Adult), which was developed for *T. castaneum* and has been applied extensively in population ecology for two decades. Density-dependent cannibalism across stages is driving the characteristic population dynamics in this model. Demographic rates (development time, juvenile survival and reproductive rate) in Cu-exposed and control populations of *T. castaneum* were estimated from a multi-generation experiment. Each treatment included 20 replicated populations, from which we could obtain estimates on uncertainty in demographic rates. The population model was used to simulate population dynamics over multiple generations, including demographic stochasticity in reproduction and survival. The aim of this modelling study was to analyse: (1) effects of Cu-exposure on population-level endpoints such as long-term abundance and risk of (quasi-)extinction; (2) sensitivity of the population-level endpoints to toxicant effects in the different life-history stages; and (3) the role of demographic uncertainty for the population-level toxicant effects. Analysis of demographic rates from the simulated population time series show that density dependence dampened the long-term Cu effects e.g. on reproductive rates. Nevertheless, sensitivity analysis indicates that Cu effects on reproduction were more important for the overall population responses than changes in larval or adult mortality.

WE 008

How population models can make better use of existing toxicity test data in environmental risk assessment: a case study with musk xylene

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In this paper we use musk xylene as a case study to analyze the extent to which typical ecotoxicity test data sets produce data that can be used in population models to produce value-relevant outputs. Our analyses show that population models can be used to integrate and interpret data for different organism-level endpoints that are typically measured in standard toxicity tests. Thus, standard toxicity tests can, in principle, provide information that can feed into value-relevant assessments of chemical impacts. However, in order for standard toxicity data to be useful in this context, several changes need to be made in the way that tests are prioritized and in the way that the data are reported. In addition, there needs to be agreement on the criteria that population models have to fulfill in order for them to be acceptable as a basis for risk assessment and management.

WE 009

Energetics affects toxico-kinetics: a case study of uranium toxicity on the metabolism of zebrafish, *Danio rerio*

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Toxicity data on effects of uranium on zebrafish *Danio rerio* show conflicting results. Low environmental concentrations induce effects on reproduction in one study while hardly affecting reproduction at the same concentration in another study. Uranium reduces growth in juvenile zebrafish but not in adults. Uranium is hardly eliminated during depuration in studies where individuals are not set up as reproducing groups, but eliminates considerably when individuals are observed to reproduce. Uranium impacts survival of exposed juveniles but not in adults. Finally, the ultimate concentration after 20 days of exposure differs by a factor two in two separate studies using the same exposure condition.

We combine all of the above observations into a single study and analyze the data using a single coherent framework: Dynamic Energy (DEB) budget theory. We run first model simulations using a DEB model applied to zebrafish to understand how exposed individuals deviate from baseline metabolism and second we incorporate a toxico-kinetics module which specifies uranium internalization. The apparent discrepancies are explained using the following principles which follow from DEB theory: (i) toxico-dynamics affects uptake, (ii) food level affects effects, and (iii) energetics (dilution by growth, elimination through reproduction) affects toxico-kinetics. The interpretation of all data sets becomes straightforward in this context and results are no longer conflicting. Laboratory experiments under controlled conditions give mechanistic insight into the mode of action of uranium. Care must be taken however in the interpretation of the results and especially when extrapolating to field conditions where food is sometimes limiting and rarely constant in its availability.

WE 011

Effects assessment of boron in the freshwater environment

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Boron is a naturally occurring trace element that is essential to a variety of organisms. This poster will describe the available data considered in developing a freshwater PNEC for boron according to the REACH framework. Data screening based on reliability and relevancy criteria (Klimisch score) was used to select the high quality chronic toxicity data for PNEC derivation of boric acid and sodium borates. In total, 72 reliable individual chronic NOEC or EC10 values were selected for 24 different species (covering fish, amphibians, invertebrates and algae). Species mean toxicity values have been calculated and varied between 2.9 (for the fish *Micropterus salmoides*) and 60.0 mg B/L (for the higher plant *Lemma minor*). These data supported the development of a species sensitivity distribution (SSD) and estimation of the HC5 value. The best fitting distribution resulted in a HC5 of 4.1 mg B/L. A similar HC5 of 3.3 mg B/L was calculated using the conventional log-normal distribution. Analysis of the remaining uncertainties revealed that the endpoints addressed in the boron database for aquatic species are of appropriate duration and of high quality. Moreover, the database cover the appropriate range of taxonomic groups as mentioned in the ECHA Guidance Document. However no reliable mesocosm data are available but several field studies have been reported in freshwater locations experiencing high boron concentrations either seasonally or within a relatively small geographic range. These field data suggest that aquatic organisms, even those considered sensitive, can tolerate such conditions. The proposed freshwater PNEC is based on the best fitting HC5-50 value using an AF of 2 resulting in a value of 2,02 mg B/L. This value, based on added boron concentrations, is therefore taken forward to the risk characterization.

WE 012

Physical, biological and chemical methods for classification of arsenic and manganese bearing wastes and soils in Western Georgia and health-risk assessment

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Vast amount of industrial, toxic and potentially toxic waste (about 70-80 million tons of arsenic, manganese, zinc, barium, iron and coal wastes and slag, containing heavy and noble metals) is accumulated in Western Georgia. This causes morbidity of the residents, impacts the quality of environment and hinders tourism in the state. Development and related growing load on the natural environment affects quality of air, drinking and surface water, cause soil contamination and hinders production of ecologically clean agricultural products causing deterioration of the human health. The situation is aggravated by the fact that amount of the toxic waste is growing by 1-2 million tons annually. These wastes are not classified and without any treatment are disposed on poorly managed landfills and in decaying storages. The 'hot spots' need immediate and effective remediation.

During recent 3 years numerous physical, biological and chemical methods for express-testing and eco-friendly treatment, recycling and remediation (microwave treatment, chemical and biological leaching, stabilization-solidification, phyto-remediation) were developed in Georgia giving the opportunity to create and offer benchmarks for the adequate arsenic and manganese waste classification system and to rank the contaminated soils. Systematized data on the content of arsenic and manganese in soils and industrial wastes of Western Georgia regions contaminated by these metals, new methods of testing and remediation, as well as proposed basic standards for classification and ranking of wastes and soils are presented. The results of health-risk assessment carried out in contaminated regions are also given and analyzed in this paper showing alarming prevalence of numerous dangerous diseases (skin spots, skin cancer, tumors, respiratory diseases, allergies, hereditary abnormalities, nerve and behavioral disorders, etc.). Recommendations on developing the classification system as well as on treatment and remediation methods and prevention/decontamination emergency measures are formulated. It is shown that microwave pre-treatment greatly improves the efficiency of demonstrated methods, and the use of high-power pulsed generators leads to a multiple increase in the effect compared with continuously operating generators of the same average power.

WE 013

Risks assessment around a lead-batteries recycling company using ecotoxicity tests

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With the rise of sustainable development, rehabilitation of brownfield sites located in urban areas has become a major concern. Remediation of contaminated soils is therefore a strong aim with the development of bioremediation and tools for risk assessment. Actually, criteria for landfilling are based on leaching tests that represent the mobilized fraction and therefore may present a risk in case of transfer. However, ecotoxicological tests to guide the landfill are not yet currently used. Nevertheless, their use seems very relevant to assess the actual toxicity of the leachate and better reflects the reality of effects on living organisms.

Standard leaching tests (EN 12457-2) asked by European authorities to guide landfilling were first performed on ten samples of soil taken from a site recycling lead-acid batteries. This experiment determines the fraction potentially be mobilized and therefore poses a risk if transferred. Physico-chemical parameters from all samples were also determined (pH, lead total concentrations, etc.). Toxicity of leachate was evaluated by *Daphnia magna* (OCDE 202) and *Alivibrio fischeri* (*Vibrio fischeri*, ISO 11348) bioassays. Besides, four modified *Escherichia coli* stains with luminescence modulated by heavy metals were also used to assess the toxicity of these pollutants.

Results shown that although total lead concentrations can be very high (up to 40 000 mg / kg), only a small fraction was solubilized. Chemical analysis and ecotoxicological bioassays were well correlated. This study highlighted the use of modified bacteria strains sensitive to metals will be useful tools for the end-users for environmental monitoring of contaminated sites.

WE 014

Does phosphorus limitation increase the toxic effect of silver on aquatic fungi and leaf litter decomposition?

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Forested headwater stream functioning is intimately linked to leaf litter decomposition ensured by microbial decomposers, mainly aquatic hyphomycetes, which transfer allochthonous carbon to higher trophic levels. Evaluation of this process is increasingly used as an indicator of the state of ecosystems, both in terrestrial and in aquatic conditions. Yet, impacts of contaminants on this process, as well as effects of environmental conditions on the response of this process to contaminants, remain scarce. Interactions between the toxic effects of metals and phosphorus (P) level, limiting element which is one of the main drivers of detritus decomposition, have never been tested. We carried out a microcosm study with a consortium of eleven hyphomycete species to verify if 1) the decomposition process under metallic stress (presence of silver, Ag) could be, at least partly, overcome when organisms benefit of high phosphorus availability, 2) these effects being mediated by changes in aquatic hyphomycete community structure. Fifteen combinations of metal and nutrient conditions, i.e. 5 concentrations of silver nitrate (0, 0.1, 1, 10, and 100 µgAg.L⁻¹) and 3 concentrations of phosphorus (20, 100 and 1000 µgP.L⁻¹) were tested, each treatment being replicated 4-times.

Litter decomposition and fungal biomass were increased by P-level. Toxic effect of Ag on decomposition was significant only at the highest concentration independently of P-level. On the contrary, P and Ag-level had a significant interaction on fungal biomass. Analyses of fungal community structure via PCR-DGGE showed that both P-level and Ag concentrations shaped microbial communities, without significantly altering species richness.

This study clearly shows that toxic effects of Ag contamination on fungal community structure are more pronounced under P-limitation. This limitation is often encountered in headwater streams ecosystems that could make them more sensitive to metal stress. Despite alterations of community structure, P-limitation remained the most important driver of leaf litter decomposition, excepted under very high Ag concentrations. Using leaf litter decomposition as a functional indicator of state of ecosystems seems thus more sensitive to nutrient availability than to level of metal contamination, excepted at very high metal concentrations.

WE 015

What's causing toxicity at a zinc - copper mine site?

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Introduction

Hudson Bay Mining and Smelting (HBMS) operates a mine in Flin Flon, Manitoba CA. The mine produces Zn and Cu concentrate. Zn and Cu rich effluent from the tailings pond discharges into Ross Lake, which has been found to have extremely low populations of biota. pH in the lake averages 6.5 with occurrences of low pH. At low pH free ion Zn is more soluble and hence bioavailable. With high Zn and Cu concentrations, and low pH, it is unclear what is causing toxicity in Ross Lake.

Goals

- Determine source(s) of toxicity in Ross Lake
- Measure and model bioavailable fractions of Zn and Cu
- 2. Materials and methods

MODELING

- Speciation calculations were performed using WHAM and measured water chemistry
- LC50 was estimated using site specific data in the Biotic Ligand Model (BLM)

FIELD SAMPLING

- Free ion Zn and Cu was measured using Diffusive Gradient in Thin Film (DGT) membranes
- Water chemistry was collected at locations around Ross Lake in October 2010 and July 2011
- 3. Results and discussion
- WHAM estimates of free ion metal ion show good agreement with Zn and Cu concentrations measured with DGTs
- Zn²⁺ concentrations are consistently lower than LC50 calculated by BLM, Zn not a source of acute toxicity
- High Ca concentrations ameliorate Zn toxicity (Santore et al., 2002)
- Cu²⁺ concentrations are consistently higher than LC50 calculated by BLM, Cu a source of acute toxicity
- pH not likely influencing Zn or Cu toxicity

Conclusions

Cu, not Zn likely causing toxicity
Low pH likely to also stress organisms, but not due to increasing Zn solubility

Recommendations

Explore the effect of metal mixtures on toxicity using this data set and newly available models. Determine if zinc and copper are having a synergistic effect on Ross Lake.

Acknowledgements and References

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WE 016

The effects of pH on acute and chronic toxicity of Pb²⁺ on *Daphnia magna* and *Ceriodaphnia dubia* and Biotic Ligand Model (BLM) development

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We investigated the individual effects of pH on the chronic toxicity of Pb²⁺ on *Daphnia magna* and *Ceriodaphnia dubia*. Until now the effects of pH on the acute and chronic toxicity of water flea species were not clear. This inhibited the development of a chronic BLM for this group of species. The effects of pH on the toxicity of lead were tested during 48 hours acute tests and a 21 and 7 days chronic reproduction test for *D. magna* and *C. dubia* respectively. The pH experiments were conducted with 4 pH levels (6.4, 7, 7.6 and 8.2). Results of the acute tests for both species showed that there was limited effect of pH when considering the dissolved lead fraction. When corrected for speciation however there was an increasing toxicity effect of the free metal ion (Pb²⁺) at higher pH. Preliminary results of the chronic test for *C. dubia* suggest that there were no effects of pH on the toxicity of lead. Initial results of the chronic test with *D. magna* showed an increasing toxic effect with decreasing pH. Finally based on the results we are developing a BLM for the *Daphnia* genus.

WE 017

Impact of incorporation of bioavailability on risk assessment of metals in Japanese surface waters

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It is generally accepted that the bioavailability and toxicity of trace metals in surface water to aquatic life depend on the speciation of metal, not on total or dissolved metal concentration.

Metal speciation is a function of water chemistry including temperature, pH, organic content, and the compositions and concentrations of ions and solid phases in solution. To develop efficient and effective methods of assessing and managing the risk posed by metals to aquatic life, it is important to determine the effects of water chemistry on the bioavailability of metals in surface water. For this reason, we have employed the technique called diffusive gradients in thin-films (DGT) in the study of several Japanese rivers, including meta-contaminated rivers adjacent to abandoned mines, to gain information on the bioavailability of metals in Japanese water systems. The measurements of labile metals as determined by the DGT technique suggest that the biologically available fractions of metals in urban rivers are relatively low compared with those in rivers adjacent to abandoned mines. The fractions of Zn and Cd appear to be higher than that of Cu. An inverse relationship was found between labile- Cu fraction and dissolved organic carbon (DOC). Our result suggested that large amount of Zn and Cd occurred as hydrated ions or small inorganic complexes in water of the rivers studied, whereas most of the Cu occurred as Cu-organic complexes. Moreover, we have examined the impact of incorporation of bioavailability on risk assessment of metals in various Japanese surface water types and to identify what types of Japanese surface waters and metals required to consider bioavailability when assessing risks on aquatic life.

WE 018

Delivering a practical methodology to account for metal (bio)availability in the water framework directive - case studies

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Significant scientific progress has been made for assessing the risk of metals in surface waters in recent years, both at a technical and regulatory level. The current state of the science has recently been incorporated into the new EU Technical Guidance for deriving Environmental Quality Standards (EQS) under the European Water Framework Directive (WFD). The guidance supports the use of a tiered compliance assessment regime for metals that incorporates bioavailability and recommends using Biotic Ligand Models (BLMs). This approach has been endorsed by the EU Scientific Committee on Health and Environmental Risks (SCHER). The bio-met project has developed an online resource (www.bio-met.net) that includes a user friendly BLM tool (the bio-met bioavailability tool) and associated guidance for simply determining the bioavailability of copper, nickel and zinc in European freshwaters and undertaking compliance assessment against a bioavailability based EQS. This poster describes a series of example case-studies conducted across EU Member States that compare the EQS compliance picture using the bio-met bioavailability tool compared to conventional (i.e. hardness-banded) EQS.

WE 019

Transformation/dissolution testing of metals and inorganic substances according to OECD guidance 29: technical challenges & experience

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Within the framework of the European REACH- and CLP regulations, data are needed to characterise the ecotoxic potential of metals and metal substances. The ecotoxicity of soluble inorganic metal compounds depends on released metal species and the composition of the medium. Furthermore, ecotoxicity scores may be read-across based on dissolved metal concentrations and molecular weight conversions. To facilitate read-across of ecotoxic effects to sparingly soluble metal substances, it may become necessary to investigate the rate and extent to which these compounds can produce soluble ionic and other metal bearing species in aqueous media according to OECD Series No. 29 (2001). A test system that meets the specifications of this OECD protocol has been successfully implemented to examine the transformation/dissolution behaviour of a range of metal substances, including redox-sensitive metals. The aqueous test media are based on reconstituted water prepared according to ISO 6341 with pH values in the range of 5.5 - 8.5. The OECD protocol requires exact and comprehensible procedures (e.g. accurate loadings, temperature monitoring, performance of mass balance at test end, and tests were performed in accordance with GLP requirements. In addition to a strict execution of the experimental test procedure, methods for the quantification of dissolved metal ions and species were developed and applied. Element-specific determinations by ICP-OES or ICP-MS were used and validated by appropriate QA/QC measures, including the analysis of certified reference materials, fortified samples, reagent and method blanks and recalibration samples. If different redox-species were formed during a test, dedicated coupling methods, i.e. liquid chromatography coupled to ICP-MS, were developed and successfully applied to separate metal species in different oxidation states. Due to redox equilibrium processes, the stabilization of the formed redox-species immediately after sampling was critical. Differences in transformation/dissolution were observed in response to varying loadings, solution pHs, exposure times and particle surface areas. Our results furthermore emphasize the need to perform the test with sufficient replication as well as the benefits of testing samples with narrow particle size distributions. The relevance of these findings for read-across and the respective classification and labelling will be discussed as well as strengths and weaknesses of the current OECD protocol.

WE 020

The critical surface area-toxic units approach to the hazard classification of metals and alloys

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In the search for standard, reproducible and reliable methods for the aquatic hazard classification of alloys under the GHS (Globally Harmonized System of Classification), the Critical Surface Area-Toxic Units (CSA-TU) approach offers a dependable way to derive acute aquatic hazard classification proposals based on laboratory T/D (Transformation/Dissolution) data. The CSA is the surface area loading of a substance, usually a metal, alloy or sparingly soluble inorganic metal compound, to an aquatic medium that delivers a selected acute or chronic ERV (ecotoxicity reference value) to the medium at a specified time. To derive a GHS hazard classification, T/D data, consisting of dissolved metal concentrations in an aqueous test medium, are compared to acute and chronic ERVs. In this presentation, we use worked examples to show how the CSA TU approach can be applied to derive acute GHS hazard classification proposals for nickel-silver, ferromnickel, Monel, brass and Inconel alloys, and Ni and Co metals. To derive an acute hazard classification proposal for nickel-silver alloy from the pH 6 T/D data, we compared the 168-hr concentrations with a relevant ERV among 29, 120 and 70 µg/L for Cu, Ni and Zn, respectively, but because of the disparities in the reactivities of the alloy components and in the ERVs, it was difficult to select one 168-hr metal concentration over another for comparison with an ERV. However, calculating the 168-hr TUs for each component and plotting these as a function of total surface area loading enables the derivation of a CSA for nickel-silver from which can be calculated a minimum specific surface area of 5.88x10⁵ m²/g above which, and a maximum critical diameter of 11.5 mm below which, nickel-silver would classify as acute GHS. We show how the alloys can be compared on the basis of the CSA required to reach TU = 1, revealing that the order of reactivity was nickel-silver>ferromnickel>Monel>brass>Co metals>Ni metals>Inconel. For Ni and Co metals, we show how extrapolating 168-hr concentrations for 10 and 100 mg/L loadings to 672 hr can be combined with existing 1 mg/L 672-hr T/D data to yield their respective chronic CSAs. The CSA-TU approach is valuable in aquatic hazard classification because a CSA-TU dataset need be generated only once for a given alloy from a particular production method and may be used repeatedly to classify various specific surface areas and particle sizes of the alloy as they are produced.

WE 021

Transformation/dissolution of lead substances for environmental classification

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For environmental classification under the UN GHS and its equivalent European CLP system, a metal or poorly soluble metal substance is tested according to a transformation/dissolution protocol (TDP) developed under the framework of the OECD to determine metal availability in a standard solution, and then the outcome of the TDP testing is compared with ecotoxicity reference values (ERV) of the metal for the hazard identification in the aquatic environment. The TDP was tested on lead in the form of metal powder (diameter: 75 µm), metal compounds (lead distearate & trilead dioxide phosphonate), and high-purity metal massive (99.99%) as available on the market. In general, the assays were performed in mass or equivalent surface loadings of 1, 10, and 100 mg/L at pH 6, 7, and 8, as appropriate, for screening (24 h) and/or full tests for acute (7 d) and chronic (28 d) classifications. Lead metal powder showed the solubility (> 3000 µg/L) that was above the ERV for lead. The two lead compounds

showed significantly different dissolution rates for lead. For massive lead, wire samples were mounted in epoxy resin and submitted to simulated environmental conditions of aging for 28 days before the initiation of TdP testing in the OECD media. The aging process included 3 cycles of 30-minute watering per day at 20°C under a 16/8-hour light/dark cycle. Lead being a soft metal, the step for polishing the surface was challenging due to implantation of polishing materials into metal matrix. A method consisting of grinding steps followed by chemical etching (75% glacial acetic acid & 25% hydrogen peroxide) was developed to overcome this problem. Interestingly, the aged and non-aged samples showed similar dissolution of lead for 7d and 28d time courses, suggesting that the corrosion layer formed during aging was labile. Additionally, the application of TICKET-UWM model successfully demonstrated the rapid removal of lead from the water column (> 70% in 28 d) and insignificant remobilization from anoxic and oxic sediments, proving compliance with rapid degradation under the GHS. This paper primarily presents TdP studies with brief information on the environmental hazard classification for lead.

WE 022

Transformation/dissolution of nickel metal and sparingly soluble nickel compounds

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T/D testing can be used to derive appropriate hazard classifications under the Globally Harmonized System of Classifying and Labeling Chemicals (GHS) and the Regulation (EC) No 1272/2008 on Classification, Labeling and Packaging of substances and mixtures (CLP). Several nickel substances underwent T/D testing in the context of REACH and GHS to evaluate the extent of transformation and dissolution in order to determine the appropriate hazard classifications. Results were compared to the existing harmonized classifications reported in the 1st ATP of the CLP regulation. T/D testing indicated that in some instances the harmonized classifications were appropriate, while in others, the harmonized classifications were either over- or under-protective compared to the appropriate hazard classification derived from the T/D data.

WE 023

Classification methodology for metal concentrates

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The application of the EU CLP-regulation on single substances or mixtures of pre-defined amounts of single substances, has proven to be relatively straight-forward. This is, however, not necessarily the case for complex compositions of naturally occurring substances. Metal ores and concentrates mainly contain the mineral of commercial interest, but may also hold small quantities of impurities that could trigger classification.

Applying a methodology that accurately takes into account the presence of such classified impurities (e.g., Pb) is a challenge as these substances are predominantly incorporated into a mineral structure with limited bioavailability. Because of this very important bioavailability aspect, any classification based simply on the elemental composition of an ore and concentrate may generate a significantly over-stringent classification.

This presentation elaborates on the impact of different methodological strategies on the final outcome of a classification exercise, using molybdenite concentrates as an example.

It can be concluded that due to the complex, mineralogical composition of metal ores and concentrates a simplistic, elemental-based classification approach should be avoided as it assumes maximum bioavailability for each element that is present in the concentrate. The mineralogical approach, on the other hand, is based on the classification of each mineral, and a more refined and realistic classification can be obtained if bioavailability data are available for the individual minerals (e.g., TdP data for specific minerals). Bioavailability data may justify the removal or modification of a classification for a specific mineral.

By conducting a TdP test on the metal concentrate, a complete picture of the bioavailable fraction of each element can be obtained, and this information can be used to derive a relevant and realistic classification of the concentrate.

RA08P - Escape from the Ivory Tower – Environmental sciences should impact public and policy

WE 025

Science journalism - how to raise public awareness of environmental problems?

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Communication of results is a central aspect of scientific work. However, scientists exchange their ideas mainly between their specific communities. This has, of course, advantages for stimulating in-depth discussions about particular scientific problems, but disadvantages when we think about how to communicate our results to the open public. The most important method of communicating scientific results is through publications in scientific journals. However, the feedback in mainstream mass media to such publications is very poor. Hence, once scientists are interested to increase their visibility they should think about appropriate publication formats.

Even though the field of "science journalism" has emerged over the last decade there is still a gap between the scientific community and the open public. One of the main reasons is that, based on a survey of the U.S. National Science Foundation in 2001, more than 90% of scientists think that journalists do not understand the nature of scientific research. Surprisingly, almost 80% of journalists agree. The solution of such a problem requires a long-term strategy and central aspects of such a strategy could be (i) the education of the next generation of scientists through implementation of "science journalism" courses directly into the study programs of the natural sciences (such as the environmental sciences);

(ii) the establishment of new formats for the dissemination of knowledge such as an European University TV Channel as Internet channel and as TV broadcast, which will be fed by the Media Departments of the participating Universities. A pilot project is currently running (<http://eutv-university.eu/>).

This would open new job perspectives for the students in different fields such as the mass media or research institutions (e.g., the German Research Foundation offers the opportunity to apply for subprojects on public relation in the bigger funding schemes such as the Collaborative Research Centres), and would offer new publication opportunities for scientists. In the long run, such a strategy should increase the quality of scientific reports in the mass media.

WE 026

Establishment of a transparent ethical codex for scientific communication and publication

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In times of "publish or perish" and "struggle for funding" scientists are under extreme pressure.

Scientific communication and publication exerts a key-role in the life of a scientist, however clear and transparent standards on European or even global level do not exist. Driven by own experiences in ecotoxicology and recent public scandals there is a pressing necessity of a re-evaluation of existing tools to guarantee ethical correctness in science and particularly in publishing.

A transparent and clear guidance document from the start of a scientific study until its publication is presented as discussion basis for the development and implementation of European or even global harmonized standards. Several topics will be illustrated, such as a) authorship, e.g. the rules for co-authorship, order of authors, honorary authorship, authorship after leave of institute, retraction of manuscripts; b) citation, e.g. citation of "friends" versus citation of both first and important previous studies in the resp. research field, degree of change in a text passage or figure to be sufficient to be regarded as own contribution, plagiarism and redundancy, anonymisation and presentation of data; c) review processes (manuscripts, research proposals), etc. An action plan to develop and implement a new ethical codex for scientists in ecotoxicology on both national and international level in a truly interdisciplinary task force is proposed.

WE 027

How to relate information efforts in environmental science to specific audiences in order to generate maximum impact

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When environmental scientists communicate their work to the public they should recognize that their 'language' might not be readily understood outside the community where it should generate impact for environmental health. This problem is known as the 'foreign correspondent issue' and can be taken into account by means of audience-specific language and content.

During daily work environmental scientists not only use a multitude of field-related terms when, e.g., talking to colleagues, writing manuscripts or reading literature, but they also think in a way clearly influenced by their occupation. Within the community, this 'language' is readily understood and, moreover, a key to effective communication. However, as scientific findings leave the ivory tower, they require translation into common words in order to be easily perceived by the public and generate impact. But it might not be sufficient to just find regular words for technical terms. Equally important is to consider, e.g., the different knowledge, interests, opinions and desires of the various possible recipients - to put it short: the respective audience's culture.

This presentation aims to identify the most relevant audiences for research results from environmental sciences, their key characteristics and their possible function within science communication. We will address the questions: At whom to aim? Whom to reach? How do we think? What do we seek for?

WE 028

Keys to smart home diffusion: a stated preference analysis of smart meters, photovoltaic generation, and electric/hybrid vehicles - suggestions for LCA applications from the field of economics

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Awareness of global environmental problems and the requirements for renewable energy utilization are increasing. In light of the March 11 earthquake and the Fukushima nuclear crisis, a radical reconsideration of Japanese energy policy is now being discussed. Given these circumstances, the development of a smart grid in electricity systems has raised hopes of meeting goals for climate change, energy competition, and the safety of systems and technology. This leads to many economic questions related to incentive policies for operators to implement such technology.

Smart grids can be classified into two systems: the upstream power supply system and the downstream power demand system. This paper deals with the latter, specifically concerning residential demand for smart equipment to make up smart homes. In particular, we investigate the future diffusion of advanced or smart meters (SM) that measure electric consumption hourly, photovoltaic (PV) generators that are installed on residential rooftops, clean-fuel electric vehicles (EV), and hybrid electric vehicles (HEV). The implementation of these technologies in Japan is imminent, and policy is needed to prepare for smart home diffusion. This paper will conduct a conjoint analysis of the future diffusion of SM, PV, EV, and HEV using the results of an online survey administered in March 2011. Through this estimation of consumer future demand and expected greenhouse gas emissions, this paper suggested a connection between economical demand analysis and LCA research.

WE 029

PRIMO'S next - advanced student's workshop on the fundamentals of science, environment and health

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"Biomarkers are the answer! But what is the question?" After many ecotoxicology conferences we had the slight sensation that students were lacking creativity and losing the ability of asking good questions.

To discuss these premises, we organized 2 editions of the 'International School on Marine Ecology and Ecotoxicology', now called PRIMO's Next, gathering a group of 30 students and 15 instructors from 15 different countries, for 10 days in October 2010 and 2011, in Brazil.

The result was outstanding! The high level of technical classes and discussions about science activity and communication invited the students to think about their own work, from the observation of nature and the proposal of a question to the most adequate technique and the communication of the results, in a way they never did before. The significant improvement on their presentations can be observed comparing the platform presentation in this section from students who attended the school with the students' seminars videos recorded at the school and available on youtube.

From now on, we are proposing to organize 'PRIMO's Next' meeting aiming to prepare the next generation of PRIMO attendees to make PRIMO better and better. This 'school' would take place every two years, in different developing countries, in between PRIMO meetings.

WE 030

The students lab 'Fascinating Environment' - A holistic approach for improving academic teaching and education

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Here we introduce the Students Lab "Fascinating Environment", which was established at RWTH Aachen University in 2009. The students lab was founded to strengthen education and training in environmental sciences by providing state-of-the-art analytical and bioanalytical facilities and meanwhile has matured into a holistic approach to academic teaching.

More than 80 % of the lab equipment became available thanks to sponsoring by more than a dozen industry partners. The idea behind the cooperation with the industries was to provide a university platform for interested companies to present their products, whereas simultaneously benefiting from the utility of sponsored equipment for education and research. This approach has resulted in a large number of bachelor and master theses and also several international peer-reviewed scientific publications.

The students lab furthermore maintains a close cooperation with the Institute for Teaching Methodology in Biology at the RWTH Aachen University. Several school adaptations of ecotoxicological test systems are currently being developed by future teachers with the aid of the students lab to sustainably integrate ecotoxicology at the level of secondary school-education and early interest pupils in environmental sciences. A recent project in the students lab has adapted the fish embryo test (FET) with the zebrafish (*Danio rerio*). The adaptation of the laboratory test protocol includes suggestions on how to successfully conduct the test directly at schools, portrays special project days that take place at the students lab on a regular basis and includes a general introduction into work at an academic institution.

As of winter term 2010/11 the new project FILM started in association with the students lab. The aims of FILM are (a) to capture scientific results on film, (b) to plan and produce short movies about scientific test systems, (c) to process both for a broad variety of possible audiences and (d) to comprehensively and unambiguously present one's own research in a filmed interview situation. The project was included into an already existing skill course system for students of biology and ecotoxicology.

WE 031

Developing entrepreneurship in Russian environmental science

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Problem of effective communication between "Ivory tower" scientists on the one hand and public, business and policy-makers on the other hand is a barrier for sustainable development of Russia. National academia has the capacity to develop effective environmental technologies and solutions which could provide the opportunities to achieve "win-win" results: allows to get both environmental and economic effects. After the collapse of the USSR, process of transfer of research-based innovations from academia to economy suffered serious decline and nowadays implementation of innovations. There is a need of stimulating of entrepreneurial mindset among academics and students, student involvement through entrepreneurship education programs, developing infrastructure, incubators and government support programs.

There are several hypotheses were developed, which concern opportunities for entrepreneurship in environmental science and promotion of cleantech innovations in Russia.

- Russian environmental science has potential to develop cleantech innovations, primarily, in natural sciences that were competitive during the Soviet times, including biology, chemistry and physics.

- Natural science professionals, who could develop innovations don't usually possess entrepreneurship and management skills to commercialize it.

- There is no appropriate infrastructure for transfer of innovations, lack of number in business incubators, technology transfer offices, legislation and laws do not provide the sufficient level of intellectual property protection

- Experiences of the past show that government played key role in development of innovations. Nowadays, innovations mostly associated with ineffective budget money distribution and realization of political goals, rather than economic and social ones.

- Scientists have lack of motivation to participate in transfer of technologies, which were developed in governmental organizations since scientists would not get sufficient financial reward.

- Potential future innovators and inventors, who study in Russia would rather go to the western countries, where more appropriate environment for the development.

- There no appropriate education and learning opportunities for entrepreneurs who can participate in transfer of knowledge.

WE 032

Sustainable training concept on chemicals risk assessment in Serbia

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The Serbian Chemical Agency (SHemA), as competent authority for chemicals management on national level, plays a crucial role in the establishment and enforcement of the legal framework in this field. Capacity building in order to prepare SHemA's staff for responsibilities within the European chemicals legislation is imperative. Currently, SHemA staff is undergoing a very intensive training related to chemical safety, with special accent on chemicals risk assessment provided within the present IPA 2008 Twinning project and project implemented by Swedish Chemicals Agency (KemI). The objective is to support the successful implementation of the national legislation on chemicals management and thus contributing to the protection of human health and of environment by improved practices for the safe use of chemicals and biocides in Serbia. In terms of approach, trainings are oriented mainly towards lecturers with practical exercises, but it is planned as a next step to apply approach of learning-by-doing and training-on-the-job. In order to create a modern interdisciplinary universities' program related to chemical safety, in line with the best EU practices, with a special accent on chemicals risk assessment is under consideration to launch master-level study designed for professionals working in governmental authorities, industry and academia.

In order to ensure a sustainable education structure after the conclusion of the current projects, it is relevant to consider, within the budget planning regarding prospective SHemA staff training, the eventual necessity of involving also external sources, i.e. for foreign university experts for specific areas, study visits to EU Member States evaluation bodies, EC training and information seminars, commercial training courses, etc. The establishment of a sustainable training concept on chemical safety will set the base for a long term improvement of chemical management in Serbia.

WE 033

Risk and uncertainty: bioavailability based environmental quality standards for metals

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The environmental regulator for England and Wales, the Environment Agency, is in the process of redefining environmental quality standards (EQSs) for copper and zinc in terms of a permissible bioavailable concentration value. Whereas the consideration of bioavailability improves the ecological relevance of EQSs, assessing the extent by which compliance with EQSs expressed in terms of bioavailability is achieved is complex since bioavailability cannot be measured directly and requires tools, such as the Biotic Ligand Model (BLM), to convert the bioavailable value into a measurable dissolved concentration. BLMs require, as a minimum, pH, dissolved organic carbon (DOC) and calcium (Ca) concentrations as inputs, however, little data on the environmental concentrations of DOC are available and consequently, there is uncertainty in the extent by which water bodies in England and Wales are likely to comply with future standards. Compliance is important, not least of all because it provides an indication of environmental risk, but also because it informs decisions on the degree to which sewage effluents are required to be treated. Since the management of sewage treatment capacity requires a long planning horizon, it is useful to understand, in advance, the impact of new regulatory regimes in order to facilitate planning. However, for the reasons given above, this has only been possible at a limited scale. In order to quantify the potential impact of bioavailability based standards on compliance, a Monte Carlo approach

was applied to generate a distribution of EQS values for each water quality monitoring location in England and Wales based on probable combinations of pH, DOC and Ca. For sites that were missing water chemistry data the characteristics of its nearest neighbour for which data was available were applied. The results indicate that, at an aggregate level, the absolute number of monitoring locations at risk of failing to comply with the EQSs as they may become, was unlikely to change, however, a redistribution of locations at risk appears likely and suggests that, from a water company perspective, there are likely to be winners and losers. The risk assessment results and an overview of stakeholder responses to the results and the approach are provided.

WE 034

Old news or real risks? Bisphenol A under scrutiny

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Having the daily news on Bisphenol A (BPA) in mind, it becomes clear that this substance still rises concern and seems to be a never ending story - scientifically as well as politically. It is used in many articles such as plastic bottles made of polycarbonate, coatings of water pipelines made of epoxy resins or cash receipts made of thermal paper. Environmental compartments may be exposed via sewage water treatment plant effluents from paper recycling, water pipelines, industrial processes or by leaching from plastic products made of polycarbonate. It is used widely dispersive and is produced with a high tonnage of many manufacturers in Europe.

Despite the huge amount of generated data and accomplished risk assessments for BPA, it is still controversially discussed how to assess this substance. It is well known for its endocrine effects on aquatic organisms at very low concentrations. However, there are different interpretations of animal tests and disagreements on the assessment of its endocrine activity. Additionally, some monitoring studies indicate that environmental concentrations might be higher than expected based on existing data. It is questioned whether all exposure pathways are known and to which extent other uses contribute to a relevant environmental exposure. Because of these uncertainties the question rises how to soundly regulate Bisphenol A. UBA proposed a comprehensive substance evaluation under REACH in order to clarify if more or other data is needed and how Bisphenol A should be regulated in future. Possible consequences might be a restriction of certain uses or the identification of BPA as substance of very high concern due to its endocrine disrupting properties.

This presentation aims at giving a structured overview on the challenges mentioned and the steps towards a solution for Bisphenol A from an environmental perspective.

WE 035

Risk management of substances of very high concern: first challenges with the authorisation process under REACH

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The Authorisation process is one of the major Risk Management Options for Authorities under REACH. This is a three step process:

- Identification of Substances of Very High Concern (SVHC, started 2008)

- Inclusion of Substances in Annex XIV, the Authorisation List (first recommendation in 2009, first Annex XIV beginning 2011)

- Applications for Authorisation (awaiting first applications)

The poster will present objectives, some statistics and challenges faced during the first years of SVHC identification and inclusion of substances in Annex XIV.

63 out of 78 dossiers, submitted so far for SVHC identification, refer to CMR substances. The poster will also show some statistics about public consultations.

The Substance identity is a challenge in some cases, particularly for

- Substances with multiple identifiers

- Substances without clear identifiers

- UVCB substances

Substances identified as SVHCs and included in the Candidate List, are prioritised and recommended for inclusion in Annex XIV by ECHA. The REACH regulation outlines three criteria for priority setting in Art. 58(3):

- PBT or vPvB properties; or

- Wide dispersive use; or

- High volumes.

ECHA and the MSC, which gives its opinion to ECHA's proposal, agreed that regulatory effectiveness needs also to be considered. Each of these criteria has its issues that ECHA take into account in a two tiered approach, a scoring exercise and an argumentative approach.

- PBT/vPvB: As intrinsic properties of the substances have already led to their identification, this criterion receives about half the impact than the other two criteria.

- The wide dispersiveness has been broken up in two sub criteria, wide spread uses and the possibility of releases. This does not aim to evaluate risk as such, but the potential for exposure.

- The main issue with the volume is to identify the volume in the scope of authorisation requirement, as others are not taken into account.

- Regulatory effectiveness is particularly important for groups of substances with same hazard or with UVCB substances.

WE 036

Timbre expert system as information platform and communication channel for innovative and widely applicable strategies, technologies and solutions for contaminated sites risk assessment

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Risk-based approach for sustainable management of contaminated sites and brownfields produced in the last years an abundance of regulations, strategies, guidelines, tools, documented case studies, assessment technologies, as well as risk communication methodologies and tools, which are too often not used in their entire potential. The non-visibility of already available, useful and innovative methodologies and tools for environmental risk assessment and in general for sustainable management of contaminated sites is the reason why risk related information and opinions among experts, decision makers, stakeholders and the general public are not exchanged and communicated using the best available technologies/approaches and decision support strategies and tools. This situation makes the success in potentially contaminated sites regeneration unsatisfying in terms of financial, eco-efficiency or social acceptance.

The 7th Framework Programme project timbre (tailored improvement for brownfield regeneration in Europe) aims at providing contaminated sites' owners, local authorities and stakeholders with a web-based expert system that can be at the same time a user-friendly collector and provider of all available information related to environmental risk assessment and sustainable management of contaminated sites for experts, stakeholders and decision makers involved in the risk-based management of contaminated sites and brownfields. The first step for the development of the timbre expert system consisted in the definition of a shared decisional framework for the assessment and sustainable management of contaminated sites and brownfields which is composed of the main phases of the risk-based regeneration process (e.g. site characterization phase, environmental risk assessment and communication phases, etc.). The process to develop the framework has been enriched with a strong collaboration with the timbre case studies stakeholders who have been involved in the rehabilitation framework development through a participatory process which comprised the organization of local workshops and the filling in of an appropriate questionnaire for evaluation of the contaminated sites regeneration framework. The active involvement of stakeholders represents a strong guaranty that the scientific process will produce useful and shared results, which will be properly communicated between all the actors involved in the risk-based contaminated sites and brownfields regeneration process.

WE 037

REACH and other regulations: information and communication on chemicals

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For the implementation of the REACH Regulation (Registration, Evaluation and Authorisation of Chemicals), the Italian Ministry of the Environment, Land and Sea has the task to give information about the risks of chemicals.

Among the initiatives promoted by the Ministry, we highlight the e-bulletin "Sostanze chimiche - Ambiente e Salute" (Chemicals - Environment and Health), the "Lista delle sostanze vietate (o in restrizione)" - (List of banned or restricted substances) and the Ministry's official website.

1. The e-bulletin deals with activities and news related to REACH, other Regulations concerning chemicals and risk mitigation measures. The aim of this newsletter is to provide, through a simple language, adequate information for the public on risks and safe use of chemicals.

The e-bulletins already published have been presented at the 3rd National Conference on REACH, which was held in Rome on November 14, 2011.

The e-bulletin is available at www.minambiente.it and it's possible to receive it sending an e-mail to sostanzechimiche@minambiente.it.

2. The List of substances provides a picture of substances banned, restricted or subject to the authorization procedure under Regulation (CE) n.1907/2006 (REACH), Regulation (CE) n.850/2004, Regulation (CE) n.1005/2009 on substances that deplete the ozone layer.

The list is designed as a tool to facilitate the consultation of information about 1,100 substances of very high concern for the environment and human health. The list is currently available on the Ministry's website.

3. The Ministry's website contains two sections "REACH" and "Environmental risk assessment" where it's possible to find information about chemicals under REACH Regulation, Plant Protection Products and Biocides.

Information on REACH:

http://www.minambiente.it/home_it/menu.html?mp=/menu/menu_attivita/&m=REACH.html&lang=it

Information on "Environmental risk assessment":

http://www.minambiente.it/home_it/menu.html?mp=/menu/menu_attivita/&m=argomenti.html/Valutazione_del_Rischio_Ambientale__VRA_.html&lang=it

These sections are periodically updated and they include "Activities and documents", "Databases", "Information for the public", "European and national legislation", "Events" and information from "European Chemicals Agency (ECHA)".

These initiatives address:

- General public

- Environmental and consumer organizations

- Regional Environmental Protection Agencies
- Regions
- Unions and professional associations
- School, University
- Research Institutes

WE 038

The international panel on chemical pollution, IPCP

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Hazard and risk assessment of chemicals is a highly complex task, because there are thousands of chemicals and a wide range of effects in numerous species that need to be assessed. Therefore, careful evaluation and interpretation of both the available scientific knowledge and the existing data gaps and uncertainties is essential. This evaluation and interpretation should be focused on the needs of decision makers outside the scientific field. Because every single chemical may pose highly complex questions for the assessment of exposure and effects, additional capacities for work at the interface between science and politics are highly desirable. Here we present a new institution working at this interface, the International Panel on Chemical Pollution, IPCP. The IPCP focuses on chemical pollution problems of global relevance and is a platform where academic scientists can jointly evaluate their findings and collaborate on the transfer of these findings to decision makers. In several existing interface institutions in the field of chemicals assessment, scientists are called in as individuals and/or representatives of their institutions. Often, scientists from academia, government and, in some cases, also industry work together in these interface institutions. Taking a different approach, the IPCP is a platform primarily for academic scientists who want to share their knowledge about certain aspects of chemical pollution problems and want to join a global network that supports them in conveying their knowledge to decision makers at the national and international level. We present the objectives and work approach of the IPCP, also in comparison to other interface institutions, such as the International Programme on Chemical Safety, and provide an overview of the current activities of the IPCP with a focus on communication of scientific results to the public.

WE 039

PREPARE knowledge exchange initiative: Pharmaceutical Release into the Environment under Pandemic and Regional Epidemics

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PREPARE is a Natural Environment Research Council-funded Knowledge Exchange Initiative which is a multi-disciplinary network aimed at providing a foundation of scientific understanding on the fate and effect of widespread pharmaceutical use during a pandemic and epidemic. By bringing together industry and the research community, it is anticipated that the Initiative partners will contribute to a thorough assessment of the risks, a more thorough assessment of the known-knowns and an informed determination of the knowledge gaps. It is the aim of PREPARE to not only identify novel hazards but also to spark innovative solutions and coordinate the research to fill knowledge gaps.

PREPARE has held three workshops that focused on the human and environmental risks posed by widespread antiviral and antimicrobial use during an influenza pandemic. The issue is that during the course of an influenza pandemic, large quantities of drugs are projected to be used to treat cases of influenza and influenza-associated complications. With few exceptions, a large proportion of the ingested pharmaceuticals are excreted from the human body in a biologically-active form to ultimately enter WWTPs and rivers. The antibiotic-component of the pharmaceutical response can be expected to challenge and non-uniformly inhibit the growth of microorganisms within WWTPs on which their treatment effectiveness depends. Whilst this is predicted to reduce the overall organic removal of a WWTP, the greatest risk is the loss of nitrifying microorganisms. Inhibition of the microorganisms responsible for nitrification may lead to accumulation of nitrite nitrogen in the WWTP effluent, a form of nitrogen which is particularly toxic. A loss in WWTP functioning during an influenza pandemic would result in insufficiently treated wastewater entering the receiving rivers, leading to eutrophication, loss of aquatic life, and fish kill. In the case of those rivers which serve as abstraction points for drinking water, as the Thames River does for London, there are immediate implications for the quality of source water for human consumption. In summary, even if we accept the environmental hazards, it is necessary that we be aware of, and plan a response to a pandemic situation where very high drug use is prescribed. The PREPARE network is open to industrial, policy and academic stakeholders.

WE 040

WILDCOMS

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Disease and contaminants can both pose major risks to wildlife and human populations. Disease is a natural driver regulating the dynamics of wildlife populations, but some diseases warrant particular attention because they (i) cause major mortalities that lead to population crashes, (eg., VHD in rabbits), (ii) threaten wildlife species of high conservation concern (for example squirrelpox virus in red squirrels), or (iii) pose a potential threat to Man (eg., rabies, avian influenza). The wildlife Disease & Contaminant Monitoring and Surveillance (WILDCOMS) Network is a collaborative project among the major disease and contaminant monitoring schemes for vertebrate wildlife that operate in the United Kingdom. These schemes are run by various government agencies and laboratories, research centres, institutes, and academia. The overall aim is to establish a network which will foster and facilitate knowledge exchange, harmonisation towards best practice, and productive collaboration between: (i) partner organisations; (ii) surveillance schemes and end-users. It will aim to provide end-users with an integrated overview of environmental disease and contaminant risk. The specific objectives will be to develop the network and use it to address common challenges, specifically maximising dissemination of information to stakeholders and harmonisation towards common operational procedures to facilitate interaction and collaboration.

WE 041

Nyabondo integrated vector management project

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Malaria remains a major vector borne disease in many countries in sub Saharan Africa. This is true for Kenya where an integrated vector management program was implemented on Nyabondo plateau to manage mosquito populations with the main goal of interrupting malaria transmission. Malaria mosquitoes spend a considerable part of their life cycle in water. Water-based stages are immobile, hence vulnerable to control measures that target them. Brick making is the main economic activity on Nyabondo plateau. This activity leaves behind numerous pits in which water accumulates to create conducive mosquito breeding habitats. Abandoned fish ponds add to this to aggravate the mosquito problem on the plateau. We combined the biological larvicide *Bacillus thuringiensis israelensis* (Bti) and introduced mosquito-eating fish *Oreochromis niloticus* in abandoned ponds to test its effectiveness for mosquito control in Nyabondo. In addition, because most of these habitats are man-made the involvement of community members through capacity building and knowledge transfer has been implemented as a critical part to ensure sustainability of malaria control measures. Application of Bti within breeding habitats reduced 86.3% of larvae by day 1 and 95% by day 4 when compared with day 0 i.e before Bti was applied. There was a 100% reduction in mosquito larvae within active (stocked with fish) ponds when compared to un-stocked fish ponds (abandoned). Mosquito vector control needs complementary strategies that target both the adult and larval stages. The two main habitats mentioned (brick pits and abandoned ponds) are as a result of human activities and therefore the project will continue to encourage and involve the community in the control measures.

WE 042

Risk perception analysis of the use of pesticides in rural areas: a study with farmers in Bom Repouso (Brazil)

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Bom Repouso is localized in Minas Gerais State, Brazil and approximately 50% of its population resides in rural areas, producing potato and strawberry in large scale to support the national demand. However, the intensive use of pesticides by farmers has resulted in undesirable effects after 20 years. How notified by the farmers, cancer and suicide cases are increasing in this region, as such as the environmental problems. Therefore, our hypothesis is that the exposure to pesticides could be recognized as an important environmental risk factor, but farmers underestimate the consequences. Then, we tried to understand how farmers think about and respond to risk in relation to the pesticides, as well as improve the communication of risk information among lay people, technical experts and decision-makers. Methodological instruments included seminars; field studies (2009-2010) to identification of the Preliminary Assessment of Risk and interview with 50 farmers (selected among the 22 neighborhoods from the rural areas), assessing their perception about risks associated to the current production model. Preterit data (obtained in 2005-2008) were also incorporated in the research, which allow us to recognize the social-economical profile of the 1480 farmers and information about local structure. The results showed up the expansion of the agriculture, with considerable effects in social and environmental aspects, which are related to the total absence of planning in the sector and also the manner in which farmers are making their activities. In spite of the low levels of education and that almost all the respondents have never participated in a pesticide education program, farmers (70%) perceive the risks associated with pesticides. Nonetheless, their attitudes (such as the non-use of PPE, inappropriate methods of disposal of empty containers and unsafe use of pesticides) demonstrate total negligence. Also, most of them underestimate the real effect of pesticides and overestimate another one to explain the effects that were observed. Therefore, the continuous use of pesticide results from other aspects, such as cultural, besides the absence of economic and political incentive to improve another way of farming in Bom Repouso. Considering these results is possible to assume that those who promote and regulate health and safety need to understand how people think about risk, because without it, well-intended policies may be ineffective.

WE 043

Needs and opinions on risk regulation from Swedish companies using nanomaterials

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Nanotechnologies are emerging technologies, still diversifying in many scientific fields and industrial sectors. Regulatory concerns regarding the use of nanotechnologies and more specifically nanomaterials originate in lack of knowledge regarding their use and potential effects. The nanoscale properties that make nanomaterials so appealing for the development of new applications could also imply a new set of risks to health and environment, as the toxicological properties of the nanoscaled substance most likely also will change compared to the bulk material. It is important to consider future applications of nanotechnologies as well as current when discussing regulatory needs and actions. In an overview of research and development activities within Sweden, a questionnaire was sent out to nanotechnology companies. The questions covered areas such as use of nanomaterials, risk assessment efforts and the companies' views on the use of nanomaterials, business conditions and how governmental agencies should work with such issues. Previous overviews suggest that close to 120 companies are active within nanotechnology in Sweden. By using a broad set of criteria 176 companies were contacted for the purpose of this study, of which 35 responded that they were not active within nanotechnology and 48 responded that they were and filled in the questionnaire. Among these 48, four out of every five also state that they use nanomaterials. One major conclusion is that the companies are very different, regarding history, scientific area and industrial sector; nanotechnology should thus not be considered as one branch of industry. Also, assessing risk to health and environment seem not to be prioritised by most companies, although several were asking for support from regulatory agencies with risk management activities. Several companies also commented on the use of the term nanomaterial, that the importance of size is overemphasised, both by media and regulators. This, together with perceived unjustified regulatory actions was seen by the companies as an obstruction to a successful innovation climate in Sweden. The results from this questionnaire will be presented and discussed in relation the needs of regulation and communication efforts.

WE 044

Are labeled products safer?

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Around 2-4% of the German population suffer from contact allergy to fragrance compounds. In the EU, 26 supposedly allergenic fragrances must be specified on the containers of cosmetic products and washing and cleansing products if they are present above certain mass percentages in the product. Three parties (manufacturers, authorities and consumers) carry the responsibility in this kind of risk reduction strategy:

- Manufacturers must specify the "26 allergens" on the label of their products.

- Authorities should control the implementation of the labelling, at least by spot checks of the e.g. around 20 000 new or modified cosmetic products that enter the German market every year.

- Consumers carry an even bigger share: They should read the labels, know which names belong to the "26 allergens" and decide whether they want to buy and use this product containing potential allergens. Consumers need to know a lot, they should be able to make a kind of risk assessment for their private use and decide whether they want to run the risk or not. On top of that, most consumers are not aware that they carry this responsibility and assume that cosmetic products were free of hazardous substances.

The labelling of the "26 allergens" is a risk communication instrument. However, it does not communicate the unavoidable "residual" risk after all feasible risk control measures have been taken. It rather charges the general public with the tasks of risk assessment, risk control and risk management, even if their risk perception is minute or even inexistent.

Although the cosmetics industry takes over more responsibility for the use of allergenic fragrances by this "26 allergens rule" than producers of other products where no labelling is required, this mandatory labelling procedure is not suitable to guarantee consumer safety. Products need not be safer now than before the compulsory labelling, unless consumers exert their power and would no longer buy products where these fragrances are labeled, which is apparently not the case in the German market of deodorants.

Experts elaborating criteria for labelling products containing nanomaterials as risk reduction strategy could learn from the experiences made here. It must be questioned whether such a compulsory labelling alone is a suitable way for risk communication and management.

WE 045

Practical guidance for undertaking socio-economic analysis within the REACH authorisation process

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In the context of the EU REACH Regulation, Socio-Economic Analysis (SEA) is a tool for answering the question 'Is society better or worse off by allowing a particular use of a chemical?' An SEA can comprise part of an application for an Authorisation to "use" a substance listed in Annex XIV of REACH. This poster describes practical guidance on undertaking an SEA within the context of an Authorisation application under REACH, but is also applicable to wider regulatory decision making (i.e. Restrictions under REACH, medicinal products). This guidance has been developed from experience gained from undertaking a series of case-study SEA within the context of REACH Authorisation. In many instances, undertaking an SEA will be an iterative process with the analysis developing over time, as the understanding of how the supply chain will react to a refused authorisation application improves and sufficient data become available to estimate the impacts of those responses. Key messages, which will be elaborated further in the poster, include the following: The SEA process may be unfamiliar to many but does not have to be complex. An SEA must be produced for a specific use of a substance. SEA must be tailored to a particular audience (i.e. ECHA's SEAC committee); in many instances the expression "more is less" will apply. Information requirements for SEA are very different to conventional chemical risk assessment and will comprise information on the production and use of the substance, market information, information about possible alternatives, information about the impacts if authorisation is refused. A Chemical Safety Assessment (CSA) conducted under REACH will not contain sufficient information to conduct an SEA. An SEA will take time, so it is best to plan ahead even if external help is used. Information will need to be gathered within organisations, from suppliers and customers, potentially from other manufacturers (competitors) and stakeholders. Frameworks for sharing information, whilst ensuring confidentiality and competition laws are respected, will need to be established. Perhaps most importantly, SEA should be viewed as an opportunity to demonstrate the value of a product to society.

WE 046

SIMACLIM - a combination of the regional risk model and a communication tool

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The Interreg-Project diPol "Impact of Climate Change on the quality of urban and coastal waters (Diffuse Pollution)" aims to collect knowledge on the impact of climate change on water quality, to communicate and raise awareness towards this knowledge, to improve the ability of decision makers, to counteract these impacts on local and international level, and to facilitate public participation herein. The programme SIMACLIM was developed by diPol in order to facilitate these activities. It also helps decision makers when addressing complex, partly conflicting interests and when they need to balance legislation, societal, environmental and economic concerns.

SIMACLIM consists of two parts: A sensitivity model according to Vester and a relative risk model which is based on the regional risk model by Landis and Wiegers (1997). The sensitivity model is carried out by bringing the stakeholders from the region of concern together and by letting them work together on a system description of that area. Different perceptions and various levels of knowledge lead to a high level of discussion among the group. The group needs to agree on the system description, sharing a lot of knowledge while doing this, building up trust among each other. Part of the project is also to address and agree upon management objectives in the region.

These management objectives represent the input from the sensitivity model to the relative risk ranking model. The latter is intended to be used by decision makers to show the relative risks from different stressors to the identified objectives, also comparing risks to different regions. This relative risk model (RRM) that was developed by Landis and Wiegers was transcribed by diPol into an excel-based, easy to use programme in order to facilitate its use by decision makers.

With this poster, we demonstrate the application of SIMACLIM exemplarily to 2 case sites within the diPol-project.

WE 049

Selection of potential focal bird species for a refined risk assessment of pesticides in Switzerland: methodology and examples

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Pesticides are subjected to ecotoxicological risk assessments in the process of national authorization. If a pesticide does not pass the first-tier, worst-case risk assessment, a refined risk assessment is required. For birds, it is possible to refine the exposure component of the risk using "focal species". These species are, other than the fictitious first-tier species, real bird species actually occurring and feeding in the crop at the time of pesticide application. Therefore, using focal species, realism can be added to the risk assessment. The potential focal bird species for Switzerland for the main Swiss cultures were selected with a semi-quantitative method based on comprehensive Swiss ornithological literature and on expert knowledge of Swiss ornithologists. All candidate bird species were judged independently by different experts with regard to their suitability as focal species. The key criteria were i) strong association to the crop, ii) high abundance in the crop, and iii) high intake of contaminated food per kg body weight. For each main culture and growth stage, two to four focal species with different feeding habits were selected. In this presentation, the methodology behind the selection of focal species will be described, together with some concrete examples of the potential Swiss focal bird species. These focal species could be used in the future to achieve a crop-specific and more realistic estimation of the risks of pesticides in Switzerland.

WE 050

List of potential focal bird species for a refined risk assessment of pesticides in Switzerland

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If a pesticide does not pass the first step of the ecotoxicological risk assessment for birds, a further refined risk assessment is required. This involves the use of more realistic exposure estimates and the use of “focal species”. These species are, other than the fictitious first-tier species, real bird species actually occurring and feeding in the crop at the time of pesticide application in the field. Therefore, using focal species, realism can be added to the risk assessment. The focal bird species potentially relevant for Switzerland were determined by the ecotoxicology group at the Research Station Agroscope Changins-Wädenswil (ACW) based on comprehensive Swiss ornithological literature, and with the helpful input from experts of the Swiss Association for the Protection of Birds (SVS/BirdLife) and the Swiss Ornithological Institute Sempach. The suitability of bird species as focal species was judged by different experts independently. The key criteria were i) strong association to the crop (intensive access and utilization), ii) high abundance in the crop (high frequency and distribution), and iii) high intake of contaminated food per kg body weight. For each main Swiss culture and growth stage, two to four focal species with different feeding habits were selected. In this poster, the selection procedure and the whole list of the potential focal species are presented.

WE 051

Selection of focal species of birds and related exposure scenarios for different crops treated with pesticides in Norway

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The work presented here is the result of a six-year project to select Norwegian focal species financed by the Norwegian Ministry of Agriculture and Food. At the initiation of the project, a literature review covering bird species that utilize the Norwegian farmland landscape, and which species that might be susceptible to pesticide exposure was conducted (Bakken & Strann 2006). The method was partly based on a Swedish approach for selecting focal species (Wärnäck 2006). Field surveys aimed at selecting focal species for strawberries were conducted as a pilot study during the 2007 growth season (Bakken et al. 2007) and field surveys in eight fields of different crops were conducted in 2008. This field work was repeated in 2010 in eight new fields of the same crops. In 2011, ten fields of five new crops were surveyed.

The Norwegian field surveys followed the repeated line transect method. All birds sitting in, taking off from, or landing in the fields, were recorded. The birds were identified, either directly or with the aid of binoculars. Approximately 130 hours of field work was conducted between May and October each year. A total of 38 different species of birds were identified in 2008, 34 species in 2010, and 33 species in 2011.

In order to identify focal species, the frequency of observation per survey (FOsurvey) was calculated for each species. FOsurvey denotes the number of surveys in which a defined species was recorded given as percentage of the total number of surveys (EFSA 2009). This gives an approximation for the temporal consistency of occurrence throughout the study period and gives an indication of prevalence. From our field surveys, along with an evaluation of published data, the Norwegian Food Safety Authority now has a preliminary list of focal species and related scenarios for the following 12 crops/groups of crops: grassland, cereals, oilseed rape, strawberries, pulses, potato, orchards, bulbs and onion like crops, bush and cane fruit, leafy vegetables, maize, and root and stem vegetables.

WE 052

Focal bird species for non-agricultural ‘Grassland Scenario’ risk assessments

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In the new EFSA guideline for risk assessments for birds and mammals a list of crop groups is given to identify relevant scenarios for wildlife risk assessments. However, the scheme of crop groups given in the guideline is not easily applicable to a number of proposed uses such as for example non-agricultural applications. Because of different habitat structures and human utilisation it is likely that bird communities of such areas are different compared to species composition on agricultural fields. The aim of this presentation is to propose a list of candidate bird species and to present exposure relevant data for birds on golf courses that can be used in a refined risk assessment for pesticides used in grassland.

A golf course offers a great variety of different habitats for wild birds. Based on the results of published bird surveys, a total of 799 breeding pairs of 88 different species, were recorded on 6 golf courses in central Europe. The blackbird, white wagtail, great tit, blue tit, greenfinch, blackcap and the chiffchaff were the most characteristic and stable elements of the bird community on golf courses, and were thus determined as the main candidates for focal bird species.

None of the species required in the GD (EFSA, 2009) for risk assessment in grassland is among the main candidates for focal bird species on golf courses based on literature. Therefore, further field observations and surveys will be conducted on golf courses in south western Germany in spring 2012 in order to verify candidate bird species for higher tier risk assessments.

The use of plant protection products on the various parts of a golf course is different. Pesticides will most often be applied in local treatments such as on greens and teeing grounds and on some spots on the fairways where e.g. mosses and weeds are considered to cause a problem. Therefore, we aim to identify the main foraging areas of birds, to better understand the exposure of birds to plant protection products on golf courses.

WE 053

Relevant focal bird species in Slovakia used in refined pesticide exposure assessment

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According to revised Guidance document on risk assessment for birds and mammals, published in EFSA Journal 2009; 7(12):1438, focal bird and mammal species used in risk assessment of Plant Protection Product (PPP) are real species that actually occur in the crop when PPP is being applied. Selected species should cover all other species from the feeding guild highlighted at the screening level at Tier1 of risk assessment.

In our literature review we primarily focused on bird occurrence in Slovakia that can be potentially used as a focal species in risk assessment. We reviewed recently published articles or books.

On the basis of the proposed scenarios in the updated guidance document published by EFSA in 2009 and our literature review, we focused on a total of 14 bird species as potential focal species to be used in risk assessment (*Alauda arvensis*, *Emberiza citrinella*, *Anser fabalis*, *Anser anser*, *Perdix perdix*, *Motacilla flava*, *Parus caeruleus*, *Erethacus rubecula*, *Fringilla coelebs*, *Serinus serinus*, *Carduelis carduelis*, *Carduelis cannabina*, *Turdus merula* and *Sturnus vulgaris*).

We suggest to add the Grey Partridge (*Perdix perdix*) to the list of focal species as insectivorous bird species. The chick's diet predominantly consists of insects in the first three months of their life. Risk assessors use Grey Partridge as medium herbivorous bird and the importance of insects in the chick's diet is not taken into account in risk assessment! Therefore if the PPP is applied from beginning of April till beginning of June, we suggest to use the Grey Partridge as an insectivorous bird and after that time period as a medium herbivorous bird species.

The use of the Yellow Wagtail (*Motacilla flava*) as a focal bird species in refinement of potential risk (proportion of a bird's daily diet obtained in habitat treated with pesticide and composition of diet obtained from treated area) is questionable, because of its rare occurrence in Slovakia. We suggest to replace Yellow Wagtail with Skylark or Grey Partridge (April - June), where possible.

On the basis of our literature review we fully support to use 13 of them, one species is of limited value for risk assessments (*Motacilla flava*).

Acknowledgement - This work was supported by the National Reference Laboratory for Pesticides UVMF Košice, Slovakia.

WE 054

Shift in crop preference by some focal farmland bird species during the breeding season in The Netherlands

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European farmland bird populations have been in a steep decline since the 1960s. Agricultural intensification is one of the main factors which has driven these declines.

Intensive use of pesticides is one of the many factors that is covered by agricultural intensification. Several studies have shown that pesticide use can have direct and indirect effects bird survival or reproductive success of birds. In order to assess effects of pesticides on birds in an appropriate way detailed knowledge of crop preference and crop use by focal bird species is crucial for higher tier risk assessment. This presentation will discuss the crop preference of two important farmland birds in arable systems in the Netherlands: the skylark (*Alauda arvensis*) and the yellow wagtail (*Motacilla flava*). Skylarks showed a preference for winter cereals during the first stage of the breeding season (April - early May). After this period spring cereals were much more preferred than other crop types. At the end of the breeding season (late June - July) a wide variety of crops was used by skylarks, but cereals were basically avoided. Yellow wagtails showed a strong preference for winter cereals during the first half of the breeding season. Although winter cereals were also used during the second half of the breeding season, numbers of territorial birds were much lower. During the second half of the breeding season most yellow wagtails were found in potato fields. The results of these studies show that crop preference by birds often changes during the breeding season as a result of the development of the crop and associated changes in vegetation cover within an arable landscape with different crops. Pesticide applications in crops which are strongly preferred by birds are most likely to have most effects on these birds. In the first place because of possible direct effects on the birds, but also due to indirect effects as a result of reduced food availability. Therefore, results of detailed crop preference studies on birds, including temporal changes in crop selection, should be used in risk assessment procedures of pesticides.

WE 055

Bird focal species for pesticide risk assessment in orchards in Spain

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The current bird and mammal risk assessment scheme for pesticides in Europe (EFSA Guidance document, 2009) considers specified “indicator species” at the initial Screening Step and “generic focal species” at Tier 1 assessment. These “indicator species” or “generic focal species” are not real species but are considered to represent worst-case scenarios and hence to be protective of other species occurring in that crop. In order to conduct a refined risk assessment it is necessary to identify “focal species” i.e. species

that actually occur in the crop in question and that are protective of other species.

Field studies were conducted in citrus plantations, olive groves and stone fruit plantations in Spain to identify the relevant bird focal species in these crops. Standard methodology for the “transect method” as detailed in Appendix M of the EFSA Bird and Mammal Risk Assessment Guidance (2009) was employed. This involved skilled ornithologists walking transects across the relevant crops and recording all birds within an “in-crop transect band” of 25m to either side (or width of crop field if narrower). Transects averaged 500-600m long with a total transect band area of 53-70 m² per crop. Three surveys were carried out, timed to cover the main pesticide application periods but also focusing on the bird breeding season in the spring.

Candidate focal species were identified as those with a “Frequency of Occurrence per field” (FOfield) of $\geq 20\%$ i.e. those species which were recorded in at least 20% of surveyed fields. Further selection was based upon feeding guild, bodyweight (smaller species generally at higher risk) and abundance. Recommended focal species are given in the poster. An analysis of olive data showed four bird species, the serin (*Serinus serinus*), the hoopoe (*Upupa epops*), the blackbird (*Turdus merula*) and the song thrush (*Turdus philomelos*), were statistically significantly more common in olives with weedy ground cover compared to olives with bare soil between rows following cultivation.

WE 056

Farmland bird communities vs crop specific focal species in a Mediterranean landscape: The case of arable crops in Hellas

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In Europe, in the scientific regulatory guidance documents developed in order to conduct risk assessments for birds and mammals for plant protection products (PPP), the potential risk for the avian community is identified, apart toxicity, on the basis also of a theoretical exposure via the use of crop specific ‘focal species’ (FS) in various BBCH scales. The FS concept means one or group of species, having spatial and functional requirements effectively defining environmental limits for the protection of other species present in the crop.

Field size, land structure, its use and management and characteristics of the surrounding non-crop areas, crop rotations can influence the bird fauna and the way birds use the agricultural fields. It can be difficult to assess the relationship between them as the mobility of birds allows them to use the available habitat on a larger-scale than that which might be assumed when looking for such associations. The presence of farmland bird species is influenced by the availability and characteristics of alternative habitat types and therefore specific crops and their bird populations do not function as isolated patches. The interrelation between non-crop, crop areas and crop types as foraging habitats for birds will be influenced by the relative size of the fields and of off-crop areas. Such interrelations may be complex in agricultural landscapes characterized by small fields of different crops with diverse off-crop areas as hedgerows or bushes between the fields. Such landscape occurs frequently in Mediterranean countries, more than in other regions of the EU. For such Mediterranean landscapes, it is necessary to obtain more detailed knowledge about bird use of different habitats before defining the most appropriate FS that can be proposed.

The aim of this study is to present data on bird FS from a typical Mediterranean agricultural landscape in Hellas (Kopaida plain) with such diversity of rotational crops and off-crop habitats.

The methods used for bird recording were “line transect” (2 years) and “point counts”.

Investigated parameters included the qualitative composition of the bird community, the frequency of occurrence, dominance and abundance of species present. The poster will propose a list of bird FS for arable crops used as an ecological niche by birds, assigned to foraging guilds, diet guilds and size classes. Their use in a refined risk assessment for PPP is discussed further in the final poster.

WE 057

Exposure evaluation for wild mammals in ornamental fields in the Netherlands

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An ecotoxicological risk assessment must be performed prior to authorization for all plant protection products in the EU. For birds and wild mammals, “generic focal species” scenarios are proposed as initial steps in the Guidance Document of EFSA for “Risk Assessment of Birds and Mammals”. As a second step, a refined ecotoxicological risk assessment can be conducted based on real focal species for the respective exposure scenario. For many major crops (e.g., cereals) such information is available in the literature or from targeted field studies, but little is known for a range of minor crops, such as ornamental fields.

We aimed to determine focal species in tulip and lily fields in the Netherlands by setting up a trapping grid with life traps for three trapping nights. We typically found Wood Mice in and around the field, and Bank Voles only in forested surroundings. Additionally a few Common Voles were trapped.

Based on these results we recommend that Wood Mice should be considered as focal species for both tulips and lilies. They were found directly in the field, on the edge of it and in the woods. Bank Voles were exclusively caught in the woods and can be ruled out for risk assessment as they do not enter the actual fields. Common Voles were only found in low numbers in the edge of tulip fields but not in lily fields. This suggests that there is no stable population at potential risk in either type of ornamental fields.

We also investigated whether herbivorous Common Voles are interested in lily or tulip onions as food. We designed a laboratory feeding experiment with Common Voles and fed them tulip and lily onions parallel to their standard food. We separated them by gender and discovered that due to social behavior female voles seem to feed a larger amount of the onions than male. Lily onions were strongly avoided by both genders. This result agrees with our field observations in that lily fields were even less used by Common Voles than tulip fields in the Netherlands.

WE 058

Bats at risk? - Bat activity in the agricultural landscape and insecticide residue analysis of bat specific food items

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Although bats are reported as being threatened by pesticides they are currently not considered in the European Union pesticide risk assessments. The reason for that contradiction is probably related to the scarcity of information on bat activity in pesticide treated fields and the pesticide residues on their food items.

We surveyed bat activity in an agricultural landscape and detected high activity levels of the common pipistrelle a foraging habitat generalist at several agricultural sites. Besides, airborne forager and bats that take part of their food by gleaning arthropods from the vegetation were recorded frequently. In a further step we recorded bat activity and measured pesticide residues on bat specific food items following applications of two insecticides in an apple orchard. The initial value and the decline of the pesticide residue pattern were found to depend on the arthropod type, their mobility, their surface-weight ratio, and the mode of action of the applied pesticide. The highest initial residue values were measured on foliar-dwelling arthropods. By performing the toxicity-exposure ratio approaches of the current pesticide risk assessment no acute dietary risk was found for all recorded bat species. However, a potential reproductive risk for bat species that include foliar-dwelling arthropods in their diet was indicated.

Considering the potentially higher sensitivity of bats compared to other mammals due to their ecological trait our results emphasises the importance of adequately evaluating the risks of pesticides to bats.

WE 059

Risk assessment for birds and mammals in Mediterranean countries. Do we need a different approach?

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The most recent scientific regulatory guidance document to conduct risk assessments for birds and mammals for plant protection products, issued by EFSA (2009), designates a tiered assessment framework.

If an active substance, and its associated product and use, fails the earlier stages (screening step and tier 1) of the tiered approach, it is possible to further refine the exposure element of the risk assessment by using crop specific ‘focal species’ present at BBCH stages at the moment of the application. For birds and mammals a ‘focal species’ is a real species that actually occurs in the crop when the pesticide is being used and is considered to be representative of all other species from the feeding guild that may occur in the crop at that time. Whereas several studies have been conducted in typical crops of central temperate Europe, less information is available for specific crops in Mediterranean countries.

New regulation 1107/2009 replacing Directive 91/414/EEC includes the concept of the zonal evaluation, and the subsequent mutual recognition of authorizations in order to guarantee the commercialization of PPP in the EU and avoid duplication of work. From the experience gained from the majority of active substance evaluations, the risk assessment on birds and mammals requires higher tier assessments, which leads a considerable higher workload and expertise for risk assessors, regulators and notifiers.

Although SMS countries face almost the same crops and weather conditions, there may be difference on perspectives as well as on protection goals. The basic characteristics of the Mediterranean agronomy and agro-environment (e.g. high diversity of environments, the intensive rotation in some crops), may facilitate/refine further the risk assessment on birds and mammals.

Here, we suggest objective criteria for selecting suitable focal species to improve the interpretation of the risk assessment in Mediterranean agricultural landscapes and provide quality indications to identify protection goals and risk management decisions for Mediterranean countries in order to facilitate the development of a registration work-sharing programme.

WE 060

Focal species and their ecological behaviour in pesticide risk assessment in the Netherlands

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The ecological behaviour of bird and mammal species is regularly used in refined risk assessments of pesticides in Europe. Also in the Netherlands applicants often choose this route to address risks identified in the first tier. There is as yet no harmonisation between EU countries on the choice of focal species per crop, nor on the required conservatism in choosing PT and PD values.

To aid the discussion in the session on focal species of birds and mammals and their ecological behaviour for refined risk assessments of plant protection products in Europe, this poster will provide a review of focal species with PD and PT used in risk assessments of pesticides in the Netherlands in the past years and considerations on the assumptions behind these choices.

WE 061

Experiences with the application of the EFSA Birds and Mammals guidance

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The EFSA Bird and Mammal guidance document was first published in 2009 to supersede SANCO/4145/2000. The various assessments within the guidance have been expanded and updated based on more recent information/data. Since its use, in both new assessments and updating previous applications using the old SANCO guidance, it has become apparent the impact of the changes to the outcome and interpretation of the risk assessments. Focusing on the most commonly used assessment of dietary risk, the differences between the two guidance documents are substantial, although still maintaining the tiered approach.

Comparison of the step approach and selection of crop group/species scenarios provides a clear understanding as to the factors driving the assessment and how potential differences can occur in the assessment outcome. The updating of applications to the EFSA bird and mammal assessment reveal the impact of the new guidance can generally provide more favourable conclusions in demonstrating an acceptable risk. However, there are also examples that show little difference in the overall outcome and even instances where the EFSA guidance highlights an unacceptable risk which was not apparent under the old SANCO guidance. The EFSA guidance utilises the 'Generic Focal Species' at the Tier 1 level based on more representative data than the old SANCO 'Indicator Species' and therefore failure at this stage can require greater consideration for refinement than under the old SANCO guidance. This makes the selection of the 'Focal Species' in refinement particularly critical and as further data becomes available, PD and PT factors need particularly consideration as they play a more influential role in demonstrating safe use.

WE 062

Ecological context of long-term avian risk assessments: UK spring seed treatment example

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Long term avian risk assessments for seed treatments have a relatively high failure rate. Refined risk assessments may include focal species and associated dietary parameters (e.g. literature or field data for PD, PT). Compound specific seed residue decline data may also be used. Despite such refinements, however, the risk assessment may still indicate potential for concern.

One way forward is to place the risk assessment into an ecological context. For spring and summer applications especially, concern centres around exposure of birds during the breeding period (EFSA, 2009). Risk assessments also tend to be conducted on a one-field basis, equivalent to exposure of birds across the agricultural landscape. For relatively minor crops, however, this may not be the case.

Here, we show how information on the timing of breeding activity and timing of application (chronological aspect) may be combined with information on cropping density in selected focal regions (spatial aspect) to estimate the proportion of breeding activity in a focal species to which the long term toxicity/exposure ratio (TERLT) would apply. For a spring barley seed treatment in the UK, we show that the TERLT would apply to approximately <1% of breeding activity in the Skylark (for which the refined dietary TERLT was <5). Also, using breeding data, we propose an alternative focal species for which breeding would coincide to a greater extent with the exposure window (i.e. worst-case timing).

Overall, therefore, assessing ecological context allows for added realism in risk assessments.

WE 063

Assessing avoidance in a series of modified laboratory and cage tests with birds

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According to EU procedures for the registration of plant protection products, potential risk for the avian community is identified, apart toxicity, on the basis also of a theoretical exposure. Bird risk assessment scenarios include intake via feed, generally considered to be the most important exposure route, and represent a realistic worst case assessment where the exposure scenarios are selected to reflect a situation where the total daily feed is contaminated. Avoidance is a factor that could be used to refine the risk as it may be a significant factor that reduces actual exposure. However, there is still significant uncertainty on the actual impact of avoidance to minimize exposure under laboratory and field conditions. In some cases, strong avoidance shown by captive birds failed to protect wild birds.

This report describes three basic test designs to determine the avoidance behavior of birds and their contribution to exposure to pesticides. There are different options to quantify the degree of avoidance. The determination of avoidance of treated food is possible either by a "no-choice test", in which only treated food are offered ad libitum during the exposure period or by a "two-choice test", in which both treated and untreated food are offered to the birds at the same time.

In this study we attempt to present a sequence of methods that takes into account the avoidance behavior in the evaluation of slightly modified 5 days "no-choice test", 3 days "two-choice test" and a proposed modified 3 days semi field ("cage test") method with birds in order to evaluate the food avoidance behavior.

This battery of tests could investigate the question whether there are consistent differences between the three possible designs but also provides information necessary to understand the intrinsic properties, possible learning abilities but also other factors that may contribute to the overall avoidance response to a pesticide. It also provides enough evidence to decide whether risk is substantially reduced or not by avoidance in the field.

A case study is presented and a refined risk assessment for plant protection products is discussed further under the light of the most recent scientific regulatory guidance document to conduct risk assessments for birds and mammals for plant protection products issued by EFSA [EFSA Journal 2009, 7(12): 1438]. The use of avoidance testing in the context of current European regulations for plant protection products will be discussed.

WE 064

Risk regulation for farmland bird and mammal species by accounting for indirect effects of pesticides

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The current state of scientific knowledge indicates that indirect effects of plant protection products (PPPs) can significantly contribute to the risk for non-target species in agricultural landscapes. Considering the legal requirements of the new EU pesticide legislation to avoid the occurrence of unacceptable effects on the environment including biodiversity and to attain a sustainable use of pesticides it is obvious that indirect effects cannot be ignored in the risk regulation of PPPs. One of the essential prerequisites for the development of efficient risk management strategies for indirect effects is the identification of the species concerned, especially their ecological requirements, with focus on food availability and potential interferences with the timing of PPP applications.

We conduct a comprehensive literature review on effects of PPPs on a range of bird and mammal species that occur in German arable landscapes, with special focus on indirect effects and the species' ecology. From this data we develop a sensitivity index to assess the potential risk a species is facing from effects of PPPs. We then use this index to calculate vulnerability indices that compare and evaluate the influence of different crop types or seasonal and regional variations in PPP applications. The vulnerability index, based on species-specific features, allows for making potential and actual hazards of pesticides visible. Results from this evaluation enable the definition of focal species in German agricultural landscapes with a specific focus on indirect effects and highlight the necessity of comprehensive risk assessment strategies.

Indirect effects of PPPs threaten populations of wildlife species in arable landscapes. The identification of umbrella species is found to be an essential prerequisite to derive effective measures which compensate or minimize the risk from indirect effects for a broad suite of affected species. We here represent a tool to further enhance existing risk management strategies in order to achieve the overall aim of a sustainable use of PPPs.

RA10P -Global Mercury: Bridging science and policy

WE 066

Human risk through consumption of European sea bass contaminated by mercury: (re)definition of edible parts

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Juvenile specimens of *Dicentrarchus labrax* (European sea bass) were collected at three different sites along an anthropogenic mercury (Hg) contamination gradient (reference - R, moderate - L1 and highly contaminated site - L2) at Aveiro lagoon (Portugal). Total Hg (T-Hg) was measured in muscle and kidney, and organic Hg (O-Hg) was determined in muscle. In order to evaluate the human risk through consumption, the weekly and daily ingestion were also estimated and compared with the Provisional Tolerable Weekly Intake (PTWI) and with the Reference Dose (RfD). Both muscle and kidney T-Hg levels revealed significantly higher values at the highly contaminated site relatively to R. The obtained muscle and kidney T-Hg values were compared with the values set by the European regulations for marketed fish, revealing that kidney exceeded the maximum established limit at L2. This evidence reinforces the need to redefine the concept of edible tissue, creating the possibility to include potentially "accumulator" tissues that are not removed during cooking procedures. The Hg (T-Hg and O-Hg) weekly ingestion was higher than the PTWI in the contaminated sites and O-Hg daily ingestion rates were higher than the RfD in all sampling sites. In agreement, the consumption of sea bass from the contaminated sites may induce risk to the consuming population, especially to children and pregnant women. This reinforces the need to design regulations that include different tissues, besides muscle, and to invest in advisory campaigns where the specific risks and benefits for each population are highlighted.

WE 067

Hg bioaccumulation in shoots of the macrophyte *Elodea nuttallii* in the field and in the microcosm

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1. Introduction

Mercury (Hg) is highly toxic and shows huge biomagnification in aquatic food webs. Understanding Hg bioaccumulation and uptake mechanisms is therefore a research priority. Studies in Babeni reservoir (Romania) suggested that macrophytes might participate in Hg bioaccumulation in the food chain.

2. Results and discussion

Hg bioaccumulation was measured in several macrophytes collected in Babeni reservoir. *Elodea nuttallii* showed high Hg accumulation including methyl-Hg in shoots and a broad distribution in the river. We therefore further studied this plant in the laboratory.

Tolerance and accumulation of inorganic and organic Hg in *E. nuttallii* was high. Basipetal transport of Hg from shoots to roots was predominant. Hg concentrations were higher in roots>leaves>stems and in top>middle>bottom of shoots. In shoots, 65±11% and 45±17% of Hg were found in cytosol and cell walls respectively. Accumulation was highly reduced by cold, death and by competition with Cu+.

3. Conclusions

E. nuttallii showed a high accumulation and tolerance to Hg in the field and in the microcosm. Hg in shoots mainly originated from the water column. Passive diffusion was observed in the cell wall whereas uptake in the cytosol was linked to the metabolism probably through copper transporters.

E. nuttallii does not seem to represent a major risk of remobilization of Hg from sediments to the water column but in the field herbivores may be exposed to Hg through ingestion of plant parts. Our study showed that more knowledge concerning Hg accumulation mechanisms in plants will be valuable to understand Hg bio-geochemical cycles in the environment.

WE 068

Can fatty acid trophic markers be used to determine dietary sources of mercury (Hg) accumulating in salt marsh ragworms?

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Salt marshes are naturally dynamic systems, providing essential ecological services like habitats, high productivity, mediation of biogeochemical cycles, and being a natural sink for pollutants.

Mercury (Hg) is included in the list of high priority environmental pollutants and it is still one of the most hazardous contaminants present in coastal environments. Due to the deleterious effects of Hg on ecosystem functions and services, namely through transference within the food web, Hg-contaminated salt marshes resulting from historical contamination are still a cause of concern. Knowing the fate of Hg in contaminated salt marshes is crucial to understand the repercussions that this kind of pollutant may still have within the ecosystem.

The main objective of this study is to verify if fatty acid trophic markers can be used to determine dietary sources of mercury (Hg) accumulating in salt marsh ragworms, using Ria de Aveiro as a case study.

Fatty acids are ubiquitous components of all living organisms being widely used as qualitative markers to trace trophic relationships in aquatic environments. Fatty acids can easily be related to food sources since they display taxonomic specificity, i.e., higher trophic organisms incorporate the signature of its food source with unchanged or recognizable forms.

The Ria de Aveiro is a coastal lagoon located on the northwest coast of Portugal (40°38'N, 8°44'W). In this system, Laranjo Basin is a shallow area with 2 km² that is historically contaminated with Hg and in Laranjo salt marsh *Bolbochenous maritimus* and *Juncus maritimus* may phytoaccumulate this pollutant. This phytoaccumulation mostly occurs in the belowground biomass, thus contributing to the self-remediation of the system. However, through the mineralization process, benthic fauna can still be exposed to Hg through the detritus pool.

The ragworm *Hedistes diversicolor* is a burrowing species and therefore in close contact with sediments and the Hg-contaminated detritus pool. *H. diversicolor* is one of the most widely distributed marine polychaetes and one of the few with economical value. It is an omnivorous species and a potential prey for crabs, birds and fish.

The present work tests and discuss if and how fatty acid profiles can be used as reliable indicators of dietary sources of mercury (Hg) accumulating in the ragworm *Hedistes diversicolor* in a contaminated salt marsh.

WE 069

Analysis of mercury in air and soil of mining sites in Querétaro, Mexico

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Mercury cycles in the environment are the result of its releases from various sources natural and anthropogenic. Important sources of anthropogenic releases mercury include: Artisanal/ small-scale gold mining and extraction, goldsmith shops, energy production from fossil fuels, dental amalgam fillings, use of fluorescent lamps, instruments, waste incineration (municipal, medical and industrial wastes) and landfills. Mercury mining activities have been developed in the mining site of San Joaquin, Querétaro since the colonial times and remain active in the present time. The aim of this project was to analyze the concentration of mercury in soils and air of San Joaquin, Querétaro to determine the potential risk to health by the potential liberation from soil to air, based on species Hg chemical present. The results obtained indicate that the largest percentage of Hg (above 60%) is found in the form of mercury sulfide which is very stable; it was also found the presence of elemental mercury as amalgam (under 10%). These species liberate very little mercury to air and this was corroborated since the concentration in air ranged from non detectable to a maximum of 22 ng/m³.

WE 070

Mercury in fish tissue - a comparative analysis

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For fish as a foodstuff, European Commission Regulation (EC) No 1881/2006 sets maximum levels of certain contaminants, including mercury. For fishery products and muscle meat of fish, crustaceans (excluding the brown meat of crab and head and thorax meat of lobster and similar large crustaceans), the maximum allowable concentration is 0.5 mg/kg (wet weight) and for the muscle meat of classified species of fish considered as higher predators, the maximum level is 1.0 mg/kg (wet weight).

Commission Directive 2001/22/EC sets out the analytical methodology performance requirements for analyses related to regulatory measurements connected with EC 1881/2006. Detection limits must be less than one tenth of the level of interest and quantification limits one half of that value. Accuracy must be +/- 20%.

It is possible to analyse for trace and ultratrace mercury in fish tissue by a number of techniques, including techniques based on cold vapour generation atomic absorption spectrophotometry (CV-AAS) and atomic fluorescence spectrophotometry (CV-AFS). This study compares the analytical performance of methodologies utilising acid digestion, followed by mercury determination with a new CV-AAS instrument and an existing CV-AFS instrument. Fish tissues of types falling into both categories from EC 1881/2006 were analysed and performance figures of merit established, relating the performance to the above regulations.

WE 071

Comparability, traceability and uncertainty of the results for mercury analysis and speciation in the marine environment

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Mercury (Hg) is classified as a global pollutant and is among the most toxic elements in the environment. In the environment, Hg exists in different chemical and physical forms and the conversion between these forms is the basis for its complex environmental cycling. Among the most important environmental transformation is the formation of monomethyl mercury compounds (MeHg) which have a high tendency to bioconcentrate and biomagnify in the aquatic and terrestrial food webs. Due to mercury's complex environmental chemistry that includes reduction and oxidation, methylation and demethylation, as well as partitioning between environmental compartments (air, soil, water and biota), numerous studies have been implemented to understand the major factors that govern these processes. The measurement support is not fully in line with the needs of the environmental research, particularly when mercury cycling in the marine environment is in question.

Although, chemical metrology has gained an increased importance in recent years, it is still very rarely properly addressed in papers dealing with mercury analysis and speciation. The only way to understand and use metrology in chemical measurements is to better understand the measurement process itself. Establishing traceability is comparing the value obtained by measuring an "unknown" to the value of "known" with stated uncertainties. A traceability chain is, therefore, a chain of comparisons of values to a common accepted value (for example a "reference value" carried by a reference material). Overall uncertainties cannot be evaluated without traceability chain. Traceability provides the means of placing all related measurements on a consistent measurement scale, while uncertainties characterise strength of the links in the chain and the agreement to be expected between laboratories making similar measurements. The presentation will address these topics in relation to mercury speciation and analysis in different matrices in marine environment, with the emphasis on mercury speciation in air of the marine boundary layer and sea water and provide some suggestions to progress in chemical metrology in this field, which will improve trust into the measurement results obtained on a worldwide scale.

WE 072

Status and trends of the landscape-scale mercury problem in South Florida and the Everglades

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High mercury (Hg) levels were found in Everglades fish and wildlife in the 1980s (as high as 4.4 ppm in largemouth bass, *Micropterus salmoides*, and 110 ppm in the liver of an endangered panther, *Puma concolor coryi*), prompting fish consumption advisories to protect human health. Surveys of fish in coastal waters in 1989 also found high Hg (as high as 3.9 ppm in sharks, prompting the first marine fish advisory, and 4 ppm in king mackerel, *Scomberomorus cavalla*). By the early 2000s, levels had declined in Everglades species: >50% in bass; >70% in great egret (*Ardea alba*) nestlings. These trends were heralded as evidence that emission reductions had succeeded. Yet, "hot spots" remained and, equally troubling, new ones began appearing in the Everglades. Hg remained consistently high in bass at a southern site (1.4 ppm median in 2010) while increasing 20% each year in fish from 1998-05 at a northern site. Where Hg had shown significant downward trends in bass, medians "bottomed out" during the 2000s at 0.5-0.6 ppm then began increasing; up 20% in 2011. Hg increased in panthers in one region in 2007 and in egrets at certain colonies in 2009-10 compared to mid 2000s. A state program to track trends in marine fish revealed declines in Spanish mackerel (*S. maculatus*) in 1996-02 compared to 1990-96. Yet, another study found no decline in

species from Florida Bay in the 2000s compared to 1990s. Hg levels in some individuals of various taxa exceed risk benchmarks for fish and wildlife. Multi-agency research indicates two primary drivers: very high atmospheric Hg deposition, reflecting a combination of high rainfall (30-yr. mean of 131 cm) with high Hg (2009 ann. vol.-wt. mean >12.6 ng/l); and sulfate contamination of Everglades' surface waters (up to 60 mg/l). When these drivers are overlain on the low elevation (mean elevation <2 m) gently sloping landscape, slight changes in topographic relief or water level (from rains or tides) produce dramatic variations in methylmercury production across vast wetland expanses (>9054 km²). Connectivity between ecosystems is a current research focus. The current management strategy includes: 1) advisories to protect human health ("no consumption" for 17 fish species across the Everglades and "limited consumption" for >60 species in coastal waters); 2) developing a statewide total maximum daily load (TMDL) for the freshwater system; 3) assessing the need to manage sulfur in the Everglades; and 4) developing a Gulf of Mexico TMDL.

WE 073

A preliminary assessment of Hg contamination in Lake Chapala, Mexico

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The Lerma-Chapala Basin concentrates about 10% of Mexico's human population. Lake Chapala is the largest tropical lake in Mexico and the ultimate receptor of a great variety of contaminants that are discharged through the basin to the Rio Lerma. In addition to being a major water source for the city of Guadalajara, Chapala Lake represents a major fishery and recreation resource for various communities surrounding the Lake, as well as for tourists from many parts of the country. Chapala Lake is one of the largest wintering areas for American white pelicans (*Pelecanus erythrorhynchos*), a species of special concern in the United States. We are currently conducting an in depth study to assess the impacts of Hg, other metals in general, and persistent organic pollutants on fish and wildlife of lake Chapala. Here we report the results of the analysis of Hg and MeHg concentrations in water, sediments, and three fish species, which are part of the diet of fish-eating birds and also of many human inhabitants in the region. Mean Hg concentrations in water were 0.015 ± 0.002 ng/ml, in sediments = 0.597 ± 0.190 µg/g dry weight, charal (*Chirostoma* spp) = 0.626 ± 0.065 µg/g dw, carp (*Cyprinus carpio*) = 1.597 ± 0.430 µg/g dw, and tilapia (*Oreochromis mozambique*) = 0.176 ± 0.113 µg/g dw. Carp from a nearby reservoir in San Antonio Guaracha, Michoacan had mean Hg levels of 0.396 ± 0.144 µg/g dw. The potential negative effects of these Hg concentrations on fish-eating birds and humans who also eat fish from the lake will be discussed. We will also present data on Hg in feathers from aquatic birds. This project contributes to one of the goals of the Commission for Environmental Cooperation regarding implementation of an action plan to eliminate sources of mercury pollution in North America. It also contributes to one of the seven priorities for action of the UNEP Global mercury partnership project, which is to conduct mercury air transport and fate research.

WE 074

Effect of synthetic organic compounds on the toxicity and uptake of mercury by a unicellular green alga

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Mercury is a widespread contaminant of highly concern for governments due to its biomagnification (as methylmercury - MeHg) along the food chain and its consequent impacts on top consumers, including humans. Phytoplankton is the first entry of Hg into the trophic chain and the major step in the biomagnification process. The knowledge of parameters controlling mercury toxicity and uptake by algae is thus primordial to establish relevant water quality criteria.

In natural waters, algae are exposed to numerous inorganic and organic contaminants, whose mixture can be potentially more toxic than the exposure to one contaminant at a time. Here, we are interested to study the effects of perfluorooctane sulphonate (PFOS), a synthetic surface-active compound, on MeHg toxicity and uptake by unicellular green algae. Our working hypothesis is that PFOS will increase algal membrane permeability, leading to more passive diffusion of MeHg (uptake) through the algal membrane and thus more toxicity for the algae.

To that aim, *Chlamydomonas reinhardtii* is exposed to increasing concentrations of MeHg and PFOS alone and in combination, and examined for its growth and the modes of action of the studied contaminants using a flow cytometer. Modification of membrane permeability and production of reactive oxygen species are both assessed using proper bioimaging dyes.

Uptake of MeHg by *C. reinhardtii* in presence and absence of PFOS are analyzed after short-time exposure to the studied contaminants. Intracellular Hg content is measured by an Advanced Mercury Analyser (AMA 254) after washing algal cells with cysteine. At each treatment, MeHg uptake rates are calculated and compared with each other to determine the effect of PFOS. The results obtained in this study are expected to improve our understanding of mercury-phytoplankton interactions in order to better protect aquatic wildlife.

WE 075

Levels of mercury and methyl mercury in *Larus michaellis* and *Larus audouinii* eggs from different breeding locations in the Iberian Peninsula

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We determined levels of mercury (Hg) and methyl mercury (MeHg) in yellow-legged gull (*Larus michaellis*) eggs from 8 locations in the Iberian Peninsula. These areas represent the most important gull colonies of the area and they are all declared as Special Protection Area for birds. We also analysed eggs from Audouin's gull (*Larus audouinii*), one of the most endangered gulls in the world, in three different areas of the Ebro Delta.

The main aims of this study were to evaluate i) the spatial distribution, according to site dependant anthropogenic pressure, of Hg and MeHg using *Larus michaellis* eggs and ii) differences in metal levels in *Larus michaellis* and the protected species *Larus audouinii* from the Ebro delta, the world largest Audouin breeding colony.

Hg and MeHg concentrations in the yellow-legged gulls ranged between 0.46 - 1.36 mg/Kg dry weight and 0.23-0.69mg/Kg dry weight respectively. Significant differences for both Hg and MeHg were only found between eggs from Chafarinas and the Ebro Delta (a hot spot for Hg contamination). In yellow-legged gulls, MeHg represented between 25% and 70% of the total Hg, which is lower than reported for the eggs of other species. When all individual samples were analysed, we also found a significant inverse correlation between concentrations of Hg and MeHg.

In the three areas of the Ebro Delta, Audouin's gull had significantly higher concentrations of Hg (4.64-5.37 mg/kg dry weight) and MeHg (1.5-3.0 mg/kg dry weight) than yellow-legged gull eggs (Hg 1.3-1.5 mg/kg dry weight; MeHg: 0.60-0.83 mg/kg dry weight) collected in the same locations. These data suggest that the yellow-legged gull, with an omnivorous diet and a scavenger is less subject to accumulation of Hg than Audouin's gull, a specialist epipelagic fish eater. Hg concentrations in eggs of *L. audouinii* from the Ebro Delta are amongst the highest values reported for seabirds. However, a high Hg concentration in Audouin's has not been clearly correlated with hatching failures.

WE 076

Distribution of mercury in water, sediment and fish from the Volta Lake and its major tributaries

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Concentrations of total mercury and methyl mercury were determined in fish and sediment from the waters of the Volta Lake and its main tributaries to understand their distribution in the ecosystem. Total mercury concentrations in fish ranged from 2.11 to 355.16 (mean: 75.64) ng/g wet wt. Methyl mercury concentration ranged from 1.77 to 319.48 (mean: 68.44) ng/g wet wt and accounted for, on the average 89% of the total mercury in the muscles of the fish. Methyl mercury concentrations in fish were directly proportional to total mercury concentrations with average correlation coefficient of $r = 0.98$. The relationship of total mercury and methyl mercury concentrations in fish to those of sediments from corresponding locations was fish-species dependent. Concentrations of total mercury in sediment ranged from 0.96 to 700.25 ng/g dry wt. which is lower than the IAEA threshold of 810ng/g. Water samples from the main tributaries of the Volta and the Volta lake showed total mercury concentrations of < 0.00 to 0.05ng/L. The results of this study indicate that mean total mercury and methylmercury concentrations in fish increase with increase in trophic level of fish. All the fish samples obtained from the Volta and its major tributaries had mercury concentrations below the WHO/FAO recommended limit. The low levels of mercury in the fish analyzed in this study suggest a comparatively clean aquatic environment which has not yet been impacted by mercury contamination.

WE 077

Fish brain as a critical target of mercury exposure: the importance of seasonal factors

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Mercury's (Hg) neurotoxic ability has been strongly recognized in both humans and wildlife, being brain one of the primary targets for its toxic effects. Hg neurotoxicity is widely studied in mammals but not so frequently in fish. Nevertheless, some brain disturbances have been addressed, namely brain sensitivity to Hg induced oxidative stress. In agreement, brain susceptibility to Hg pro-oxidative action was studied by evaluating the antioxidant defences modulation and peroxidative damage in the brain of wild European sea bass (*Dicentrarchus labrax*) during two different year periods (warm and cold). The selected parameters were total glutathione (GSHt) content as non-enzymatic antioxidant, catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) activities as antioxidant enzymes, and lipid peroxidation (LPO) level as a measure of oxidative damage. This research was carried out in two areas with different environmental Hg loads in the Ria de Aveiro lagoon (Portugal): a reference area, and two sites at the Laranjo area (a moderately and a highly contaminated site). *D. labrax* demonstrated a complex profile marked by toxicity signs (as CAT inhibition) and adaptability (as GR induction), indicating different toxicity thresholds, depending on the measured parameter. Moreover, the comparison of the antioxidant responses between year periods showed that the warm period was the most critical with brain's responses evidencing both adaptive mechanisms and signs of toxicity. During the cold period, no alterations in the oxidative stress parameters were observed. Although some signs of toxicity could be evidenced by the depletion of specific antioxidants responses, no lipid peroxidation occurred in any of the studied periods. The different response patterns, according to the year period, also revealed that the environmental conditions influenced the total Hg threshold able to induce or inhibit the antioxidant defences, highlighting the importance of evaluating the fish antioxidant responses in different seasonal periods.

WE 078

MeHg spatial distribution and induced Hg-methylation in settling lacustrine particles of Vidy Bay (Lake Geneva, Switzerland)

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Mercury (Hg) is one of the most hazardous heavy metals usually found in aquatic environments. Methyl mercury (MeHg), one of the toxic organic forms, can bioaccumulate and biomagnify along the trophic chain. Inorganic mercury (IHg) and MeHg have been found in the sediments and settling particles in Lake Geneva (Switzerland). Vidy Bay is the most contaminated part of the lake and receives wastewater discharges from a Sewage Treatment Plant (STP), located in the city of Lausanne. These discharges also make this area rich in bacteria. The highest concentrations of Total Mercury (THg) and MeHg in sediments are located around the STP (1.32 mg/kg and 5.2 µg/kg respectively) as compared to concentrations found further away from the source (0.17 mg/kg and 0.56 µg/kg) and also prior to the STP implementation (0.04 mg/kg for THg). With respect to settling particles, MeHg concentrations on settling particles varies greatly in the water column between the upper (75m below the surface) and lower sections of the bay (1 to 16 µg/kg), without any apparent seasonal patterns.

Methylation is shown to be carried out by sulfate and iron reducing bacteria whose metabolism transforms IHg into MeHg in lake sediments (Garcia-Bravo 2010). Moreover, the fact that MeHg concentrations in the sediment traps is, on occasion, three times higher than in the surface sediments collected close to the STP, implies that high MeHg concentrations in the sediment traps cannot be directly related to the particulate matter released by the STP. Sediment traps, exposed to an antibiotic mixture tested to kill the bacteria living on the collected particles, showed a significant difference in MeHg concentrations as compared to sediment traps without antibiotic exposure, over the same period of time.

Settling particles from the water column were filtered and analysed to rule out any input of MeHg from the water column itself. It is believed that due to the environment created within sediment traps, the growth of bacteria can be promoted, entailing methylation processes more easily than in raw sediments, as implied from a decreasing oxygen gradient observed down the sediment trap tube. In light of these results, MeHg couldn't be used as a tracer to predict the transport of particle-bound pollutants and particle resuspension.

WE 079

Critical Hg concentrations in atmospheric deposition and fish for a sensitive lake: Lake Mergozzo (northern Italy)

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Lake Mergozzo is a small (1.83 km²) basin with an average depth of 45 m characterized by low total P content and alkalinity; i.e., potentially sensitive to acidification and Hg pollution. The drainage basin of Lake Mergozzo has a limited extension (about 10 km²) which, coupled with a very limited discharge of waters into the adjacent Lake Maggiore, makes this system particularly sensitive to Hg atmospheric depositions. Analysis of 30 specimen of perch (*Perca fluviatilis*) resulted in 40% of them having Hg levels above 0.5 mg kg⁻¹, corresponding to the legal limit for fish consumption in Italy. The US EPA limit of 0.3 mg kg⁻¹ limit for protection of human health and aquatic ecosystems was exceeded in 70% of the fishes while all specimen had Hg levels above the UE environmental quality standard for biota of 0.02 mg kg⁻¹. Mercury concentrations in wet atmospheric depositions measured over one year ranged from 0.5 to 52.3 ng L⁻¹, with a weighed average of 12.8 ng L⁻¹ and an estimated annual load of 27.8 g Hg km⁻² yr⁻¹. Critical concentrations of Hg in atmospheric depositions were estimated using models developed by the United Nations Environmental Program and, depending on input parameters, were between 0.1 and 18.3 ng L⁻¹. Measured and critical concentrations being in the same range (and considering Hg concentration in perch), reducing Hg pollution in Lake Mergozzo will require substantial abatements in Hg atmospheric loads to the system.

WE 080

Geochronology of mercury contamination in deep lakes south of the Alps

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The deep, large subalpine lakes (DSL), located south of the Alps between Italy and Switzerland, are regionally important economic and environmental resources. DSL share several common morphological features (e.g., narrow and elongated shapes, steep sides, considerable depths, and most often north-south orientation), although the lithological and petrographical composition of their watersheds differs markedly because of the geological complexity of the Alpine region. Most of the DSL experienced eutrophication during the 20th century and their present trophic status varies from oligotrophy (e.g., Lake Maggiore, thanks to recovery measures) to eutrophy (e.g., Lake Lugano). To assess how regional vs. local factors influence the Hg cycle in the DSL, several sediment cores covering the last 100 to 700 years (depending on core length and sedimentation rates) were collected at suitable locations of DSL. The deepest sections of each core were usually free from human influences (pre-1850) and show that background Hg levels (0.05-0.1 mg kg⁻¹) are similar across the DSL despite differences in watersheds composition. Mercury content in the top-layers of the cores remains above background levels in all DSL and moderate to strong anthropogenic impact is observed not only in Lake Maggiore (for which a well-documented history of Hg pollution exists), but also in lakes Orta and Como. Analysis of the complete Hg profiles is being undertaken and will elucidate how Hg deposition in DSL responds to changes in trophic status and regional climate patterns.

WE 081

Mobility and reactivity of mercury deposition to the litter layer of a Chinese subtropical forest: results from a study including addition of a stable Hg isotope to novel in-situ exchange devices

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A novel "exchange meter" (EM) approach for assessing the fate of mercury deposition to the litter layer of a forest ecosystem has been implemented to a full year study 2010-11, divided into 6 consecutive 2-month sampling periods at Mt. Dinghu biosphere reserve, South China. Briefly, the EM device includes an open cylinder that is arranged so it seals up two layers of Hg passive sampling discs (Pure silver 0.8 µm mesh filter) at the bottom. The applicability of silver discs to trap Hg in precipitation was confirmed in laboratory tests with respect to blank, collection efficiency and long-term stability. Above the Ag layers is a nickel net placed holding natural litter homogeneously spiked with ²⁰²Hg. Five replicate EMs in two clusters were set-up at the field site to represent a clearing and an under canopy plot respectively. For each sampling period and EM, a 15 g sample of freshly prepared litter spiked with 150 ng ²⁰²Hg was applied. Subsequently, the exposed litter and silver discs were replaced and brought back to laboratory for analysis. The samples was analyzed by RA-915⁺ Zeeman Mercury Spectrometer and ICP-MS for total Hg (Hg-tot) and Hg isotope composition (²⁰²Hg) respectively. The Hg flux from litter to run-off water through leaching (F_{run-off}) is assessed specifically by the fraction of ²⁰²Hg spike transferred to the silver discs, while the corresponding volatilization flux from litter to air (F_{vol}) is calculated as the difference between the net loss flux of ²⁰²Hg spike in litter and F_{run-off}. Net deposition flux over the sampling period is in-turn assessed by the initial and final Hg-tot content.

So far the samples collected during 2010 have been processed. The material analysed allow us to draw some conclusions. A significant fraction of the Hg spike added to the litter fall matrix was labile towards reduction to volatile Hg⁰ inferred from the Hg flux to air (F_{vol}-18.5-23.6 ng m⁻² h⁻¹) with maximum values during the November-December period. On the contrary, Hg present in the run-off water was in comparison consistently low (F_{run-off} ~0.8-2.5 ng m⁻² h⁻¹). In general, the effect of a forest canopy on the fluxes was insignificant.

WE 082

Regional scale characterization of mercury contaminated site

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Centuries of mercury mining in the Idrija mercury mine, Slovenia, resulted in elevated levels of Hg compounds in all environmental compartments. Nowadays, significant amounts of Hg from contaminated surfaces in the area are being emitted to the atmosphere while rivers are spreading it towards coastal area in N Adriatic, making the problem of mercury contamination in the Idrija Hg-mine region not only of local but also of regional concern. The distribution and behaviour of mercury in this area in various environmental compartments is relatively well documented. It is agreed that the vulnerability of the ecosystems depends mostly on the transfer of Hg from the geosphere to the biosphere. Thus, formation and bioaccumulation of methylmercury is the most critical point of environmental quality in this mercury contaminated site. However, due to dispersed pollution, an integrated risk-based assessment at regional scale is needed for management purposes. In this contribution environmental impact of historic Hg mining was assessed in the Idrija River catchment draining the area. An integrated approach was used, taking all the environmental compartments and major processes of mercury cycle into account. Mercury data were integrated into Geographic Information System (GIS) and combined with other relevant environmental data. Mercury fluxes between various compartments were established by combining different models (WCS mercury tool and Erosion Potential Model coupled with newly developed GIS emission model) adopted for site-specific conditions. The GIS was employed to spatially model erosion of contaminated soils, surface runoff, atmospheric deposition and mercury emissions from contaminated surfaces. In this way, accounting for Hg entering and leaving the system, its mass flows, flux rates, and turnover times were identified. Moreover, spatial distribution and significance of most polluted sites were assessed by producing pollution-risk maps which might help focus resources in making decisions regarding mitigation and remediation strategies in the area.

WE 083

Appreciating the benefits vs. risk of fish consumption in Italy: Media representation vs. total Hg content in hair and selected fish products

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Translating scientific evidence about health benefits vs. potential risks from fish consumption into information readily understandable by the general public is not an easy

task. The general public usually recognizes the existence of benefits and risks connected with fish consumption, but often has very poor knowledge of the specific benefits and risks, of the contaminants of concern, and of the 'risky' types of fish products. In the case of mercury, scientific knowledge increasingly confirms that, in the absence of local pollution factors, very high Hg content is limited to a few top-predator species. Given this situation, the mass media have the important task to correctly pass on the available scientific information to the public for a correct choice (in terms of species and quantity) of the fish products to be preferred. To evaluate to what extent such 'transmission' is taking place, we assessed how the benefits vs. risk of fish consumption are represented in the two main Italian broadsheets. In parallel, we collected hair samples and information on dietary habits from volunteers residing in various areas of northern Italy. Finally, we compared Hg content in some fish products available on the Italian market and crossed-checked these results with the existence of relevant fish advisories. Our socio-scientific approach shows that further work is needed to translate scientific evidence on Hg-related health risks into an appropriate communication framework.

WE 084

The use of isotopic tracers to study transformation potentials and subcellular localization of Hg species by a sulfate reducing bacteria

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Microbial Hg methylation is a key process in the Hg cycle controlling the MeHg concentrations in the aquatic environments. Despite our improved understanding of the microbiological mediated Hg methylation in the environment and the identification of the involved bacteria, we have only a vague idea of the underlying metabolic mechanisms and of the parameters that control the efficiency of that transformation. Because methylation extent can be controlled by both cellular uptake and reversible demethylation pathways, the use of multiple isotopically labelled Hg species may provide additional mechanistic insights of such processes. In this work, a pure sulfate reducing strain (Strain BerOc1. D. caledoniensis) was incubated in the presence of isotopically labelled mercury species (199Hg(II) and 201MeHg) under controlled anaerobic conditions. The analysis of the exogenous spiked and formed Hg species in the different subcellular fractions (periplasm, cytoplasm, membranes) by GC-ICPMS has allowed to quantify simultaneously methylation and demethylation potentials of the isolated strain, to determine the differential reactivity of the spiked and formed Hg species and to assess their localization at the subcellular level. A second incubation with two other isotopic tracers (198Hg(II) and 202MeHg) was performed on each isolated subcellular fraction in order to determine their potential to transform Hg species. In parallel, Hg species associated to cytoplasmic and extracellular fractions were also investigated by size exclusion chromatography-ICPMS. The results have demonstrated the differential binding capacities of Hg species with biomolecules and may contribute to the elucidation of metabolic pathways involved in Hg species transformations.

RA12P - Health and environmental risk assessment of pesticides and biocidal products

WE 085

Assessment of ecotoxicological combination effects of mixtures of pesticides and biocides

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Pesticidal and biocidal products often contain mixtures of active ingredients and/or mixtures with inert. Moreover, the use of tank mixtures and serial spray applications leads to mixture exposure in the environment. Risk assessment furthermore has to address that mixtures change their composition after release into the environment. This project (funder: German Federal Environmental Agency) assesses the relevance of combined effects from such exposure and develops options of how environmental risk assessment could account for it.

The relevance analysis is based on product analysis and application practices, for which authorisation information are utilised. To identify options for environmental risk assessment a review of current experimental evidence in the analysis and prediction of mixture toxicity has been performed and existing regulatory schemes have been collated.

Various mixtures are found in about one fourth of pesticidal products, with a bias towards herbicides and fungicides. For biocides the picture is less clear; their composition is more diverse and both products and markets are changing with the progression of product authorisation. Ecotoxicity data provided during the authorisation process often deal with individual ingredients only, especially when it comes to chronic or higher tier effect data. It can be demonstrated that components activities and product effects are not necessarily identical. Moreover, other substances than just the active ingredients can be shown to contribute to a products' toxicity. Thus, explicit considerations for combined effects seem necessary.

Empirical evidence shows that combined effects from mixture exposure have often been demonstrated. Two models, concentration addition and independent action, are generally considered valuable to calculate expected mixture responses. Both are based on simple assumptions of non interactive, 'additive' combined effects and their applicability has been investigated in many bioassays and diverse chemical classes. With some noteworthy exemptions they seem suitable for regulatory use. Options for regulatory implementations were investigated and will be discussed. Also, results from a recent workshop on options for inclusion of mixture assessment in the biocide authorisation will be presented.

WE 086

Simultaneous environmental exposure of biocides - potential underestimation in the environmental risk assessment

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According to Article 10(1) of the Biocidal Products Directive 98/8/EC (BPD) concerning the placing of biocidal products on the EU market for the inclusion of active substances in Annex I, IA and IB cumulative effects from the use of biocidal products containing the same active substances should also be taken into account, where relevant. This refers to the risk assessment of an active substance contained in different products of the same Product Type (PT) or of different PTs. Additionally, the Technical Notes for Guidance on Annex I inclusion states that for the first evaluation of the active substance the applicant (in the dossier) and the Competent Authority (in the report) should consider what combination of exposures to the active substance from all the representative uses is realistically possible. Currently, there are no EU-wide harmonised technical guidelines available for such environmental exposure assessments. Therefore, guidelines on data requirements and methodology for conducting cumulative environmental exposure assessments are required. A previous project developed an approach for cumulative environmental exposure assessment: i.e. providing information on applied technical terms, definitions of cumulative and aggregated risk assessments, and technical guidelines in other regulatory fields or in the human exposure. Now a harmonised guidance document shall be elaborated to ensure an unobstructed assessment of cumulative risks from biocidal products throughout the EU. Legal aspects on data requirements, data confidentiality and potential options of the regulators shall be enlightened.

The poster will present the outcome of the discussion on the previous project's proposal for a methodology for conducting cumulative environmental exposure assessments in regulatory context as well as initial findings of the current project and first proposals.

WE 087

Risk mitigation measures for the authorisation of disinfectants - developing guidance for harmonisation

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The European Biocidal Product Directive 98/8/EC requires that biocidal products may only be authorised when they have no unacceptable effects on human or animal health and on the environment. If an unacceptable risk to the environment has been identified during the risk assessment, it may be possible to reduce the risk to acceptable levels by imposing "risk mitigation measures" (RMM). However, at European level a harmonisation of possible RMM is missing. Due to the short deadlines foreseen for mutual recognition of biocidal product authorisations this is a critical point because a harmonisation of RMM is difficult to realise at this stage.

In a preceding research project the RMM proposed by stakeholder were analysed on the example of wood preservatives and insecticides. The efficiency and practicability of these RMM were evaluated (<http://www.uba.de/uba-info-medien-e/4053.html> > <http://www.uba.de/uba-info-medien-e/4053.html>). In the follow-up project, again sponsored by the German Federal Environment Agency, the RMM for disinfectants are currently assessed and appropriate solutions for reduction of identified risks will be presented. The aim is the development of product-type specific "guidance documents", with precise proposals for environmental RMM and a set of standardised RMM-phrases which shall be discussed and harmonised among European Competent Authorities in 2012. The first proposal will be presented and discussed on this poster. It is suggested to distinguish between general RMM and specific RMM. General RMM should reflect the application of best practices, best available techniques, good housekeeping, and general hygiene requirements. Thus, general RMM cover preventive measures which are triggered by the hazards of the ingredients (biocidal active substance and additives) and contribute to a sustainable use of disinfectants. Specific RMM should be risk based and thus derived from the environmental risk assessment. They will be prerequisite for the authorisation of biocidal products with identified unacceptable risks for environmental compartments or biota. Nevertheless, reasonable exemptions could be laid down to reflect special national requirements.

WE 088

Toxicity of polyDADMAC, KCl and alumina to *Corbicula fluminea* and non-target aquatic species

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Invasive bivalves such as the Asian clam, *Corbicula fluminea*, have been widely recognised as an ecological problem and are also responsible for damaging impacts on

freshwater-dependent industries due to their biofouling activity. Depending on the way industries interact with freshwater ecosystems, the chemicals currently available for biofouling control may have high toxicity to non-target species, and hence improved methods with enhanced environmental acceptance are required. This rationale triggered the integrated approach followed in the present study. Three chemicals particularly suitable for use as control agents in the highly regulated drinking water treatment industry were tested: (i) the cationic polymer polydiallyldimethylammonium chloride (polyDADMAC), (ii) potassium chloride and (iii) alumina. In order to determine the molluscicidal activity of these chemicals mortality rates amongst adult Asian clams were assessed following long-term exposure. As a second stage of the study, the environmental toxicity of the chemicals was addressed using the 72-h growth inhibition of the planktonic microalgae *Pseudokirchneriella subcapitata* and the 48-h immobilisation rate of the cladoceran zooplankton *Daphnia magna* as indicative endpoints. The results showed that polyDADMAC was the most efficient biocide against the Asian clam. At 2 and 10 ppm the polymer produced 100% mortality after 840 h and 552 h of exposure, with LT_{50} values of 497 h and 284 h, respectively. Exposures to KCl and alumina lasted for more than 1400 h, but 100% of mortality could not be achieved; estimated LT_{50} for these chemicals were higher than those estimated for polyDADMAC. As for the environmental selectivity of the biocides, polyDADMAC has shown the highest toxicity to *D. magna* with a 48-h EC_{50} of 12 ppm, followed by alumina and KCl. The same order in toxicity was observed for *P. subcapitata*, where a polyDADMAC 72-h EC_{50} of 0.2 ppm was recorded. In summary, polyDADMAC is the most efficient biocide against *C. fluminea* while KCl is the least efficient. Although polyDADMAC presented a relatively higher environmental toxicity, it is already used in waterworks in various operation stages within concentration ranges that may eventually be adjusted to ensure environmental safety, hence providing higher versatility and cost-efficiency as a potential biofouling control agent as compared with the other chemicals tested.

WE 089

Contribution of organic tin compounds to total tin levels in house dust samples from Portugal

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Organotin (OTs) belong to the most widely used organometallic compounds family with an annual production over 60 000 tons. Applications range from agriculture, marine activities and industry in products such as pesticides, bactericides, fungicides, wood preservatives, PVC stabilizers, catalysts, surface disinfectants, laundry sanitizers, among many others. Despite numerous applications it was the use of TBT (tributyltin) as a biocide in antifouling (AF) paints that accounted for their notoriety. TBT is a potent endocrine disruptor, also exhibiting immunotoxic and genotoxic capabilities towards a widespread variety of organisms. The vast amount of data on TBT environmental toxicity and persistence lead to the introduction of restrictive measures that culminated in September 2008 with the entering into force of a TBT ban worldwide. Even though organotins were banned from AF paints, they are ubiquitous in our society since many consumer products and household items have OTs in their composition. Upon degradation those consumer products will contribute to the total tin budget in the environment, being house dust a major reservoir and concentrator of such substances. Together with food, house dust has been considered an important exposure pathway for humans; however, data for this matrix is scarce. The present work describes the levels of monobutyltin (MBT), dibutyltin (DBT), tributyltin (TBT), diphenyltin (DPT), triphenyltin (TPT), mono-octyltin (MOT), dioctyltin (DOT) and also total tin (Sn) in house dust samples collected from twenty six private homes from Aveiro and Coimbra cities during 2010 and 2011. OTs, quantified by GC-MS, were detected in all analyzed samples with highly variable values ranging from 580 to 6100 ng Sn.g⁻¹ dry weight (dw). MBT, DBT, MOT and DOT were detected in all the dust samples and TBT was detected in 92% of the samples analyzed. DPT and TPT were not detected in any of the samples. The major contributor to total organotin levels (ΣOTs) was MBT (44%) followed by MOT (27%). The contribution of organic tin compounds to the total tin levels in dust was evaluated for the first time and the obtained results disclosed a huge variation between samples with levels ranging from 1% to 35% (ΣOTs/Total Sn). Possible sources of OTs are proposed and daily intake values are calculated in order to access the risk for both children and adults.

WE 090

Ecotoxicity of leachates from wood materials treated with wood preservatives

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Wood materials are often used for outdoor constructions. It is expected that with growing demand for natural and renewable materials their usage will even increase in the future. In order to protect the wooden outdoor constructions biocides (wood preservatives) are applied, and if they are released to the environment with run-off waters, this could be considered as a source of environmental pollution with highly toxic substances. Due to the high toxicity, some of the historic wooden preservatives are banned or heavily restricted e.g. creosote, pentachlorophenol. There are several possibilities to reduce the environmental pollution coming from outdoor wooden constructions, e.g., by using wood preservatives with less toxic chemicals as components where possible, by reducing contact with water, or by achieving stronger binding of the toxic substances to the wooden material. The precondition for all these actions is proper information about the presence and concentrations of toxic substances in the composition of wood preservatives.

The aim of the present research was to detect the extent of releases of toxic substances from wood materials treated with wood preservatives and to explore efficiency of paint in reduction of leaching of toxic substances. Leachates obtained from painted and unpainted wood materials treated with three different wood preservatives were tested by *Daphnia magna* ecotoxicity test (LVS EN ISO 6341:1996). All three of the researched wood preservatives were water based and targeted to protect wooden constructions in outdoor conditions. The information about the products applied was crosschecked and verified with various information sources including further testing. Ecotoxicity test results showed that all tested leachates of wood material treated with wood preservative characterizes with high toxicity to *D. magna*. Treatment of the preserved wood with paint reduces toxicity of leachates justifying painting as efficient measure to reduce leaching of toxic substances.

This study was performed in the co-operation among ESF projects 2009/0226/1DP/1.1.1.2.0/09/APIA/VIAA/080 and 2009/0144/1DP/1.1.2.1.2/09/APIA/VIAA/005.

WE 091

Biocides from façade coatings in storm water in a residential catchment area

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Houses are increasingly equipped with thermal insulation to increase the energy efficiency of heating. The plasters of these systems are often equipped with biocides - from these surfaces the respective compounds may be washed off. As rain water run-off is directly discharged into the aquatic environment via separated sewer systems, storm water overflow and rain water infiltration, discharge of storm water run-off need consideration.

Within this study the storm water run-off in a well described residential catchment area in Silkeborg (Denmark) was analyzed during a long term study in order to investigate the relevance of leaching from "natural" urbanised areas. No new buildings or freshly treated surfaces were identified in a special assessment. The samples were taken as time resolved samples from a separated sewer system, and analysed for terbutryn, cybutryn, tebuconazole, propiconazole, isoproturon, diuron, carbendazim, iodicarb, methylisothiazolinone, benzoisothiazolinone, octylisothiazolinone, dichloroisothiazolinone, mecoprop, as well as atrazine and dichlorobenzamide. The data were analysed in respect of concentrations and mass flows. Both concentrations and mass flows were very dynamic. The concentrations reached up to 300 ng/L while the mass transports reached up to a few 1000 ng/s or 35 mg/event (e.g. terbutryn, carbendazim). At least two different release mechanisms are obvious for different compounds. One might be a (first flush) wash off process of surfaces, while the other one undergoes a soil passage.

WE 092

Pesticide levels in vegetables and related health risk assessment in Central Amazon - Brazil

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Food consumption is an important route of human exposure to pesticides. In the Brazilian Amazon, intensive and incorrect use of pesticides in floodplain areas, where fresh vegetables are produced for regional urban markets is a common practice. As a consequence of that, it is possible that pesticide residues on vegetables are above of the maximum residue limit (MRL) posing health risk to consumers. Thus, the aim of this study was to assess the level of pesticide residues (deltamethrin, lambda-cyhalothrin, malathion and methyl-parathion) on vegetables cultivated in rural areas near Manaus and marketed in this city and to estimate the health risk resulting from long term and short term dietary exposure. A total of 190 fresh vegetables composite samples were collected, being 100 pre-harvest samples at plantation areas 45 post-harvest samples were collected from major markets and 45 post-harvest samples were obtained at supermarkets. The pesticide residue levels in vegetables were analyzed by headspace solid phase microextraction (HS-SPME) and gas chromatography with electron capture detection (GC-ECD). Detectable residues were found in 88 to 95% of samples, with 32% exceeding the maximum residue limits (MRL). Post-harvest samples had higher pesticide concentrations than pre-harvest samples. Health risk assessment indicated that deltamethrin, malathion and lambda cyhalothrin did not pose health risk for short and long term exposure, and parathion methyl poses chronic risk to human health. The assessment undertaken indicates a potentially high risk and represents an important step toward a more comprehensive understanding and evaluation of human health risks associated with pesticide exposure in developing countries.

WE 093

Effects of chronic exposure regimes of the fungicide azoxystrobin in outdoor freshwater microcosms

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This study focused on ecological effects of a range of chronic exposure regimes of the fungicide azoxystrobin. Azoxystrobin was selected for this study since information concerning effects of long-term chronic exposure on the structure and functioning of aquatic ecosystems is limited, especially for fungicides. The experimental site included 19 outdoor microcosms. The water volume of each microcosm was approximately 1270 liters (water depth: 0.5 m; sediment layer: 8 cm). Treatments were studied in triplicate, the controls included 4 systems. Test concentrations of azoxystrobin were 0 - 0.33 - 1 - 3.3 - 10 - 33 µg/L and were kept at a constant level between 80 and 120 % of intended nominal concentrations during the experimental period of 42 days. Responses of macrophytes, macroinvertebrates, phytoplankton and zooplankton were studied. Also decomposition of particulate organic matter was monitored.

Over the 42-d experimental period the analytically determined time-weighted average concentrations of azoxystrobin ranged from 93.5 to 99.3 % of intended time-weighted average concentrations. The study showed that zooplankton and especially copepods and Daphnia group longispina were the most sensitive groups for a chronic exposure of azoxystrobin.

Macrophytes did not show significant treatment-related effects as also was the case for macroinvertebrates, phytoplankton, decomposition of organic matter and water quality parameters. Based on the responses of the copepod population a consistent NOEC was calculated at the 1 µg/L-treatment level. At the zooplankton community level at concentrations up to and including 10 µg a.i./L no consistent treatment-related effects were found. Most treatment-related effects observed were not followed by recovery since the chronic exposure regime was maintained until the end of the experiment. The chronic threshold value was approximately a factor of 10 lower than was found for a single application of azoxystrobin in an outdoor microcosm study (CTgB, www.ctgb.agro.nl). The safe threshold values for chronic exposures at the population and community level suggest that the first-tier value for chronic exposures ($44 \mu\text{g/L NOEC for Daphnia magna}/10$) = 4.4 µg/L is not protective for chronic effects at the population and community level.

WE 094

Highest tier? EPAT analysis in aquatic risk assessment of plant protection products

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For standard risk assessment conducted during the EU review of plant protection products (PPP), the concentration of active substances in water bodies adjacent to a single field is calculated using the surface water model FOCUS SWASH. For higher tier assessment, micro- and mesocosm studies or probabilistic approaches (e.g. SSD - Species sensitivity distribution) can be used. On the exposure side more realistic PEC calculations by means of mitigation measures such as buffer zones or drift reducing nozzles or the use of PEC_{low} values for chronic endpoints may considerably reduce the risk. However, for the FOCUS D scenarios - in particular for D2 which has to be considered in winter cereals and winter oilseed rape - only limited mitigation measures are available as drainage is the main entry path. In such cases, an analysis of the exposure profile in water and sediment may give valuable information for a successful estimation of risks to aquatic organisms.

For that reason the exposure pattern analysis tool EPAT was used to characterise the exposure pattern of various active substances in surface water. The main focus was set on specific compounds that enter the aquatic environment via drainage. By this, potential uses of EPAT as a higher tier refinement tool for acute and chronic risk assessment is presented and critically discussed.

WE 095

Integrative approach for the risk assessment of freshwater reservoirs influenced by intensive agricultural activities: a case-study of Alqueva reservoir (South of Portugal)

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Freshwater reservoirs located in intensive agricultural areas are more vulnerable to chemical 'stressors', such as pesticides. So, the characterization of these water bodies for prevalent pesticides is extremely important, once most of these compounds are used in an indiscriminate way by farmers and induce toxic effects in species of aquatic ecosystems and benthic communities.

The purpose of this study was to evaluate the dynamic of pesticides and its correlation with the toxicity values obtained in the Alqueva reservoir, an important source of water irrigation and supply (South of Portugal).

Water and sediments samples from the Alqueva reservoir were analysed considering: the chemical profile (pH, temperature, dissolved oxygen, conductivity, chloride, calcium, magnesium, sodium, phosphorus, Kjeldahl nitrogen, ammonia, nitrite, nitrate, biochemical oxygen demand, organic matter and trace elements), the pesticides analysis by on-line solid phase extraction-liquid chromatography-electrospray-tandem mass spectrometry (online SPE-LC-ESI-MS/MS), and the ecotoxicological effects (luminescence inhibition of *Vibrio fischeri*, 24-h mortality test with *Thamnocephalus platyurus*, 48-h immobilization/mortality assay of *Daphnia magna*; 6-day mortality/ growth inhibition of the crustacean *Heterocypris incongruens*).

The pesticides more frequently detected in the water column were atrazine, bentazone, MCPA, mecoprop, metolachlor and terbuthylazine, and in sediments diuron, terbuthylazine and chlortoluron. The highest levels of these pesticides were quantified in the water column. The areas (sampling stations) most polluted by pesticides were Sra. Ajuda, Lucefécit and Álamos, located in the northern and in the middle of the reservoir, respectively. Considering the toxicological results we can conclude that the species most sensitive to the environmental samples from the Alqueva reservoir were the bacteria *Vibrio fischeri* and the crustacean *Heterocypris incongruens*. Further, the results showed that some locations at the Alqueva reservoir, namely, Alcarrache, Lucefécit and Álamos induce acute and chronic toxic effect in the bioindicators used. Taking into consideration the pesticides concentrations found and the toxic effects observed in each location of the Alqueva reservoir, we can establish a possible relationship between this environmental risk factor and the observed stress in the bioindicators used.

WE 096

Reducing fish use in bioconcentration studies for plant protection products

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Fish bioconcentration studies assist in determining the potential for substances to bioaccumulate. This is used for Persistence, Bioaccumulation and Toxicity (PBT) and secondary poisoning assessments. International data requirements for plant protection products include triggers for determining bioconcentration factor (BCF) values, generally where bioconcentration might be expected, e.g. for substances with an octanol-water partition coefficient ($\log K_{ow}$) > 3 and that are stable in water. Bioconcentration tests are time consuming, expensive, and use large numbers of animals (a minimum of 108 per study). Alternative methods that replace, reduce or refine the use of fish for BCF testing would therefore be of value in improving efficiency, reducing costs and supporting animal welfare considerations. Currently the OECD Test Guideline for BCF testing (TG 305) is being revised to include the possibility of reducing the cost and number of fish used, when this can be done without compromising the BCF determination. One potential modification that would substantially reduce fish use is to use only one exposure concentration, instead of the two currently required. Analysis of 55 studies on plant protection product active substances demonstrates that BCF values for whole body, edible and non-edible tissues do not significantly differ between these low and high exposure concentrations. One exposure concentration could therefore be employed for BCF testing of many substances, reducing fish use by one third. We recommend this modification is included in the upcoming revisions of OECD TG 305.

WE 097

Setting up of validated procedures for the monitoring of the non-intentional effects of the control methods on man and the environment

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The control of noxious or vector mosquitoes (Diptera-Culicidae) is an activity for which the socio-economic, health and environmental stakes are high. In France, given the importance of the surfaces concerned, this public health mission was entrusted to specialised public operators. Recent events, such as chikungunya epidemic in La Réunion, the spread of introduced tropical *Aedes albopictus* in Europe, causing the first chikungunya epidemic in Italy, have led the national and European authorities to become well aware of the increased need for the evaluation of the efficacy, and the health and environmental risks pertaining to the current control strategies and to have at their disposal decision-making tools in these domains. In such a context, a Life+ project, Control of noxious or vector mosquitoes: implementation of integrated management consistent with sustainable development (IMCM LIFE ENV/F/00488) was proposed in order to demonstrate integrated methodologies and techniques applied to Integrated Pest Management modified for mosquito control. This project involves five public operators in Metropolitan France (EID Méditerranée, EID Rhône-Alpes, General Council of Southern Corsica) and overseas (General Councils of Martinique and Guyana). One of the main objectives of the IMCM project is to evaluate the non-intentional effects on Man and the environment that can result from these control methods. This task proposes the application of complementary methods for environmental risk assessment (in aquatic and terrestrial compartments) of control methods and for biocide exposure risk assessment for applicators under real conditions, using different simulations of control treatments in temperate or tropical zones. Ultimately, these procedures will be fully integrated into a sustainable approach to environmental monitoring (observatory). The methodologies applied have been validated during previous collaborations.

WE 098

Pesticide residue dynamics in passion fruits grown in Colombia: field trials, modelling, and human health risk assessment

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To evaluate the exposure of pesticides from the cultivation of passion fruits and subsequent human health risks, we combine several methods: (i) experimental field studies including the determination of pesticide residues in/on passion fruits, (ii) dynamic plant uptake modeling, and (iii) concepts of human health risk assessment. Eight pesticides (azoxystrobin, difenconazole, mancozeb, tebuconazole, deltamethrin, imidacloprid, thiacloprid and thiamethoxam) were applied to passion fruit plants cultivated in the region of Cali, Colombia. Pesticide concentrations were measured periodically in passion fruit samples from the time of application until harvest. Residues in both whole fruits and fruit pulp only were analysed using gas- and liquid chromatography. Measured concentrations were compared with predicted residues calculated by using a dynamic and plant-specific pesticide uptake model, namely dynamiCROP. The model takes into account the time between pesticide application and crop harvest, the time between harvest and final consumption, the amount of spray deposition on plant surface, uptake processes, dilution due to crop growth, metabolism in plant material, and loss due to food

processing (peeling). Measured and modelled residues correspond very well ($r^2=0.88-0.99$), with all predictions falling within the 90% confidence interval of the measured values. A mean error of 43% over all studied pesticides was observed between model estimates and measurements during the complete sampling period. The fraction of pesticide applied on the field during plant cultivation that eventually is ingested by the consumer is on average $10^{-4}-10^{-6}$, depending on the overall time between pesticide application and ingestion and the processing step considered. Dynamic model calculations and intake fractions via passion fruit consumption based on experimental data corresponded well for all studied pesticides deviating less than a factor 1.9 from each other. Pesticide concentration in fruits measured at harvest time were all below the European MRL and the MRL set by the Codex Alimentarius, and therefore does not indicate any violation of international regulatory thresholds.

WE 099

Risk of pesticides in the air for organic farming

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Over 750 million olive trees are cultivated worldwide, about 95% of those are in the Mediterranean region. Turkey accounted for over 9% of world production and is 4th biggest producer in the world. Over 24% of olive oil production comes from Province Aydin in Turkey. Pesticides are not or seldom used in olive trees, so Province Aydin is very suitable for organic olive oil production but passive accumulation of pesticides via short and long range transport of pesticide poses risk for olive trees and organic farming. The olive gardens in province Aydin were divided to 150 zone by help of GIS and the samples were collected in 2007 and 2008. The leaves were extracted and analysed by GC/MS/MS. The results showed that 19.33 % of olive gardens was found as unsuitable for organic farming, although 80.67% of this area was suitable for organic farming process. A map was created to ensure possible organic farming gardens in the province of Aydin.

WE 100

Multiple contaminations of organochlorine pesticides and heavy metals around the river region of Daye Smelter, China

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Numerous researches have been conducted on heavy metals contamination around the river region of the Daye Smelter in China. This study is showing multiple contaminations of organochlorine pesticides (OCPs) and heavy metals may occur in this region. Soil, vegetable, river water and sediment samples of the irrigation system around the Daye Smelter were collected for analyzing OCPs and heavy metals (As, Cd, Hg, Pb) in 2009. It was found that HCHs, DDTs, CHLs were the main OCPs residual compounds. The concentrations of HCHs were higher than DDTs in most vegetable, soil and water samples. Composition analysis indicated that lindane (CHCH) and dicofol-DDT may be input in this region recently. Relatively high concentrations of heavy metals were also found in the region, and their concentrations were generally higher in the Donggang River than in the Xigang River. Local residents may suffer from the health risk because of the multiple contaminations.

WE 101

Using an ecosystem services approach to enable pragmatic environmental decision-making: a case study on citrus production and insecticide use

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The findings of this pioneering proof-of-concept study delivered a case study to inform discussions between scientists and policy makers in pesticide regulation where an ecosystem services approach can inform risk management decisions.

An ecosystem services framework has been developed and is applied to a key insecticide for use in citrus growing in southern Spain. For more than 40 years, farmers in the region have relied upon an organophosphate insecticide as an important component of a pest control management strategy. The use of the pesticide limits the occurrence of blemishes on the surface of citrus fruit caused by red scale (*Aonidiella aurantii*), greatly affecting the marketability of the produce and the income earned by farmers at harvest time.

A spatial model was developed to evaluate the use of the insecticide in three citrus orchards in the Valencia Region. Specifically, the results of the evaluation were used to determine: (a) changes in ecosystem services in the absence of insecticide use; (b) the influence of other management actions on ecosystem services arising from the discontinued use of the insecticide; (c) the effect on wildlife posed by the discontinued use of the insecticide; and (d) the possible temporal profile of changes in ecosystem services over a 50 year period such that strategies might be developed to minimise changes and enhance or preserve current ecosystem services and functions for future generations.

Comparison of the scenarios against the baseline condition indicates the importance of the insecticide to commercial citrus production. In the event the use of the OP insecticide is discontinued, some ecosystem services and income are projected to decline. If use continues and potential impacts are offset with a mitigation action such as a vegetated conservation area, ecological services are projected to increase compared to baseline conditions, while preserving income and both amenity and carbon sequestration values.

Future directions for the project are defined.

RA14P - Landscape ecotoxicology and spatially explicit risk assessment

WE 104

Ecotoxicology and macroecology - time for integration

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Despite considerable progress in ecotoxicology, it has become clear that this discipline cannot answer its central questions, such as, "What are the effects of toxicants on biodiversity?" and "How the ecosystem functions and services are affected by the toxicants?". We argue that if such questions are to be answered, a paradigm shift is needed. The current bottom-up approach of ecotoxicology that implies the use of small-scale experiments to predict effects on the entire ecosystems and landscapes should be merged with a top-down macroecological approach that is directly focused on ecological effects at large spatial scales and consider ecological systems as integral entities. Analysis of the existing methods in ecotoxicology, ecology, and environmental chemistry shows that such integration is currently possible. Therefore, we conclude that to tackle the current pressing challenges, ecotoxicology has to progress using both the bottom-up and top-down approaches, similar to digging a tunnel from both ends at once.

WE 105

Effects of landscape heterogeneity and sub-lethal pesticide exposure on dispersal of *Asellus aquaticus*

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Different testing strategies are applied for the ecological risk assessment (ERA) for pesticides to understand potential environmental side effects of pesticide application and, also recovery there from. Rarely, however, do testing regimes account explicitly for the impact of animal movement and its relevance for recovery from adverse effects.

In the case of *Asellus aquaticus*, and other aquatic macroinvertebrates (e.g. *Gammarus pulex*), recovery by immigration of individuals from uncontaminated sites is an important factor for re-establishing population densities after pesticide exposure. However, not much information on movement patterns of aquatic macroinvertebrates is available nor which factors influence their dispersal in which way.

Therefore, we performed video tracking experiments to derive information on movement behaviour of individual *A. aquaticus* under different conditions. We found that differences in preferred turning angle range, resting times, and movement velocity were exhibited under different testing regimes. Especially, exposure to different concentration levels of the single pesticides showed levelled effects on overall animal activity; resting times increased with increased exposure concentration and directionality of movement paths was found to decrease at the same time. The findings imply that currently applied techniques could lead to unrealistic estimations of recovery timeframes if environmental heterogeneity and/or pesticide residue effects are not accounted for.

An individual-based model, MASTEP (Metapopulation model for Assessing Spatial and Temporal Effects of Pesticides), has been developed at Alterra (Wageningen, The Netherlands) and is used to estimate the combined allogenic and autogenic recovery of *Asellus aquaticus* after exposure to an insecticide. This model will be used to investigate how our experimental findings translate to larger time and spatial scales when extrapolated to the landscape level. We will study in particular how environmental factors influence dispersal rates and thus time to population recovery after pesticide exposure.

WE 106

Does water network structure matter for the simulated recoveries of aquatic macroinvertebrate populations after pesticide stress?

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Little is known about the influence of spatial heterogeneity on the ecological risks of chemicals. Population effect models are interesting tools to be used in this question because they provide a cheap and fast, though simplified method of assessing large-scale effects. Individual-based population effect simulation models such as MASTEP are spatially explicit, but their spatial environment consists of a single linear circular connected waterbody. This study aims to evaluate if and how different levels of spatial complexity influence the impacts of hypothetical pesticides on populations of aquatic macroinvertebrates. The MASTEP population model for *Asellus aquaticus* was used and combined with the spatial structure of a typical Dutch ditch network. For each of the included water bodies, concentration time curves were calculated. A local MASTEP model was connected to each of the sections of the water body network. Exposure concentrations were linked with effects by calculating daily mortalities of individuals

following a stochastic death approach. Different scenarios were simulated in 10 replicates each, and the recovery times have been calculated as the number of days after the input of the pesticide, that population abundances in treated scenarios need to reach 95% of the level of the abundances in respective control simulations. Conclusions from a series of simulations show, that the influence of the water network complexity and of spatially explicit vs. non-explicit settings obviously depends on the toxicity of the compound. This is quite well understood, when the mechanisms of recovery are taken into account. Recovery can occur via i) recolonisation from unaffected ditches and ii) local regrowth of survivors. High toxicities lead to “wipe-out” events in the downstream section, so local regrowth only plays a minor role in the recovery. In this case the connectivity of the affected sections of the water network to unaffected parts is decisive for recovery. The lower the toxicity is, the more is local regrowth contributing to recovery and hence the spatial structure is less important. The consideration of spatially explicit settings lead to lower recovery times for compounds with high toxicities. A lower number of unaffected influents lead also in a spatially explicit setting to lower recovery times. It remains to be analysed how in a spatially explicit setting multiple applications in space and time influence the recovery times.

WE 107

Spatial temporal exposure assessment in networks of small surface water bodies

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In Germany the dominant surface water types near agricultural fields are small streams and ditches. These water bodies are especially vulnerable to pesticides.

In this work we present a prediction model for the pesticide exposure in networks of small surface water bodies. The tool can be used to analyse pesticide exposure patterns and indicate water segments of potential concern. The tool is configurable to develop and simulate management strategies which avoid the pesticide pollution of surface water bodies.

For the computational analysis we established a discrete and topographic node network with an resolution of 2.5 meter per segment. The modeled nodes are derived from the natural river flow via spatial data bases. Each node has a degree of ingoing and outgoing arcs which route the incoming and outgoing water stream from and to the next node. Further characteristics for river sections like width and depth of a stream and average flow velocity are calculated and can be configured in the network data model.

Every single node can be assigned with load information which consist of loading time, the specific substance and the loaded mass. The initial load information is assessed by a probabilistic approach for drift and a run-off model which was linked to a GIS evaluation on entrance points and run-off pathways from critical source areas. The load is routed through the modeled network in dependence of the average flow velocity. For each segment in the network the concentration level will decrease and the length of the pesticide wave in flow direction will increase. This behavior is simulated by calculating the longitudinal dispersion. For each node the resulting concentration of a substance is summarized over all passing concentration levels.

For three catchments in North-Rhine-Westphalia the time varying concentration was assessed and analyzed over a complete vegetation period on segment level considering the realistic pesticide input form surveyed application strategies. Based on such results it is possible to analyze and compare different scenarios of risk management as for example the implementation of fixed non crop zones or wetlands.

WE 108

A new approach to landscape representation in agent-based models for pesticide risk assessment

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In pesticide risk assessment there is an increased need to model processes in which spatial structure has crucial importance. Among them are those related to dynamics of chemicals in the environment - the domain of fate modelling, but also ecology and behaviour of animals especially important for population modelling. The majority of spatially explicit population models and, to our knowledge, all agent-based population models use a single approach to represent space - a grid. When landscape is represented as a grid it is split into multiple cells of the same size and shape (often square or hexagonal), each changing its state independently. However, in certain cases this approach is not optimal. It may be difficult to tell what size of cell is sensible for the model purpose and best for incorporating available data on species ecology. Therefore we have developed an alternative approach to space modelling based on field-scale space units.

We have developed a spatially explicit, agent-based modelling framework SkyPig for modelling bird populations. Currently SkyPig incorporates two species: skylark and woodpigeon. The purpose of both models is risk assessment of pesticides in higher tiers. In this study we focus on modelling exposure using the skylark model.

The most of the ecological and agricultural data relevant for bird population models describes no variation within fields, which have various sizes. Therefore we modelled the natural landscape structures like agricultural fields as the basic units of landscape. They are implemented as separate objects characterised by their variables and a graph of geometrical relations derived directly from GIS maps. This approach solves the problem of choosing a scale in which the data on birds foraging or nesting and crops sown may be best implemented. It also enables implementation of processes reflecting chemicals dynamics, such as pesticide application or decay. However, there are processes that apparently require finer scale, e.g. spray drift during pesticide application. A number of such processes will be discussed, and for spray drift we will present a case study to show how our approach to landscape modelling can cope with such processes.

The conclusion we reach is that our approach is very useful when reducing the data requirements of a model is desirable and that it can effectively handle processes of finer scale as well. We also suggest how this method can be effectively applied.

WE 109

Conservation biological control at the landscape scale - limits set by landscape grain and pesticide use?

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Suppression of pests by their natural enemies is considered a valuable ecosystem service, provided mostly by non-crop elements in the agricultural landscape. Conservation biological control (CBC) at the landscape scale refers to the management of the landscape in such a way that effectiveness of natural enemies is enhanced. To be effective, non-crop elements (field edges, road verges, hedgerows, ditches and other small landscape elements) have to be present in a large enough proportion, and in a spatial configuration that both promotes natural enemy persistence at the metapopulation level (e.g. by re-colonization), and guarantees the delivery of the service where it is needed (the fields).

The importance of spatial relationships at a landscape scale has been established in a large number of recent studies. Grain or mesh size of the agricultural landscape mosaic sets the amount of non-crop elements, the distance between them and the proportion of crop fields potentially benefiting from CBC. It is possible that a maximum mesh size exists above which no landscape-wide natural suppression of pests is attainable.

In a landscape with sufficient biological control, the amount of pesticides used can be reduced. However, the use of pesticides itself has a direct negative impact on potential CBC. Spraying reduces beneficial arthropod populations in the fields, but also, through spray drift, in non-crop habitats near field edges. Thus, a positive feedback exists between pesticide use and the need to spray, that is mediated by farmer and public opinion. In a fine-grained landscape mosaic where CBC may be successful, its potential will not be realized when most of the farmers spray preventively. The system, including the farmers, may have different stable states (in terms of fraction of fields sprayed) depending on landscape structure and the farmer's willingness to spray only curatively (or not at all).

We analyse the system using a socio-ecological landscape-scale model involving farmer behaviour, applying a kernel-density approach to quantify biological control exerted by non-crop elements, and spray drift equations and dose-response relationships to quantify impact of spraying on off-crop habitat. In the analysis we focus on the potential consequences of going from formerly fine-grained to modern coarse-grained agricultural landscapes, and on the role of farmer behaviour in the desired transition towards landscapes with more effective biological control.

WE 110

Are body condition and somatic indices related to trace elements accumulation in small mammals?

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Body condition and somatic indices are widely used in ecology as estimators of wild animal health status, because they are hypothesized to be representatives of individual health and fitness. The potential confounding factors influencing these indices, when used as in situ markers of toxicity, are however poorly known. The aims of this study were then (1) to assess whether individual (age and gender) and environmental (landscape) variables influence body condition index and somatic indices and may thus act as confounding variables for the interpretation of indices in an ecotoxicological framework, and (2) to investigate the relationships between these indices and trace metal (TMs) concentrations in small mammals. In autumn 2006, 859 wood mice (*Apodemus sylvaticus*) were trapped along a soil pollution gradient (3 pollution levels), in four different landscape types (agricultural land, shrubland, urban area and woodland) around the former smelter of “Metaleurop Nord” (Northern France). Concentrations of lead (Pb) and cadmium (Cd) were measured in liver and kidneys. Scaled-mass body condition index (SMI) and somatic indices (scaled liver index SLI and scaled kidney index SKI) were computed. High levels of TMs were found in small mammals, indicating both an important transfer in food webs and a potential risk for these organisms. Relationships between indices and individual variables showed that the SMI varied according to both age and gender ($p < 0.05$, partial R^2 of 0.08 and 0.02%, respectively). SLI and SKI were influenced only by age ($p < 0.05$, partial R^2 of 0.06 and 0.23, respectively). Landscape, which has only been scarcely studied previously in ecotoxicology, significantly influenced the indices, individuals living in woodlands and agricultural lands exhibiting significantly higher SMI and lower SLI and SKI compared to those living in shrublands. After taking into account those confounding factors (relevant individual variables and landscape), we failed to detect any influence of TMs on body condition and somatic indices. Those results suggest that the use of these indices as toxicity markers may lead to misinterpretation if not used with caution. Further studies are still needed to better identify and quantify the role of ecological factors such as habitat or stresses (parasitism, predation, etc.) on those indices, and to conclude about body condition and somatic indices relevance in ecotoxicology, alone or combined to other toxicity markers.

WE 111

Exposure and ecological risk assessment of herbicides in Japan: risk calculation approaches focusing on space- and time varying herbicide exposure

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Concentrations of herbicides used in paddy rice fields exhibit large spatial and temporal variations in Japanese rivers. Several models can treat space and/or temporal variabilities in exposure and effect assessments. For exposure assessment, a GIS-based multimedia modeling system (Grid-Catchment Integrated Environmental Modeling System; G-CIEMS) has been developed for predicting spatiotemporal dynamics of chemical concentrations in Japanese rivers. For effect assessment, Dynamic Energy Budget toxicity (DEBtox) models have been developed as tools that connect temporal change in exposure concentrations with effects on organisms. In this study, we developed an

integrated ecological risk assessment (ERA) approach that combines the output of G-CIEMS and DEBtox model simulations. The applications of the approach to preliminary ERAs of five major paddy herbicides (pretilachlor, butachlor, simetryn, esprocarb and mefenacet) in Japan were also conducted. First, risk calculations focusing on spatial variabilities in exposure data conducted by creating ecological risk maps. Hazard quotient (HQ; HQ = maximum concentration at the site / EC50) was used as a risk index and the values of HQ values were graphically presented on GIS-based maps. Second, risk calculations focusing on temporal variability were conducted by using DEB tox models for algae. The parameters of the DEBtox models were estimated from standard standard ecotoxicity tests for algae by applying Bayesian methods. The loss of biomass under the temporally varying exposure concentration was used as an ecological risk index. Our risk calculations created risk maps that graphically represent risk from herbicide in Japan. For example, the risk map suggested that the risk of pretilachlor is especially high in the middle-east area in Japan. The DEBtox model simulations suggested that herbicides can severely decrease algae biomass for about four weeks. Our results suggest that pretilachlor is the highest risk factor among five paddy herbicides.

WE 112

Urbanization, land use, and river water quality in Chongqing, 1997-2010

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The purpose of this paper is to describe a preliminary investigation into the relationship between river water quality and urbanization together with associated land use changes in a megacity that is rapidly urbanizing and developing economically. We examined Chongqing municipality in China as a typical case to quantify its land use change and urbanization, and reveal the relationship between these factors and river water quality. We examined the period from 1997 to 2010 during which Chongqing urbanized significantly. In 1997 Chongqing, which is located in the southwestern region of China, was designated as the fourth Municipality administered directly by the central government besides Beijing, Shanghai and Shenzhen. It is the largest municipality in China in terms of area and population, and most of the Three Gorges Reservoir lies within it. In this study we analyzed data on long term land use changes and river water quality in the megacity using correlation analysis and regression analysis in order to explore the relationship between river water quality and changes in land use and urbanization. The data on river water quality were obtained from 23 cross sections of the three main rivers (Yangtze, Jialing and Wu) as well as 98 cross sections of 53 second-level tributaries, as found in annual reports by Chongqing Environmental Protection Bureau. Data on land use change were collected from Annual Reports of Chongqing Land Use Change Situation issued by the Chongqing Land and Resource & Housing Management Bureau. The results of this study revealed the relationships between river water quality and land use changes associated with urbanization. It is concluded that there is an urgent need to conduct comprehensive land use planning for protecting water quality in this rapidly developing megacity. This conclusion probably also applies to many other cities that are in a similar condition.

WE 113

Mapping risk reduction of pesticides at the landscape scale: an ecosystem service approach

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Pesticides, in particular insecticides, have adverse short- and long-term effects on freshwater communities. They prevent the achievement and maintenance of a good chemical and ecological status of surface water bodies, as aimed for by the EU Water Framework Directive (WFD). The recovery of community structure after exposure to insecticides is facilitated by the presence of undisturbed upstream stretches that can act as sources for recolonization. Hence, landscape elements such as forests or pastures indirectly provide an ecosystem service. The ecosystem service consists of buffering the impact of pesticides on water quality regulation, breakdown of leaf litter or outdoor recreation of streams, which are related to aquatic biodiversity. This leads to three questions: i) which areas face the highest potential exposure by pesticides and the greatest resulting ecological risk, ii) which areas are most effective in buffering the ecological risk, and iii) in which areas would additional measures to mitigate ecological risks be most effective.

We predicted the potential exposure of small streams to insecticides (run-off potential - RP) and the resulting ecological risk (ER) for freshwater fauna on the European scale using a spatially explicit model. Then, the service provided by the landscape (reduction in ER) was calculated by comparing the ER of a landscape lacking completely recolonization sources with that of the actual landscape.

33% of all stream sites in cultivated areas of the EU-25 countries were predicted to fail the requirements of the WFD for only slight deviation from undisturbed conditions due to run-off of insecticide. In Italy, northern Spain, southern France, and northern France/Belgium 'reduction in ER' by existing recolonization sources was estimated to be above 50%. Likewise England, southern Germany, Slovakia and parts of the Czech Republic showed high service values. The overall pattern of the ecosystem service followed the pattern of predicted RP and ER. However, there was not always enough recolonization area present to compensate for high-risk potential.

Hence, there is a great potential to improve the ecological quality of agricultural streams to comply with the WFD. This could be achieved by enhancing the ecosystem service provided by the landscape, that is, by establishing new and protecting existing nursery habitats for aquatic species. The maps compiled in this study depict those areas where such measures would be most effective.

WE 114

Applicability of mitigation measures for surface water in Italy: the case study of the Lombardy Region

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The authorization process of plant protection products (PPPs) provides a risk assessment of surface water contamination, which has the main purpose of addressing the impact of PPPs on surface water quality and on aquatic ecosystems.

If the risk assessment, performed according the supported GAP, show an unacceptable risk for aquatic systems, mitigation measures have to be applied to reduce the concentration of PPPs which can reach water bodies and, affect aquatic organisms.

Due to the absence of data at national level for Italian landscape and considering the increased importance of mitigation measures in PPPs, as also underlined by the Sustainable Use Directive, new investigations have to be implemented to select the proper mitigation measures that better fit the environmental and agricultural characteristics of Italy..

The aim of this study is to evaluate the applicability of mitigation measures in the Lombardy Region, combining the use of GIS and statistical approaches (multivariate analysis) to identify critical combination of mitigation buffer for water bodies versus the regional arable land.

These multivariate analysis are based on a set of parameters, such as elevation, geo-pedological soil conditions, slope, distance between field and water bodies, which are considered representative of the Regional arable lands.

Results of this study will provide a starting point to the development of an helpful decision maker tool, in order to select the most appropriate mitigation measures at local level.

Improvements of data availability at National level, would allow the results of this work to be extended to the entire Italian agricultural landscape.

WE 115

Off-crop risk mitigation for non-target arthropods

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Lower tier risk assessments for plant protection products frequently detect a risk in the off-crop areas for insecticides applied in agricultural fields. A reality check of such risks is difficult to perform, as toxicity assessments are based on easy to rear beneficial organisms. Furthermore calculation of the exposure for the off-crop area are unsure, and trigger validation is based on in-crop vs laboratory comparison of effects on only two beneficial arthropods. As refinement studies for off-crop areas are rarely available, regulators want to issue use restrictions, i.e. the use of risk mitigation measures.

At the Escort 3 meeting it was concluded that different off-crop areas comprise different protection goals, that "ecological function" is a primary factor for structuring protection goals for non-target arthropods, and that an off-crop risk assessment should meet national agricultural environmental schemes. Possible risk mitigation options include buffer zones, wind breaks (e.g. hedgerows or tree rows), drift-reducing application techniques (nozzles, tunnel sprayers), and changing application rate, period and interval. However, suitable and accepted measures to reduce the risk have not yet been defined.

We present options for risk mitigation measures, which consider Swiss agricultural schemes and land-use. The planned procedure involves four main steps:

- 1) Categorisation of off-crop habitats
- 2) Defining protection goals for categories of off-crop habitats
- 3) Adjusting risk assessment procedures to consider categories of habitats and protection goals
- 4) Define and evaluate risk mitigation measures.

WE 116

A major product-linked stewardship campaign promoting user take-up of low drift nozzles in the UK

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Risk assessments for aquatic organisms for Plant Protection Products in the EU are based on spray drift deposition values for conventional nozzles. Using these drift values, Predicted Environmental Concentrations are derived, and compared with data on toxicity of active substances to aquatic organisms. In the UK, chlorpyrifos is a key active substance for pest control in fruit and arable crops. In some cases there are no alternative products. The buffer zones needed when using conventional nozzles are greater than accepted under UK policy. The technology of low drift nozzles means that the chlorpyrifos can be applied within the UK buffer zone limits - offering a pragmatic solution for maintaining environmental protection - whilst continuing the availability of this useful product for UK growers. In October 2011, Dow AgroSciences, Makhreshim Agan UK, and Headland Agrochemicals launched the 'Chlorpyrifos. Say No to Drift' campaign. The launch was at the National Fruit Show in Kent, and was kick-started by the offer of 200 free sets of low drift nozzles (Albuz TVI) to UK fruit growers, by registering on the campaign stand or on the campaign website (www.saynotodrift.co.uk). For arable crops, most growers already use low drift nozzles - but the key for the campaign is to ensure that these nozzles are always used when applying chlorpyrifos. Full engagement with the growers and agronomic advisors is essential to explain the importance of the nozzles, working towards the overriding aim of the campaign which is to have 100% of applications of chlorpyrifos in UK made through low drift nozzles in 2012. To ensure that the low drift nozzles provided the necessary drift reduction a field spray drift

study was commissioned by the three companies in an orchard in Kent (run in summer 2011). The study showed around 95% drift reduction using a tower sprayer and 85% reduction using an axial fan sprayer compared with the standard (axial fan and conventional nozzles). Hence, the study provided a strong technical foundation for the campaign recommendations. The response of the grower has been tremendously positive. At the National Fruit Show, in just two days, growers for over 50% of the UK apple area (culinary and desert) had signed-up to receiving and using low drift nozzles when applying chlorpyrifos.

WE 117

Site-specific risk assessment based on direct toxicity assays of an abandoned pyrite mine in Spain

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Risk assessment methodology has been recognised as a powerful tool for the decision-making process in contaminated sites management. The risk to environment varies considerably with the site specific conditions. Therefore, the assessment of the impacts of contamination has to be evaluated on a case by case basis. Abandoned mines are quite common and may show high levels of environmental contamination. In a previous study performed in our laboratory, risk assessment methodologies were used to assess the area surrounding an abandoned pyrite mine in Bustarviejo (Madrid, Spain). The potential environmental risk on this site comes mainly from the tailings with high concentrations of arsenic and metals such as copper, cadmium and zinc leading to significant contamination of soil, plants and waters at the streams. This evaluation was based on total metal concentration of metals in soil and waters and it allowed identifying severe impacts on soil and aquatic organisms as well as the necessity of refining the risk. The bioavailable fraction and metal interactions were considered key factors for a more realistic approach. Toxicity assays performed with contaminated environmental samples allow integrating the effects due to contaminant mixture and help in evaluating the risk associated with exposure to bioavailable substances present in a soil. This paper details an improved procedure for site specific ecotoxicological risk assessment, based on the use of bioassays. Soil samples were selected to represent zones of high, medium and moderate concentrations of soil metals. Samples were tested at different soil concentrations (12.5, 25, 50 and 100% test soil/soil (w/w)) using a soil microcosms denominated "Multispecies Soil System" (MS-3). This system consisted of soil columns coupled to a leachate collector system that allowed assessing toxicity to several interacting organisms (microorganisms, plants and earthworms) being exposed simultaneously. The leachates were collected and tested for toxicity using representative aquatic organisms (i.e. algae and daphnia). Moreover, the bioaccumulation of contaminants in earthworms and plants was determined as a measure of the bioavailability of metals in soil. Data gathered with this study will be used to refine the previous evaluation of risk based on total soil metal concentration in order to get a more realistic risk assessment.

This work was funded by Madrid Community through ELADES Project S2009/AMB1478.

WE 118

Analysis of the status-quo of riparian zones between agricultural land and surface waters in Germany

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Surface waters are especially sensitive to pesticides exposure. Directive 2009/128/EC commits EU Member States (MS) to take appropriate measures to avoid pollution of surface water, such as the establishment of buffer and safeguard zones, planting hedges along surface waters etc. Before taking such actions in an efficient and appropriate way the status-quo of riparian zone between agricultural land and surface waters need to be assessed nation-wide. As there is no spatial database in Germany that provides such information, an approach was developed to give a most precise estimate on community level.

The approach is based on spatial data of the land parcel information system (LPIs) for a precise description of the agricultural land. The database is part of the Integrated Administration and Control system and exists in all MS. Information on the surface water network stem from the German authoritative topographic database ATKIS. For the assessment a zone of 10m directly adjacent to the top of the water body embankment is analyzed in 1m steps.

With GIS methods both datasets are spatially related. Although the dataset stem from different data models a good degree of consistency is achieved as both datasets are derived from aerial orthophotos.

Results show that small water bodies are the dominant type of water body in close distance of up to 10m to agricultural fields (91%). Assuming the establishment of a general 3m no-crop buffer zone 54% of this goal would be already achieved (for 5m no-crop buffer zone, 45%; 10m no-crop buffer zone, 28%).

WE 119

SYNOPSIS-WEB, an easy-to-use online tool to assess the potential aquatic and terrestrial risk of pesticides on field level

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The use of pesticides in agriculture causes environmental risks that must be carefully managed. One overall goal of the current German National Action Plan (NAP) on the Sustainable Use of Plant Protection Products is to reduce the environmental risk by 25%. Within the NAP the risk indicator model SYNOPSIS is used to assess trend of the terrestrial and aquatic risks of pesticides in agriculture. The current indicator model is applied for different modes of action. SYNOPSIS-TREND is used to assure the tracking of the risk trends on national level on yearly basis with annual sales data. SYNOPSIS-GIS is applied for regional risk analysis and the detection of hot spots with field based surveys on pesticide use and extended GIS based datasets on land use, slope, soil types and climate. In this study a third application level, SYNOPSIS-WEB was developed in order to make this indicator available as an easy-to-use online tool, which can be used by farmers, advisors and stakeholders for risk analysis and comparison of single pesticides application strategies on field level in Germany.

In the web application the user can select a certain field on a detailed map, choose a crop to be treated and upload the pesticide applications to be tested. The field selection will automatically extract the environmental parameters which are necessary to assess the aquatic and terrestrial risk from a central spatial database. This database was established by merging information from an extended geographical dataset on land use (ATKIS), a digital soil map (BÜK1000), a digital elevation model (DGM25) and a set of 430 climate stations and >2000 precipitation stations (DWD). Furthermore a database of all registered pesticides in Germany (<https://portal.bvl.bund.de/psm/jsp/>) was linked to the tool from which the user can choose the products to be applied. To upload an application the application date and rate have to be added by the user. SYNOPSIS-WEB then calculates and displays the risk indices which can be downloaded on product level as XML-files. In addition SYNOPSIS-WEB can be accessed via a web-service to be imported into other tools e.g. farm management software.

RA15P -Linking exposure to effects in environmental risk assessment

WE 121

How to identify the metabolic mechanism of action (mMoA) from toxicity data? Comparing species and effects in a DEB framework

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Mathematical modeling in ecotoxicology contributes to understanding effects of toxicants at the individual level and allows extrapolating these effects to the population level.

Moreover, models enable us to compare effects of different toxicants across species, and facilitate extrapolation to other environmental situations.

Ecotoxicology would benefit from a general framework to integrate and compare toxicity data across all organisms and chemicals. Dynamic Energy Budget (DEB) theory provides a conceptual framework that explains how organisms allocate energy from food into growth, reproduction, and maintenance. The same framework can be applied to all organisms; inter-species differences are expressed as differences in parameter values. Effects of toxicants can be seen as disturbances from the control situation, i.e., as changes in a parameter value. The modified parameter thus constitutes the predominant mechanism of action of the toxicant on metabolism (a metabolic mechanism of action, or mMoA). For each mMoA, a specific combination of effect patterns on measurable endpoints exists, e.g. effects on growth, reproduction, development, feeding, and respiration. Although several studies have shown the potential of understanding toxic effects in a DEB framework, standard toxicity data usually do not contain sufficient information to accurately determine the mMoA. Some mechanisms show similar patterns of effect under standard test conditions, so that uncertainties remain. Identifying the appropriate mMoA is of particular importance for extrapolation to the population level.

The aim of this study is to provide information about which endpoints need to be assessed to be able to identify each possible mechanism of action as determined by the standard DEB model. We use Monte Carlo simulation techniques to simulate all possible mechanisms of effect in several species to determine which endpoints reveal the most unique deviation from the control. We determine the simplest set of (simultaneous) observations on different endpoints at different parts of the life cycle and experimental conditions which should allow discrimination between each toxic mMoA.

WE 122

A tiered modeling approach for simulating toxicokinetics of baseline narcosis in aquatic organisms

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More than a century ago Meyer and Overton pioneered concepts related to baseline narcosis: a general, non-specific mode of toxic action. These principles resurfaced with the development of quantitative structure-activity relationships (QSARs) for aquatic toxicity and bioconcentration. The data and models have shown that for neutral organic chemicals a relatively constant whole body residue of about 2-8 mmol/kg wet weight (ww) is associated with mortality in acute exposures (96h LC50). The critical body residue (CBR) approach in environmental risk assessment is considered advantageous, particularly if mitigating factors associated with differences in exposures can be quantified, for example, using mass balance models. Many industrial chemicals are expected to exhibit a baseline narcotic mode of action. A tiered modelling strategy is developed that progresses from "simple models" to "more sophisticated models" based on the availability of data to parameterize the model. For physical-chemical properties, the simple model requires only the octanol-water partition coefficient (KOW) and molar mass (M), whereas the more sophisticated model utilizes more refined partitioning information to quantify chemical partitioning to target and non-target compartments (e.g. membrane-water partition coefficients, protein-water partition coefficients, etc). Metabolic biotransformation rate information for the parent chemical is also required for both models. The modelling framework allows the option to use empirical kinetic

data preferentially over default model calculations. Model output includes chemical concentrations, fugacities, chemical activities, as well as the time-course of toxicity and the critical residues for the toxicity tolerance distribution. Uncertainty estimates are provided for model calculations. Differences in the input and output of the “simple” and “sophisticated” models are illustrated. These models are under-going evaluation and testing with existing and new toxicity data and are intended to facilitate the application of laboratory toxicity testing data for environmental risk assessment.

WE 123

Higher-tier assessment of exposure and effects of pesticides on aquatic non-target organisms

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The use of mathematical models in ecological risk assessment (ERA) is a promising way for increasing the level of ecological realism in ERA. In addition, it offers the extrapolation from single species effect studies to multi species and various scenarios for which the risk needs to be estimated.

Non-target organisms like green algae or lemnaceae are of particular importance for the aquatic risk assessment of herbicides, since they are often the most sensitive groups of species, play functional roles in aquatic ecosystems, and on the other hand, have a high potential for recovery from short-term pesticide effects.

The exposure of pesticides in surface waters is estimated using various models (e.g. FOCUS TOXSWA) taking different routes of entry into account. Pesticides can enter water bodies via spray drift + runoff, or + drainage in a time-variable scale, resulting in exposure profiles of different height and duration.

It is not clearly defined how exposure assumptions in effect studies cover the exposure profiles occurring in realistic worst-case scenarios for runoff or drainage entries with respect to peak duration, height and area under the curve.

This presentation aims to provide insights into how ecological modelling can improve ERA.

WE 124

Explanatory power of peak and time-weighted average concentration for effects of pesticides as observed in semi-field experiments

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Laboratory and semi-field experiments usually do not provide insight in the effects of time-variable exposure to aquatic organisms. This issue was emphasized in a recent EU ELINK workshop on linking exposure and effects in the aquatic risk assessment procedures for pesticides, and resulted in recommendations for addressing time-variable exposures, and when to use peak or time-weighted average (TWA) concentration. The aim of present study was to compare the effects as observed in cosm experiments with peak concentration of the exposure profile as well as the TWA21d concentration using different sensitivity endpoints. For this purpose, a literature review was performed of empirical PERPEST database which has been built by performing a review of freshwater model-ecosystem studies evaluating the effects of pesticides. Peak exposures of single and multiple application of pesticides were derived from the publications and its corresponding TWA21d concentrations were calculated. In order to allow comparison of studies with different insecticides, we expressed the exposure concentrations as toxic units (TU). TU were calculated by deviding the concentrations evaluated in the cosm study by the Hazard Concentration 50% (HC50). Different grouped endpoints were selected from each microcosm and mesocosm study and responses were assigned to effect class. In case of peak exposure to the organophosphate insecticide chlorpyrifos, clear effects (Class 3) on “Microcrustaceans” are regularly reported at exposure concentrations between 0.1-100 Peak-TU, while in the range 0.1-1 Peak-TU, slight effects (Class 2) are observed. When expressed as TWA-TU, these threshold concentration ranges are 10 times lower. The data presented for microcrustaceans and chlorpyrifos show a clearer dose-response relationship in case of TWA exposures, when compared to peak exposure. On the poster results will also be presented for other insecticides and they will also be aggregated for mode of actions and chemical groups.

WE 125

Behavioural analysis on *Daphnia magna* exposed to different pollutants: possible contribution for water quality assessment?

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Immobilization test on *Daphnia magna* is one of the most used standardized test for water quality monitoring. However, this test does not show intermediate toxic effects prior to the death of daphnia at the end of the 48 hour exposure. Behavioural tests may provide sensitive early responses and therefore have been proposed as non-destructive early warning system. Several online behavioural biomonitors are being used throughout Europe to control water quality. However, it is currently unclear how to derive robust ecotoxicological endpoints from observation of daphnia behaviour, how to quantify the sensitivity of these endpoints and finally how to use these endpoints in the context of environmental risk assessment.

The aim of the study is (i) to assess how behavioural responses can be used as ecotoxicological endpoints and (ii) to compare the sensitivity of standard versus behaviour endpoints and (iii) to analyze how behaviour endpoints can contribute to water quality assessment.

For this purpose, we used a new image analysis system, which allows the simultaneous tracking of up to 240 daphnids in 24 exposure chambers (10 daphnia per chamber).

In this system, toxic substances with different modes of action were tested at several concentrations and replicates. Different parameters (swimming speed, distance between organisms, number of active organisms) were monitored during the whole exposure time of 48 hours. This exposure time was chosen in order to directly compare results with endpoints of the standard tests.

Behavioural endpoints are presented as function of concentration and time for each substance in order to understand changes in behaviour induced by toxicant and to predict the time of effect onset. Results are discussed with regards to possible utilisations and limitations of behavioural endpoints for early warning system in water quality control.

WE 126

Linking Selenium exposure to physiological, biochemical and morphological responses in three species of salmonids - use of weighted logistic regressions to compare sensitivity of endpoints

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Coal mining, uranium mining and irrigation of selenium (Se)-rich soils contributes to loading of Se into surface waters. Although Se is an essential element, it becomes toxic at concentrations slightly above those required for homeostasis. Fish are susceptible to Se toxicity and species-specific sensitivity to Se has been documented in adult fish and also in fish embryos and larvae. We have gathered physiological, biochemical and morphological data from several species of salmonid fishes (rainbow trout, brook trout, cutthroat trout) exposed to selenium either in mine pit lakes located on reclaimed coal mining sites, streams draining these areas, or in fish exposed to Se through diet or water in the laboratory. Specific sets of data, including plasma hormone concentrations, liver lipid peroxidation, liver GSH content, liver glycogen reserves, and condition in adult fish, and incidence of malformations (craniofacial, spinal, edema and fin fold), weight and swim up success in fish larvae, are regressed against tissue Se using weighted logistic regression to identify response thresholds. Response thresholds are then compared to determine the most sensitive endpoint, for use in risk assessment and diagnosis of Se-caused adverse effects. (Funded by MITHE-SN, Alberta Conservation Association, NSERC).

WE 127

Assessment of tissue-specific accumulation and effects of cadmium in a marine fish fed contaminated commercially produced diet

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Commercially produced fish diet is now widely used in fish farming but it often contains elevated levels of cadmium (Cd). However, the adverse effects on fish are poorly understood. In this study, farm-raised marine grunts *Terapon jarbua* were fed Cd-contaminated diet or exposed to waterborne Cd for four weeks. Tissue-specific Cd bioaccumulation and its effects were subsequently examined. We found that Cd was accumulated in different fish tissues (digestive tracts, gills or livers). At the end of the exposure, Cd accumulation peaked in the fishes' livers (5.0-6.3 µg g⁻¹), followed by the digestive tracts (0.83-3.16 µg g⁻¹) and gills (0.27-2.74 µg g⁻¹). Endpoints such as the survival rate, specific growth rate, condition factor, and superoxide dismutase activity were not significantly affected by Cd exposure. In contrast, metallothionein (MT) induction and subcellular Cd distribution indicated that there were possible sublethal effects of Cd exposure. MT was induced in response to Cd accumulation, but it returned to the control levels after a longer exposure period, except for hepatic MT induction resulting from waterborne or low dietary Cd exposure. The Cd percentage in the heat-stable protein (HSP) fraction increased over exposure time, and it accounted for more than 57% Cd in the fishes' livers and 80% Cd in their digestive tracts by the end of the exposure period. Overall, although Cd in commercial fish diet did not have significant lethality to *T. jarbua*, sensitive responses, such as hepatic MT induction and subcellular Cd distribution, revealed that the Cd-induced storage and detoxification in *T. jarbua* may increase fish's tolerance to toxic metals.

WE 128

Implementation of bioavailability in the effects assessment of lead in the terrestrial environment

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At present, the risk assessment of lead (Pb) for soil in the European REACH registration file is based on a fixed generic predicted no effect concentration (PNEC_{soil}) for all soils. This provisional PNEC_{soil} was derived from the 5th percentile of the distribution of the chronic NOEC values for several plants, invertebrates and microbial processes. Although bioavailability and toxicity of metals can be strongly affected by the variation in soil physicochemical properties, the available Pb data did not allow establishing a relationship between soil properties and Pb toxicity to terrestrial organisms. As a result, toxicity data were not normalized for differences in soil properties. In order to address this issue and close data gaps compared to other metals (e.g. Cu, Ni and Zn), a research project was initiated aiming at building bioavailability models for Pb and understanding Pb toxicity in experimentally aged soils. Toxicity of Pb was measured for 7 endpoints (2 plants, 2 invertebrates and 3 microbial endpoints) in 7 soils covering

a wide range of soil properties (pH, organic matter content, texture and cEC). In addition, toxicity of Pb was compared in 3 soils under 3 conditions: i) freshly spiked with PbCl₂, ii) freshly spiked, leached and pH corrected, and iii) freshly spiked and aged for 5 years. Variation in soil properties significantly explained variation in Pb toxicity for some endpoints (e.g. nitrification), but for other endpoints (microbial respiration, *Folsomia candida* reproduction) Pb toxicity was not affected by soil properties. Leaching and aging of soils attenuated Pb toxicity significantly, confirming higher toxicity in freshly spiked soils compared to soils equilibrated under field conditions. The data were used for the derivation of a refined correction factor for leaching and aging effects. This poster describes the implementation of the new data and bioavailability models in the terrestrial effects assessment and derivation of a PNEC_{soil} for Pb.

WE 129

Field validation of cell viability as a biomarker of metal exposure in soil invertebrates

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Invertebrates such as arthropods and mollusks are often used in toxicity assessments of metal polluted environments, as they are known to bioaccumulate metals. It is also well known that they exhibit various sublethal toxic effects as a result of such bioaccumulation, for example reduced cell membrane integrity. In a previous study we investigated the potential use of the trypan blue exclusion assay to demonstrate the effects of metals on hemocyte viability of woodlice. The aim of the current study is to test the field application of this technique. Millipedes (*Ommatoiulus moreleti*) and snails (*Helix aspersa*) were collected from a metal smelter, as well as from an unpolluted botanical garden. Hemolymph was drawn from each animal and the percentages viable hemocytes were determined by means of the trypan blue exclusion assay. The animals were then digested with nitric acid and metal concentrations determined with an ICP-AES. Results generally showed higher metal concentrations in animals from the metal smelter, with concomitant reduced cell viability. This suggests that cell viability, using the trypan blue exclusion assay, may possibly be successfully used within a suite of biomarkers, as a general indication of stress.

WE 130

Linking subcellular metal partitioning to the particulate and dissolved exposure pathways and chronic effect of copper in two deposit feeder organisms

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In toxicity assessment, increasing importance is being placed on determining tissue metal residues-effect relationships that result from environmental exposure. Toxic effects are elicited when a critical dose of a chemical is reached in one or more sensitive compartments of the organism. However, it is well recognised that, following uptake, metals may partition within different subcellular tissues, be detoxified or excreted, but only the metabolically active fraction of metal contributes to toxicity. The present study investigates the links between the subcellular metal exposure and toxic effect to reproduction of the epibenthic amphipod *Melita plumulosa* and growth of the bivalve *Tellina deltoidealis* exposed to copper-contaminated sediments with varying properties (particulate organic carbon (POC) content and/or fraction of fine particles). The subcellular partitioning of copper was determined for both organisms and residue-effect relationships investigated. The results highlighted: i) strong correlation between total copper bioaccumulation and both POC-normalization copper concentration of the <63 µm sediment fraction and copper concentration in overlying water for both organisms (*M. plumulosa* $r_{\text{sed}}=0.87$, $p<0.001$; $r_{\text{OW}}=0.84$, $p<0.01$; *T. deltoidealis* $r_{\text{sed}}=0.93$, $p<0.001$; $r_{\text{OW}}=0.96$, $p<0.001$); ii) significant correlation between the metabolically available fraction of copper and normalized sediment copper concentration for both organisms (*M. plumulosa* $r=0.79$, $p<0.01$; *T. deltoidealis* $r=0.67$, $p<0.05$); the correlation was quite weaker for *M. plumulosa* between the metabolically available fraction and copper concentration in the overlying water ($r=0.64$, $p<0.5$, $df=9$) but stronger for *T. deltoidealis* ($r=0.92$, $p<0.001$); iii) strong linear relationship between the compartment of copper biologically detoxified within *T. deltoidealis* and both the %-POC/silt normalized sediment copper concentration ($r=0.91$, $p<0.001$) and copper concentration in the water phase ($r=0.85$, $p<0.001$); iv) strong correlation ($r=-0.83$, $p<0.01$) between sublethal effect and metabolically available fraction for *M. plumulosa*. Copper associated with the particulate phase has been demonstrated to be the major exposure route and potential cause of the toxicity for *M. plumulosa*. However, in *T. deltoidealis*, particulate and dissolved exposure routes seem to play both an important role in copper uptake. These results provide further insight into the interaction between tissue residues and chronic effects.

WE 131

The impact of contamination by phophogypsum on the structural and functional diversity of the soil microbial community in a deposition area (Sfax, Tunisia)

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The phosphate industry is an important economic activity in Tunisia. Nevertheless it originates several tons of phosphogypsum (PG) (5 tons of PG per 1 ton of phosphoric acid) which is a waste-by product of the production of phosphorus fertilizers by a wet-acid method. Usually PG is dumped in stockpiles, exposed to weathering conditions and contributing for the spread of contaminated dusts, causing serious environmental and health problems. In this work we studied the impact of contamination near the PG pile area (Sfax, Tunisia) on the structural and functional diversity of the soil microbial community. With this purpose we choose five transects in the area, where four soil samples were collected per transect. A reference soil (REF) was collected a few kilometres from Sfax. The soils were characterized comprehensively, i.e. a set of physical and chemical parameters (pH, conductivity, salinity, organic matter, holding water capacity, particle size distribution) and total metals content was measured. Moreover, the bacterial communities of soil samples were analysed through PCR-DGGE for 16S rDNA. The BIOLOG assay was performed to assess the functional diversity of soil samples. The environmental and molecular data were analysed by multivariate statistical analysis. A Principal component analysis of environmental data showed a clear separation of the REF soil from the other soil samples mainly based on its organic matter, clay-silt and iron contents. Two other soils were set apart, from the others based on the salinity or on lead, zinc and uranium contents. A Redundancy analysis was made in order to perceive the influence of environmental variables in the DGGE profiles of different soil samples. There was a great variability within samples, and, in general, the environmental factors analysed had a low contribution for this variability. Once again the genetic profile of the REF soil was clearly separated from that of the other samples. The BIOLOG assay showed generally lower substrate utilization for sites presenting higher contamination. Similarly, the microbial community in REF soil and in soil S1.4 (nearby the PG pile and salt marshes) evidenced higher metabolic rates mainly associated with the consumption of carboxylic acids, carbohydrates, amines and amino acids. Overall, the integration of PCR-DGEE and BIOLOG data proved to be a valuable approach for the identification of variations in the diversity of soil microbial communities under PG contamination.

WE 132

Exposure of honey bees and other pollinating species to pesticides

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Plant Protection Products or PPPs (also called pesticides) are part of modern crop management practices. Prior to the placement of PPP on the market and their use an evaluation of the risks posed to the environment is mandatory worldwide requiring an assessment of the impact of these products on the agricultural environment, and among others on arthropod and pollinating species.

If in practice exposure to honey bees can occur both the hazard (toxicity) of the compound and also the potential exposure to the organism is then considered. The risk assessment usually follows a tiered approach whereby products of low toxicity and low risk are rapidly excluded; whereas products with a potential to harm honey bees are progressed to higher and more realistic tiers of evaluation. Consequently, it is usually not necessary to generate extensive and elaborate measures of toxicity and exposure in tier 1 risk assessments.

Many PPP are typically applied either by spray to the above ground part of plants or to soil either directly (by spray or granules) or into the soil as treated seed. A third method of trunk injection is important for the protection of trees. The method and timing application will affect if bees are exposure and if so by what routes. The physico-chemical properties can also impact exposure which may affect the distribution of the substance within plant and also the duration of exposure. In this paper, different exposure scenarios are developed with respect to method and timing of application also in relation to the properties of the substance. Various scenarios are described where bees and other pollinators can be exposed along with an indication of possible residues levels present in matrices of relevance to bees. Explanations of the exposure scenarios due to spray applications and soil/seed treatment uses are given and way in which these can be used and link to risk assessment presented. By establishing clear guidance for exposure of pollinators to PPP a more informed risk assessment process can be developed and utilized.

WE 133

Environmental assessment of a low molecular weight Phthalate Ester - DEP (Diethyl Phthalate)

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Phthalate Esters are used in a wide range of industries, with uses as diverse as plasticizers to perfume stabilisers within the fragrance industry. DEP is a relatively short chain phthalate ester, a property which is reflected by the tendency of DEP to exhibit higher water solubility and lower partitioning values than longer chain length phthalate esters. A reflection of the continued global use of DEP is the fact that DEP has been detected in several environmental matrices (aquatic and terrestrial). Consequently, concerns have been raised over the exposure of DEP to terrestrial and aquatic ecosystems as well as for the potential of DEP to inhibit functional microbial communities within sewage treatment plants (STPs). It has also been noted that DEP, based solely on its physicochemical properties, may have the potential to bioaccumulate along trophic levels and exert endocrine disrupting effects on wildlife. In light of these combined concerns, RIFM (Research Institute for Fragrance Materials) conducted a review of the available literature to determine the extent to which DEP posed a risk to the environment in terms of a PBT (Persistence, Bioaccumulation and Toxicity) and ED (Endocrine Disruption) assessment. It can be concluded that any exposure of DEP to aquatic and terrestrial organisms would be brief, as DEP dissipates relatively quickly, mainly by the degradative action of microbial consortia. When the full range of terrestrial and aquatic PNECs (predicted no effect concentrations) are analysed, these are shown to be relatively high compared to reported PECs (predicted environmental concentrations). In addition, DEP has been shown not to bioaccumulate significantly along trophic levels. Although DEP has been shown to exert endocrine disrupting activity towards fish and aquatic invertebrate species in laboratory studies, the low PECs noted are insufficient to exert risk on

these organisms. In summary, DEP does not pose a significant risk to the aquatic or terrestrial environment in terms of PBT or ED.

WE 134

Toxicokinetics model for an insecticide in rats: implications for higher-tier risk assessment

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The current risk assessment for mammals is based on external exposure measurements. Exposure to a chemical does not mean, however, that all of the dose will be bioavailable. Bioavailability, i.e., fraction of dose that reaches the systemic circulation or is made available at the site of physiological activity, as well as toxicokinetics (e.g., absorption, elimination) strongly influence the accumulated dose of a toxicant, and it is its internal concentration which makes an effect. Internal concentration is the net result of absorption, distribution, metabolism and excretion, and the toxicokinetic (TK) model is a mathematical description of these processes. We used data for an insecticide. The toxicokinetic model was parameterised using absorption, tissue distribution and excretion from a rat study with ¹⁴C-labelled material. The number of compartments (tissues) in the model and the complexity of the processes of absorption and elimination were also investigated using these data. Feeding rate scenarios and avoidance of contaminated food were taken into account in the model because they may influence the concentration of chemical in the body and the risk for animals living in natural environments.

High variability in kinetic parameters between individuals meant it was necessary to test the effects of different combinations of maximum and minimum values for absorption *ka* and elimination *ke* rate constants on the change of the concentration in the bloodstream as a function of time for each exposure scenario (i.e., LD₅₀ eaten as a bolus dose or according to three different feeding patterns). Maximum internal concentrations (*CMaxBody*) for all studied scenarios were compared. Results showed that, irrespective of *ka* and *ke* combinations, higher *CMaxBody* were reached when the LD₅₀ was given as a bolus dose and much lower when the realistic worst-case scenario was used (i.e., short feeding bouts with maximum peak ingestion rate together with breaks of low/no feeding activity). Nevertheless, the difference between *CMaxBody* reached after a bolus dose or feeding naturally depended on which combination of *ka* and *ke* was used; the lower the *ka* and *ke* the closer the feeding maximum body burden came to the bolus dose maximum body burden. The possible use of body burden model as a refinement option in bird and mammal risk assessments will be discussed. Ongoing modelling is underway and the impact of avoidance mechanism on the insecticide body burden will be also reported.

WE 135

Relevance of drinking water as a source of human exposure to bisphenol A

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A review of over 75 studies describing measurements of bisphenol A (BPA; also 4,4'-isopropylidene diphenol, CAS Registry No. 80-05-7) in finished drinking water and its source waters was conducted to characterize the occurrence and concentrations of BPA. BPA has been detected in a small fraction of drinking water and source water samples. The fraction of drinking water measurements reported as less than the detection limit is 95%, 48%, and 41%, for North America, Europe, and Asia, respectively. The highest measured concentrations in drinking water in North America, Europe, and Asia are: 0.099, 0.014, and 0.317 microg/L. The median and 95th percentile concentrations of BPA in Asian drinking water are 0.026 and 0.19 microg/L, while the median and 95th percentile concentrations in North America and Europe are limited by detection limits. In surface water/source water, the median and 95th percentile detection limits are: <1 and <1 microg/L for North America, <0.006 and <5.1 microg/L for Europe, and the median concentration for Asia is <0.0155 microg/L. In groundwater/source water the median and 95th percentile detection limits are <1 and <1 microg/L for North America, while for Europe the median detection limit is <0.001 microg/L. The 90th percentile concentration is <0.073 microg/L for Europe in groundwater/source water and is only an estimate since individual data points were not available for all studies. Oral reference doses (RfDs) for human exposure to BPA have been derived by several agencies, including USEPA, European Food Safety Authority, and Health Canada, and range from 16 to 50 microg/kg-body weight (bw)/day. The lowest RfD, 16 microg/kg-bw/d derived by Willhite et al. (2008), is based on no-observed adverse effect levels (NOAEL) of 5 mg/kg-bw/d identified for systemic toxicity in rats and mice and application of an uncertainty factor of 300. Using the highest median and 95th percentile detected concentrations of BPA in drinking water across all regions of 0.026 and 0.099 microg/L, respectively, median and 95th percentile intakes of BPA due to ingestion of drinking water by humans were calculated for several age groups. These intakes were compared to intakes calculated from all dietary sources (WHO 2010). Margins of Safety for drinking water ingestion ranged up to 41,000 among all age groups and contributed only 0.011 to 0.40% of the 95th percentile intake from all dietary sources.

WE 136

Urinary bromophenols phase II conjugates: a novel human exposure biomarker for PBDEs

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PBDEs with 4 to 6 bromine substituents have already been formally listed as POPs by the Stockholm Convention in 2009 because of their abundant presence in the global ecosystem. To monitor human exposure to PBDEs, the most frequently adopted approach is the direct quantification of selected BDE congeners and their hydroxy- / methoxy-metabolites in human tissues, for example: human plasma or serum, breast milk, and adipose tissues. However, sampling of these tissues are intrusive operations and are difficult to achieve in large-scale population-wide or nation-wide surveys. Sampling of breast milk can be considered a non-intrusive process, but samples are only restricted to lactating women of a relative narrow range of ages. It may not be able to truly reflect the level of population exposure to contaminations. Thus, to reliably assess the population exposure of PBDEs, a non-intrusive, representative and convenient sampling and analytical protocol is needed. Sampling of human urine is a truly non-intrusive process and human urine is much easier to obtain from voluntary donors, for large-scale exposure surveys. There are already numerous studies that used urinary metabolites as exposure biomarkers for different types of contaminants. It would be ideal if the presence of PBDE / PBDE-related metabolites in human urine can be used as biomarkers for human exposure to PBDEs. This would greatly facilitate nationwide and international comparisons and public health risk assessments of PBDE exposure.

Pharmacokinetic and toxicokinetic studies of BDE-47 and BDE-99 in mammalian animal models have already identified the presence of Phase II metabolites, mainly dibromophenols and tribromophenol glucuronide and sulfate conjugates, in urine samples. It is hypothesized that these Phase II metabolites of BDE congeners are also presented in human urine and their levels can be used to correlate the extent of exposure to PBDEs. If this hypothesis is validated, a more reliable population exposure assessment for PBDEs can be developed via the measurement of the glucuronide and sulfate conjugates of bromophenols in human urine. To examine this hypothesis, we synthesized a number of glucuronide and sulfate conjugates of BPs and studied the correlation between their levels in human urine and those of their apparent parent BDE congeners in human blood plasma.

WE 137

Improvement of the human respiratory risk assessment by including alkylated PAHs into the analyses

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Polycyclic aromatic hydrocarbons are very important environment pollutants. They are generated during various combustion processes and can be found at high levels at all environmental compartments. Many of them may pose severe health risk. They exhibit induction of aryl hydrocarbon receptor (dioxin-like toxicity) and either estrogenic or antiestrogenic responses, among others. For this reasons, levels of PAHs are often monitored in all environmental compartments.

Their alkyl derivatives are often produced together with the parent compounds. Even though their environmental levels are often comparable to those of PAHs and they can pose similar health risks, levels of alkylated PAHs are rarely monitored. This paper is focused on the assessment of the dioxin-like activity and estrogenicity of alkylated PAH using recombinant rat hepatoma cells (H4IIE-luc) after two different exposure time (6 and 24 h) and human breast carcinoma cells (MVLN), respectively. The atmospheric levels and associated toxic effects of alkylated PAHs were compared to those of the parent compounds in the air samples from the long-term integrated monitoring programme at the Košetice Observatory (the background site of the European Monitoring and Evaluation Programme) to show an importance of including the substituted PAHs when assessing the respiratory risks.

WE 138

Chemical synthesis, characterization and analytical method development bromophenols phase II conjugates as human exposure biomarker for PBDEs

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The occurrence of Phase II metabolites of selected BDE congeners in urines of mammalian animal models has already been well-established by numerous pharmacokinetic and toxicokinetic studies. These metabolites are mainly glucuronide and sulfate conjugates of dibromophenols (DBPs) and tribromophenols (TBPs). To the best of our knowledge, there is no literature report on the occurrence of these Phase II PBDE metabolites in human urines. It would be ideal if these glucuronide and sulfate conjugates of bromophenols (BPs) in human urines can be used as exposure markers for PBDEs. The aims of this study is to synthesize, characterize and purify selected dibromophenyl- and tribromophenyl glucuronide and sulfate conjugates as authentic and calibration standards for the development of appropriate LC-MS/MS analytical method to quantify these dibromophenol (DBP) and tribromophenol (TBP) glucuronide and sulfate metabolites in human urine samples.

Structures of the targeted glucuronide and sulfate conjugates are shown in the Figure 1. Authentic standards of the targeted glucuronide and sulfate conjugates of 2,4-DBP and

2,4,6-TBP were synthesized and purified by in-house procedures. These four BP conjugates were fully characterized by high-resolution mass spectrometry, elemental analysis and ¹H NMR spectroscopy. Their purity was validated by LC-MS with triple quadrupole tandem mass spectrometer. Also, the stability of these conjugates in human urine under various preservation conditions were investigated.

WE 139

A survey of the traditional food consumption that may contribute to enhanced soil ingestion in a Canadian first nation community

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An ethno-cultural survey was conducted of the Xeni Gwet'in First Nations community living in the Nemiah Valley, approximately 230 km west of Williams Lake, British Columbia. The purpose of this study was to assess and document the traditional food consumption and activities of the community that may lead to enhanced exposure to soil. The potential for ingesting soil from consuming traditionally-prepared food was also assessed through the analysis of traditional food items for tracer elements commonly found in soils. The community diet was observed to consist mainly of fish and big game, and was supplemented by berries and roots. Outdoor cultural gatherings, hunting and food gathering trips and sporting events, with their attendant potential for enhanced soil exposure, was observed to be an important facet of community life. The survey concluded that a significant portion of the Xeni Gwet'in practice a lifestyle similar to the subsistence lifestyles of other indigenous communities, where soil exposure scenarios in the order of hundreds of mg d-1 have been proposed. The assessment of a small number of traditional food items concluded that some foods, specifically roots, may have substantial amounts of soil adhering to them that would result in soil ingestion in the order of approximately 30 mg of soil per 100 g serving.

WE 140

Ecotoxicity, cytotoxicity and genotoxicity of PM10 air pollution. Correlation with chemical pollutant levels

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Air is composed by multiple environmental chemicals pollutant mixtures that may pose important adverse health effects, not only because of the effects of individual substances, but also as a result of the potential synergisms/antagonisms among them. Despite that, and because of the difficulties to assess the hazard potential of these mixtures, regulation measures to control the levels of pollutants are traditionally focused on determining the levels of contaminants individually. Air inhalation is known to be an important exposure pathway to some chemicals, such as heavy metals, organic pollutants and particulate matter. Correlation between high air pollution and adverse effects on human populations has been largely recognized, but the toxicology of these adverse effects has not been clarified. In recent years, some surveillance programs have been performed in order to monitor the air quality in industrial (close to incineration and cement plants) and urban areas in Catalonia (Spain). Data on the concentrations of metals (As, Cd, Cr, Hg, Mn, Ni, Pb, Tl, U, and V), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) as well as particulate matter (PM10), obtained by means of high-volume active sampling devices, were measured. The objectives of the present study were: i) to develop and validate a good methodology to obtain the best extraction method of air samples in order to assess toxicity tests, and ii) to evaluate the most appropriated toxicity tests to apply in this specific samples. Aqueous and organic extractions of quartz fiber filters, where PM10 had been retained, were performed in order to evaluate the toxicity of polar and non polar fractions, respectively. Ecotoxicity of extractable PM10 was performed by Microtox® bioassay utilizing *Vibrio fischeri*. Cytotoxicity and genotoxicity were determined by MTT Assay and Comet Assay, respectively, using human lung epithelial cells (A549) as target cells. Preliminary results showed that QFF extraction with a mild acid is a good methodology to obtain significant toxicity results. These air samples presented effect at cellular level but they did not present effect at genetic level. Both Microtox test and MTT assay were appropriate in order to assess the ecotoxicity and cytotoxicity of air samples.

WE 141

Consumers exposure to particle-bound PAHs at night markets in Taiwan

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This study conducts the health risk assessment for consumers exposure to carcinogenic PAHs at night markets. In Taiwan, there are more than 300 night markets that are popular and famous for offering a wide range of local delicacies and traditional snacks. Night market is a very dense outdoor cooking environment. When the concentrations of cooking contaminants are high, the cooking fumes can be harmful to both the cooks and the consumers present in the night markets.

Real-time PAHs concentration detector (Grimm 1.108, Model 130) were used to collect particle-bound PAHs with 16 different diameter channels. 13 samplings were conducted during 27 October, 2011 to 22 November, 2011. It includes 5 weekday night, 5 weekend night, and 3 background samplings. Exposure assessment were assumed that the consumers will spend 40 minutes eating dinner and 20 minutes shopping. Two different exposure concentration were estimated too. This study also used toxic equivalence factor (TEFs) to estimate the potential carcinogenic (BaPeq) for consumer exposure.

The most dominate PAHs concentration were in 2.0-3.0 µm with 14.56% for total PAHs concentration. In weekday, the average PAHs concentration were 280.14 ng m⁻³ and 178.75 ng m⁻³ for eating period and shopping period, respectively. In weekend, the average PAHs concentration were 426.68 ng m⁻³ and 298.78 ng m⁻³, respectively. Results indicate that eating area have higher concentration than shopping area, it may caused by cooking fumes emitted to neighboring area. Results show that the total average BaPeq were 83.32 ng m⁻³ and 129.90 ng m⁻³ for weekday and weekend, respectively. Recreation and diet exposure to cooking emissions in Taiwanese consumer at night markets is of health concern.

WE 142

Metal exposure exacerbates influenza infection related-lung function decrement risk for smelters

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Malnutrition is a main health issue associated with underweight, overweight, and obese groups which are concerned with the increases in the risks of morbidity and mortality worldwide. Metal particle exposure and influenza infection can both lead to lung function deterioration. The objectives of this study were three folds: (i) to construct the dose-response relationship between metal particle exposure and lung function alterations for Fe/Si-metal and Si/Mn/FeMn/FeCr smelters with various body mass index (BMI) groups, (ii) to build up the influenza infection-related dose-response profile of forced expiratory volume in 1 second (FEV1) and respiratory symptoms scores (RSS), and (iii) to assess the potential risks posed by smelting metal dust exposure and influenza infection-related RSS exacerbations among different BMI groups. The results showed that the smelting metal dust Fe/Si-metal and Si/Mn/FeMn/FeCr exposure induced FEV1 reducing rate were 0.59 and 1.11 m³ mg⁻¹, respectively. The estimated BMI-specific FEV1 were 3.41-3.56, 3.56-3.60, 3.44-3.56, and 2.70-3.44 L for underweight (BMI<18.5), normal (18.5≤BMI≤24), overweight (24≤BMI≤27), and obese (BMI≥27) groups, respectively. For Fe/Si-metal smelters, the metal exposure and influenza infection exacerbated RSS with exceedance risk at 0.5 (ER0.5) were estimated to be 0.23-0.35, 0.21-0.23, 0.23-0.33, and 0.33-0.93, for underweight, normal, overweight, and obese groups, respectively. For Si/Mn/FeMn/FeCr smelters, the estimated exacerbated RSS at ER0.5 were 0.24-0.36, 0.21-0.24, 0.24-0.34, and 0.34-0.93, respectively, for underweight, normal, overweight, and obese group. This study provides a novel probabilistic risk assessment framework to better assess occupational health risk given metal dust exposure and influenza infection.

WE 143

Novel approaches for static mesocosm studies with herbicides: linking modelled exposures with the reality of flowing water and other challenges

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A great deal of advance thought and planning is required for many substances before initiating a mesocosm study. Otherwise there is a risk of conducting a study that is of limited regulatory use. Here we will give advice and considerations for mesocosm studies with herbicides with long aquatic half-lives. This will be illustrated using example state-of-the-art mesocosm studies. It is hoped that this presentation will advance mesocosm study design and offer advice to those considering a mesocosm study. Each study required special consideration of the exposure profile in the static mesocosm system versus the reality of flowing water, and a novel design following the principles of E-Link was used.

Along with providing conservative exposure estimates, a number of other technical challenges were faced regarding the experimental design and practical conduct of the studies. The key issues faced will be discussed to drive discussion on alternative approaches for mesocosm testing.

WE 144

Time-dependent toxicity of pesticides and other toxicants: implications for a new approach to risk assessment

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A fundamental goal of toxicology is to determine safe levels of exposure to potentially poisonous substances for humans and the environment. Traditionally, safe levels of a chemical have been derived from the non-observable effect level (NOEL) estimated in laboratory toxicity bioassays with organisms which are representative of certain taxa. There are fundamental problems with the validity of this approach, both conceptual and statistical in nature. Firstly, the outdated NOEL concept is being replaced by the no-effect concentration (NEC) level, which assumes that toxic chemicals do not have any effect on a population of organisms at very low concentrations. Recent developments in ecotoxicology, however, suggest that some toxicants can produce effects at any concentration level provided their exposure time is sufficiently long. Consequently, risk assessment of these chemicals, which includes neonicotinoid insecticides, some carcinogenic substances and certain metallic compounds, may require entirely new approaches. Secondly, the traditional approach to toxicity testing is to consider dose or concentration-effect relationships at arbitrarily fixed exposure durations which are supposed to reflect 'acute' or 'chronic' time scales. This approach measures the proportion of all exposed individuals responding by the end of those fixed exposure times. However, the endpoint values obtained this way cannot be linked to make predictions for the wide range of exposures encountered by humans or in the environment, thus leading to serious underestimates of actual risk. In order to overcome this handicap, an increasing number of researchers are using a variant of the traditional toxicity testing protocol which includes time to event (TTE) methods. This TTE approach measures the times to respond for all individuals, and provides information on the acquired doses as well

as the exposure times needed for a toxic compound to produce any level of effect on the organisms tested. Consequently, extrapolations and predictions of toxic effects for any combination of concentration and time are now made possible. Examples will be shown to demonstrate that this approach is superior to current toxicological testing procedures, and has important implications for risk assessment of chemicals, particularly when the chemical has delayed toxic effects in a time-dependent manner.

WE 145

Linking fluctuating exposure concentrations to effects: a retrospective and a prospective case study

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Two case studies, a retrospective and a prospective case study, are presented in which toxicokinetic-toxicodynamic (TKTD) modelling was applied to predict effects of fluctuating concentrations on aquatic organisms.

In the retrospective case study the prediction of toxicity over time was separated from the extrapolation from one to many species and from acute to sublethal effects. A toxicokinetic-toxicodynamic (TKTD) model predicted toxicity caused by fluctuating concentrations of diazinon, measured by time-resolved sampling over 108 days from three locations in a stream network, representing urban, agricultural and mixed land use. Extrapolation factors were calculated to quantify variation in toxicity among species and effect types based on available toxicity data, while correcting for different test durations with the TKTD model. Sampling from the distribution of extrapolation factors and prediction of time-resolved toxicity with the TKTD model facilitated subsequent calculation of the risk of undesired toxic events. Approximately one-fifth of aquatic organisms were at risk and fluctuating concentrations were more toxic than their averages. Contribution of urban and agricultural sources of diazinon to the overall risk varied.

The second case study was part of the regulatory risk assessment of a pesticide. Here the objective was the prediction of mortality towards fish under fluctuating and pulsed exposure patterns derived from FOCUS-SW simulations. TKTD modelling was used to predict survival of five fish species under fluctuating exposures to the pesticide.

The General Unified Threshold model for Survival (GUTS) was used for TKTD modelling after calibration on standard acute toxicity data (96h-LC50 tests). TKTD model simulations were carried out with the original concentration profiles from TOXSWA as well as with these concentrations multiplied by a factor so that TKTD simulations resulted in 90% survival over 485 days. This factor quantifies the "margin of safety" of each combination of exposure profile and fish species and factors from all combinations of FOCUS-SW scenario and fish species can be aggregated for a probabilistic risk assessment. Survival of fish was simulated for the six FOCUS-SW exposure scenarios for which initial, lower tier risk assessment indicated a risk. The safety margins for these six scenarios and the five fish species ranged from 6.5 to 510.

WE 146

Ecological impacts of time-variable exposure regimes of pesticides on freshwater communities

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The risks of pesticides to aquatic ecosystems are often assessed by performing cosm experiments evaluating a particular exposure regime (e.g. 1 pulse application), not necessarily corresponding with exposure part of risk assessment (e.g. multiple applications). This presentation will focus on two microcosm studies which aimed to compare the effects of different time-variable exposure regimes with the same TWA concentration of the insecticide chlorpyrifos and fungicide azoxystrobin towards freshwater communities. The 1st experiment was performed in outdoor-microcosms exposed to three different regimes of chlorpyrifos; (1) a single application of 0.9 µg a.i./L, (2) three applications of 0.3 µg a.i./L, with a time interval of 7 days and (3) a continuous exposure of 0.1 µg a.i./L for 21 days, based on the same 21d-TWA. In 2nd experiment, azoxystrobin was introduced into microcosms using four different regimes; (1 and 2) a continuous application treatment of 10 µg/L and of 33 µg/L for 42 days, (3) a single application treatment of 33 µg/L and (4) a four application treatment of 15.8 µg/L, with a time interval of 10 days. The treatment levels of single and four applications were based on 42d-TWA of 15 µg a.i./L. Multivariate analyses of the chlorpyrifos, macroinvertebrate data set depicted that taxa belonging to C. dipterum, Phryganidae and Gammarus pulex showed high positive species weight, indicating that they decreased strongly in abundance after chlorpyrifos treatments. By the end of the experimental period, macroinvertebrate PRC displays the same effects magnitude for all treatment regimes, indicating that for long-term effects of chlorpyrifos the TWA is more important than the peak concentration. The PRC diagram of the azoxystrobin, zooplankton data set indicated that taxa belonging to Copepoda (Nauplii, Cyclopoida and Calanoida) and Cladocera (Daphnia longispina) are the most responsive zooplankton species observed, meaning that they are significantly reduced in all azoxystrobin treatments. By the end of the experimental period, zooplankton PRC shows the same effects magnitude for the pulsed treatment regimes, which are placed in between the chronic treatment regimes, indicating that for long-term effects the TWA of azoxystrobin could be more relevant for most zooplankton species than the peak concentration.

WE 147

Structural and functional effects of conventional and low pesticide input wheat and oilseed rape crop protection programmes in outdoor aquatic mesocosms

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This study was designed to evaluate the benefits to aquatic ecosystems of reducing pesticide inputs in crop protection programmes. Models of pesticide transfer (Ganzelmeier curves for drift; MACRO for drainage; PRZM for runoff) were used to estimate fluxes and dates of transfer for each substance under conditions of hydromorphic soils of Northern France. Two contrasted pedoclimatic conditions were considered, which correspond to drained soils of north-western France (Jailrière) and of the northeast of the Parisian Basin (Brie). Each condition was associated to two wheat and oilseed rape crop protection programmes (hereafter named 'Conventional' and 'Low pesticide inputs' which include product substitution and application rate reduction) representative of actual agricultural practices. Model estimates of the concentrations and dates at which pesticide mixtures enter water bodies were used to contaminate outdoor pond mesocosms (9 m³). Three mesocosms were used for each treatment and four ponds were kept as controls. Treatments were performed with commercial products for inputs corresponding to drift whereas active ingredients were used for inputs resulting from drainage and runoff. Water concentrations and effects of pesticide mixtures were followed from November 2008 to October 2010. They concerned water physicochemistry, microalgae (phytoplankton and periphyton), zooplankton (zpk) and benthic macroinvertebrates (bmi). Alder litter breakdown rate (litter-bag method) was used as a descriptor of ecosystem functioning. The results of this two-year survey showed that both 'Conventional' and 'Low pesticide inputs' programmes significantly altered the structure of zpk and bmi communities over the long-term, and agropedoclimatic conditions had no influence on those effects. A transient recovery of bmi was observed only in the ponds receiving the pesticide mixtures from the 'Conventional' programme. For both programmes, reduction in the abundance of shredders resulted in a significant decrease of alder litter breakdown rate, observed in Autumn and Spring. The effects of the two crop protection programmes were mainly associated with exposure to potent insecticides (pyrethroids) and some fungicides (e.g., cyprodinil). These compounds were transferred to the aquatic environment at critical periods resulting in altered structure-function relationships. This should be taken into account for improving the management of environmental risks of plant protection products.

WE 148

Emerging contaminants cause adverse health effects in native species of fish in Southern Alberta Rivers

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The present study was carried out to investigate the presence and adverse effects of emerging contaminants in the Bow and Oldman Rivers in Southern Alberta, Canada.

We provide evidence linking emerging environmental contaminants with health disruption of fish, based on ecological studies, chemical analysis, controlled laboratory experiments, and physiological/biochemical parameters. Emerging contaminants detected in the Southern Alberta rivers include pharmaceuticals, plasticizers, and other organic compounds. To investigate a link between exposures to environmental contaminants and adverse health effect in fish, we performed field studies and collected longnose dace as a suitable sentinel species abundant in most locations. The Oldman River runs through areas with significant agricultural and feedlot activities as well as certain degree of urbanization. We also sampled the Bow River at sites through the City of Calgary, which is a large urban area with three wastewater treatment plants. The findings provide evidence for the presence of emerging contaminants with estrogen-like activity in the Oldman River, based on increased vitellogenin (VTG) expression levels in male longnose dace, correlating with female-biased sex ratios in different regions receiving treated city effluents and agricultural run-offs. The results support the hypothesis that exogenous factors with estrogen-like activity resulted in sex change in longnose dace and caused genotypic males to develop as phenotypic females in the Oldman River. Controlled laboratory experiments testing the effects of chemicals detected at higher concentrations in the Oldman River, individually and as mixtures provided further support for this hypothesis. The mechanisms of endocrine disruption were investigated, using physiological, morphological and targeted transcriptomics as well as 1H-NMR metabolomics. In the Bow River, the nature of contaminants and the adverse effects on fish reproductive health and metabolism were found to be different from the Oldman River. The findings provide evidence for cumulative effects of emerging contaminants present downstream of the three wastewater treatment plants and regions close to golf courses. Overall, we provide evidence that emerging contaminants related to municipal wastewater, agriculture and large cattle operations pose a risk to the aquatic environment in Southern Alberta. This study was funded by NSERC of Canada.

RA18P - Oil spill effects and risk assessment

WE 150

Symbioses - a risk assessment tool for the oil industry in the Lofoten/Barents Sea

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There is increasing interest in oil exploration in the Norwegian arctic, in particular around the Lofoten islands off northern Norway. This area is also the spawning ground for a number of key fish species, including northeast arctic cod and haddock, Norwegian spring spawning herring. The narrow coastal shelf concentrates the fish larvae into a small area during the early stage of their life cycle. It is important to utilize the best available knowledge about the ecosystem in order to assess the risks of

potential development in the area. A range of separate models exist for the distribution of petroleum compounds, ecotoxicology, plankton, larvae and fish in the region. The SYMBIOSES project aims to integrate these into a single tool, which will examine the effects on the major fish stocks of possible oil-spill impacts on larval survivorship, and thus evaluate the combined effects of fisheries and petroleum-related activities in the area. Quantitative measures of uncertainty in the ecosystem and ecotoxicology simulation results will be explored using Bayesian probability networks. The model can then be used alongside analyses of other risks (sublethal effects, local ecosystem, seabirds, etc.) to give improved risk assessments for oil developments.

WE 151

Applying dynamic energy budget theory to estimate the impact of oil components in an Arctic food chain

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The Lofoten and Vesterålen region in Northern Norway is a biologically rich area with one of the most important breeding grounds for North East Arctic cod. After oil and gas reserves were discovered a debate has been ongoing in Norway whether the risks of oil and gas exploration on this biologically rich area are acceptable. A decision on exploration or not is postponed till 2013 and in the meantime efforts are made to quantify these risks.

A 3-year project called SYMBIOSES has been initiated to develop a holistic, integrated modeling framework for ecosystem-based risk and impact assessments of oil components, with a special focus on the effects on fish stocks. In this framework, existing models describing oceanography, oil/chemical fate, the dispersal and growth of zooplankton (the main food of fish-larvae) and fish-larvae and fish stock dynamics are coupled and extended with ecotoxicological algorithms that assess effect of oil on the key parameters of these models.

The Dynamic Energy Budget theory (DEB) will be applied to developed algorithms for the impact of oil components on the life-history processes growth, development and mortality in fish larvae. Existing experimental data on these life-history processes will be used to parameterize DEB. DEB is a mechanistic theory based on the principle that mass and energy are conservative quantities (one of the few hard laws available in biology), energy conversion process leads to dissipation, and mass and energy transport occur across surfaces. DEB describes the energy pathways in individuals. The DEB model is developed as a nonspecies-specific metabolic model that allows differences between species to be reduced to differences in the set of parameter values. The DEB model is tested for many species and deviations are rare. The DEB theory can thus be used to translate results of toxicity tests on reproduction, growth, development and mortality on the energy flow within the organism, and gives mechanistic insight in how a change in growth infers a change in reproduction and development, treating them as dependent. Furthermore, the mechanistic insight makes it possible to extrapolate results beyond the range of tested conditions, and include effects of multiple toxicants, and other factors such as temperature and food conditions.

This paper describes the proposed design plan for the ecotoxicology algorithms and presents preliminary results.

WE 152

Delayed effects of acute oil exposure on *Calanus finmarchicus* (Gunn.) reproduction

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Delayed effects of acute exposure to dispersed oil on copepod reproduction were assessed. Following a 96 hrs exposure to three concentrations of oil dispersions (0.25, 2.5 and 25 mg/L) generated from a North Sea crude oil, several reproductive endpoints (offspring production rates, egg hatching success, female reproduction participation, nauplii development) were studied in *Calanus finmarchicus*. A delay in the initiation of reproduction was observed for the group exposed to the highest dispersion concentration accompanied by a significant decrease in the number of females participating in reproduction was observed. However, a subsequent compensatory reproduction in this group, displaying reproduction rates higher than controls, after a 10-day recovery period was observed. 13 days post exposure no differences in egg production rates or hatching success were found between controls and oil-treated copepods. At the end of the experiment, the developmental stage distributions of offspring were comparable between all groups. The results indicate that, although a short-term exposure delays reproduction, copepods are capable of reproducing viable offspring after cessation of exposure. In real life, such delays may however, have ecological consequences due to relatively short periods of food accessibility (algal bloom) in cold waters. These aspects are important when assessing ecological consequences following an acute oil spill to Arctic and sub-Arctic marine environments.

WE 153

Rational application of chemicals in response to oil spills may reduce environmental damage

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Oil spills, for example those due to tanker collisions and groundings or platform accidents, can have huge adverse impacts on marine systems. The impact of an oil spill at sea depends on a number of factors, such as spill volume, type of oil spilled, weather conditions, and proximity to environmentally, economically, or socially sensitive areas. Oil spilled at sea threatens marine organisms, whole ecosystems, and economic resources in the immediate vicinity, such as fisheries, aquaculture, recreation, and tourism. Adequate response to any oil spill to minimize damage is therefore of great importance. The common response to an oil spill is to remove all visible oil from the water surface, either mechanically or by using chemicals to disperse the oil into the water column to biodegrade. This is not always the most suitable response to an oil spill, as the chemical application itself may also have adverse effects, or no response may be needed. In this article we discuss advantages and disadvantages of using chemical treatments to reduce the impact of an oil spill in relation to the conditions of the spill. The main characteristics of chemical treatment agents are discussed and presented within the context of a basic decision support scheme.

WE 154

Prediction and effects of oil dispersant application on marine organisms in Tokyo Bay

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Heavy oil spill accidents that are happening in the world. Spilled oil attacked coastal area and adversely affected on aquatic organisms. During oil spill, dispersants have been applied to disperse spilled oil quickly. However, one cannot clarify whether application of dispersant to spilled oil is an effective method or not in the environment.

In this study, the distribution of virtual spilled oil in Tokyo Bay, where many boats as well as tankers cruise, was simulated by a model using a decision making process tool for oil pollution on GIS (DOG). The oil concentrations in the water and their risks on marine fish were predicted based on the oil water accommodated fraction (WAF) toxicity on marine fish. Emulsifying oil by dispersants increase oil concentration and lead to increase of toxicity on aquatic organisms. In addition, the risk of emulsified and dispersed oil will be increased in the area of small exchange of sea water, while the risk will be decreased in the area of large exchange of sea water. Furthermore the strong wind is expected to disperse spilled oil greatly, thus the difference in oil distribution can be small between application and no application of dispersant

WE 155

Pilot scale application for monitoring and optimization of petroleum hydrocarbon bioremediation

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Petroleum contamination of sandy soils is a severe environmental problem in Libya but relatively little work has been carried out to optimize how bioremediation of such poor soil can be carried out.

The purpose of this research is to determine the potential for bioremediation of hydrocarbon-contaminated soil obtained from oil refinery field, Libya. Hydrocarbon degrading microbes are present in the soil and that nutrient addition could increase hydrocarbon transformation. Therefore it was decided to attempt pilot-scale field studies using a land-farming technique to treat contaminated soil.

Plots containing 500 kg soil (triplicates) (contaminated soil diluted with clean soil 50% volume) were set up containing different nutrient levels Urea + NPK to obtain a C: N:P ratios 100:10:1, and monitored for 90 days. Hydrocarbon levels, microbial numbers and toxicity assessment (using luminescent microbial based tests) were determined throughout the experiment. Hydrocarbon levels started at 20 733 - 22 366 ppm and diluted soil 16 000 - 17 000 ppm. Although all the land treatment units revealed a significant hydrocarbon reduction over time, the highest reduction on hydrocarbon obtained was 66 % on LTU1 ratio (100:10:1) and 63 % on LTU5 diluted soil amended with Urea + NPK ratio (100:10:1) respectively, whereas the lowest reduction obtained 34 % was on LTU4 control diluted soil. This suggests that the addition of nutrients increased the rate of hydrocarbon bioremediation.

The percentage of light inhibition obtained by this assay was in good correlation with the reduction in total petroleum hydrocarbon determined, probably because both nutrient ratios used representing the right amendment needed.

WE 156

Influence of environmental parameters on oil spill effects using Taguchi experimental design methodology

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The MIGR'HYCAR research project (www.migrhycar.com) was initiated to provide decisional tools, and satisfy operational needs, for risks connected to oil spill drifts in continental waters (rivers, lakes, estuaries). These tools aim to serve in the decision-making process once oil spill pollution occurs and/or as reference tools to study scenarios of potential impacts of pollutions on a given site. This paper focuses on the study of the distribution of water soluble components from oils in water as function of environmental parameters.

Seven petroleum oils covering a representative range of commercially available products were tested. Dynamic tests at laboratory scale were performed to study 41 polycyclic aromatic hydrocarbons (PAHs) and derivatives, among them 16 EPA priority pollutants. The coupling of the stir bar sorptive extraction (SBSE) and high performance gas chromatography mass spectrometry (HPGC-MS) was used. Limit of quantification were lower than 1 ng.L⁻¹ for each compounds. Experiments were performed according to Taguchi's experimental design methodology. Four parameters were selected: temperature, suspended solid quantity, salinity, oil:water surface ratio. Each of these parameters was of three different levels in order to be representative of continental waters. 150g of Milli-Q water were introduced in a 250mL beaker. Oils were deposited on water surface before starting a gentle magnetic stirring for 2h without vortex. The beaker was placed in thermostatic chamber and darkness to avoid PAHs photodegradation. A large variation in composition of the water soluble fraction depending on oil type was highlighted. The chemical profile of the water soluble fraction was unlike that the parent oil profile due to the different water solubilities of the various oil components. Semi-volatile compounds (naphtalenes) constitute the major part of the water soluble fraction. For Heavy fuel and Jet, all parameters have to be taken into account. For Bitumen, only suspended-solid quantity can be neglected. For Domestic fuel, Gasoline and Diesels, salinity and suspended-solid quantity can be neglected. For all oils, the water soluble fraction was minimised with low temperature. For all oils except, Bitumen and Heavy fuel, the water soluble fraction was minimised with high surface ratio.

WE 157

Hopanes and steranes in recent sediments of the Continental Shelf of Yucatan, Mexico

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The relevance of molecular markers in oil monitoring and environmental impact studies for fingerprinting is evident due to frequent oil accidents. The need for baseline studies that include oil molecular markers can clearly establish the level of impact to the environment caused by oil spill. This is the case of the continental shelf of Yucatan, which has been documented that could be affected by the Deep Water Horizon oil spill. The objectives of this study were to determine the levels, differences in distribution and abundance of these compounds, in sediments of the continental shelf of the Yucatan Peninsula. These values were compared with several Mexican oils and the Deep Water Horizon oil. As regard to the sediments samples the 17 α 21 β 30 Norhopane, 17 α 21 β Hopane and 17 α 21 β 22 29 30 Trisnorhopane were the compounds that showed that highest levels. The highest concentrations of tricyclic and tetracyclic terpanes were found on the west of the study area; this distribution is due to the proximity to the Mexican oil extraction zone, in the case of the pentacyclic terpanes the highest levels were found in the deeper zone of the study area. The abundances of hopanes in the sediments samples, the Mexican oils and the Deep Horizon oil showed significant differences. Salient compositional characteristics were: for the sediments samples, the homohopanes/C30 hopane ratio showed the lowest levels, the presence of the homomoretane which not were detected in the oils, and the absence of Tm. The Deep Horizon Oil presented a Ts/Tm ratio greater than 1, a low C29/C30 ratio, high C34/C35 ratio, presence of C30 steranes and low levels of homohopanes which were indicative of a marine deltaic environment origin. The Maya, AKM, heavy Dos Bocas and Olmeca oils had a Ts/Tm ratio lower than 1, a high C29/C30 ratio, a lower C34/C35 ratio, presence of C30 steranes and high levels of gammacerane and homohopanes, which were indicative of a carbonate marine environment origin. Finally, Istmo and light Dos Bocas oils had a Ts/Tm ratio lower than 1, a high C34/C35 ratio, the presence of gammacerane, low levels of diasteranes and a high abundance of tricyclic terpanes were indicative of an origin in a saline lake environment.

WE 158

Trace metals in recent sediments of the Continental Shelf of Yucatan, Mexico

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Oil spills around the world can affect large geographic areas. Such were the cases of Exxon Valdez, Ixtoc I, and most recently the Deep Water Horizon spill. The present study shows the distribution and baseline concentrations of trace metals in the Yucatan continental shelf, before the possible impact of the Horizon Deep Water spill. Circulation patterns of the Gulf of Mexico indicate a high probability of oil spill transport to the coast of the Yucatan Peninsula, including hydrocarbons and metals present in petroleum. Barium showed high values in the area near Cayo Arenas, site where there are Mexican oil activities, and on Holbox Island area, probably due to fertilizer use. The median observed in the case of barium was 24,894 ug/g with a maximum value of 68,24 and a minimum of 2,723. In the case of nickel, the highest concentrations of this metal were far from the coast. The median observed in the case of nickel was 0.477 ug/g with a maximum value of 4.761 and stations with values below the detection limit. Nickel as barium, showed consistency throughout the study area. For vanadium, high values were observed at both ends of the peninsula, on the west agree with the approach of Cayo Arcas oil activity area, those on the east coincide with the area of Holbox Island, high levels can be attributed to possible groundwater discharges that come from the peninsula due to the use of fertilizers and/or organisms such as sea squirts, which store large amounts of vanadium. The median observed in the case of vanadium was 0.393 ug/g with a maximum value of 3.769 and stations with values below the detection limit. Negative correlations between barium and nickel and a positive correlation between vanadium and barium were found. Barium exceeded in three stations the reference value proposed by the NOAA (AET of 48 ug/g). In the case of the values found for nickel and vanadium in the study area, none of them exceeded the reference values of the TEL and PEL. Correlations between metals and hydrocarbons are showed.

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Toxicity of mixtures of petroleum products to the marine organism *Mysidopsis juniae*

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In Brazil, after the discovery of the Pre-salt layer, a big reservoir of oil that extends from the northern Campos Basin to the south of the Santos Basin, new technologies are being sought for the exploration and exploitation of oil. Questions about the level of preparation for the exploitation of reserves at great depths are uncertain as well as the level of safety of such operations too. Environmental accidents such as the one in Gulf of Mexico (USA) and the recent one in the Campos Basin (Brazil) in November 2011, raise the alertness of the gravity of high levels of the toxic oil and its products, especially diesel and gasoline. Gasoline consists of a wide range of components, and benzene, toluene, ethylbenzene and xylene that is known to pose more hazard to the environment. Similarly for diesel, the polycyclic aromatic hydrocarbons, naphthalene and benzo(a)pyrene, they are all considered hazardous. This study evaluated the toxic effect of various concentrations of diesel gasoline ratio in the test organism *Mysidopsis juniae*, a small marine crustacean, habitat of coastal regions and a standardized test organism for toxicity testing in Brazil. In order to conduct the experiment we prepared two stock solutions of 1 liter, containing 50 mL of diesel in the first and in the second 50 mL of gasoline. To each, 950 mL of deionized water is added to complete. The solution was stirred continuously for 24 hours. The portion of fuel water soluble fuel was used for the preparation of experiments. We conducted a full factorial design 32 in triplicate, using levels of 0.5%, 2.5% and 5% (volume / volume) of two fuels from the reconstituted stock solution from seawater of salinity of 32. Five individual organisms between the age of 3 and 5 days are placed in each solution environment. The organisms were fed with *Artemia* sp nauplii. The methodology used included readings of the test for the observation of mortality with 24 and 48 hours after the exposure. At 24 hours, there was total mortality of organisms at all concentrations, independent of the proportions diesel gasoline ratio. We suggest further studies to detect the minimum levels of contamination of these fuels that can cause acute toxicity, as well as kinetics and chronic toxicity which can be of use as control measure in case of environmental accidents.

WE 160

Acute toxicity and kinetics of diesel fuel using *Mysidopsis juniae* microcrustacean

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One of the main goals of toxicology is to identify the risks associated with a known substance and determine under what conditions these risk exposures are induced. In Brazil, about 20% of the oil is converted into gasoline and 36% in diesel. Comparing to gasoline, diesel oil has larger chain length molecules which confers on it higher specific density, lower volatility and lower solubility in water. This study is aimed at analyzing the effect of different concentration of diesel oil on test organism *Mysidopsis juniae* over a period of 48 hours. In order to carry out the test, a stock solution with 50 mL of diesel oil in 950 mL of deionized water, totaling 1 liter was prepared. This mixture was stirred continuously for a period of 24 hours to achieve separation of water-soluble portion. For the toxicity trials concentrations used were 0.1%, 0.5%, 1%, 1.5%, 2%, 4% and 10% (volume / volume) prepared in reconstituted seawater in 200 mL flasks. To each of these concentrations, 5 organisms between the ages of 3 and 5 days were added. The test was performed in triplicate. The test organism used was microcrustacean *Mysidopsis juniae* (SILVA, 1979), from the cultivation of the Laboratory of Ecotoxicology at the Regional University of Joinville. Even in small concentrations, 100% mortality of the organisms occurred after 2 hours of exposure. It was suggested that further studies should be developed to identify the minimum concentration that is toxic to test organisms using lower concentrations of the soluble portions. This result can be of benefit for use as control measures to minimize environmental impact in cases of accidents involving oil and its products.

WE 161

Acute and kinetics of toxicity from soluble fraction of the gasoline used in Brazil using *Mysidopsis juniae* as test organism

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Accidents resulting from leaks and oil spills have worried the industry, the government and the civil society. Of all the petroleum products, it is important to evaluate the effects of gasoline when it gets in contact with water. The monoaromatic hydrocarbons present in gasoline such as benzene, toluene, isomers of xylene and ethyl benzene (aromatic fraction), called BTEX compounds are considered carcinogenic. In the event of a spill these are the components that will dissolve in water readily. The aim of this study is to evaluate the acute toxicity and kinetics of the toxicity of gasoline. This study was conducted at the Laboratory of Environmental Toxicology UNIVILLE - in São

Francisco do Sul. In order to carry out this test a stock-solution was prepared with 50 mL of gasoline and 950 mL of deionized water, amounting to 1 liter. This mixture was stirred continuously for a period of 24 hours in order to separate the water-soluble portion. For the toxicity tests concentrations used were 0.1%, 0.5%, 1%, 1.5%, 2%, 4% and 10% (volume / volume) prepared in seawater reconstituted in 200 mL flasks. In order to set up the test, 5 *Mysidopsis juniae* between 3 to 5 days old were placed in each flask, with reconstituted seawater. The test conditions were 32 for salinity, 12h light and 12h darkness for photoperiod and 25 °C for temperature. The organisms were fed once a day with *Artemia* sp. nauplii. The count was made every 2 hours to complete 12 hours and after which the count is made every 12 hours to complete 48 hours. Bioassays were performed in triplicate. The LC50 (48 h) calculated by probit method and was 1.97% (concentration of the soluble fraction). For the kinetics of toxicity was possible to achieve acute effects after 6 hours of test. At concentrations below 2% the effects were absent after 36 hours of testing. At 4% concentration, the effects remained until the 48 hours, while at 10% concentration mortality of the population after 2 hours of test was 100%. The use of *M. juniae* made it to be possible to evaluate the toxicity of gasoline and it is necessary that the substance is handled with care to minimize environmental risks.

WE 162

Toxicity, fractionation, identification and potential Estrogenic effects of acid extracts of oil sands process waters

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At present there is a great deal of interest in the potential environmental effects of unknown acidic organic pollutants in the process waters of oil sands, particularly those of Alberta, Canada. However, since oil sands have also now been discovered in Russia, Venezuela and the Congo, pollution from oil sands processing will likely have further reaching implications. When factored in with other potential sources, such as industrial uses and discharges, oil produced waters and biodegradation of oil spills it is clear that pollution from acidic oil-derived waste is not just a Canadian problem. Advances in the analysis of this acidic waste by methods such as comprehensive multi-dimensional gas chromatography-time of flight mass spectrometry, have made it possible for us to identify individual components of the acid extracts and this in turn has enabled us to conduct targeted toxicity tests, both of individual acids and of simple mixtures of 40 acids. The latter confirmed concentration addition by non-specific narcosis. By applying thin layer silver ion chromatography to fractionate the acidic extracts we have also recently been able to tentatively identify steroidal type acids within oil sands process water; these may account for observed endocrine-disrupting activity. A complementary approach is to synthesise these acids in order to aid positive identification and to permit relevant toxicity testing. This synthesis is currently in progress.

RA22P -The use of rodenticides, a nagging issue on effectiveness and risks

WE 163

Risk mitigation management for rodenticides

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Risk mitigation management for rodenticides

1. Introduction

Rodenticides are regulated in Europe as biocides or as plant protection product regulated under Council while in North America are regulated under restrictive federal legislation. Some of second generation AVKs, are known PBT-compounds, which would normally restrict their use: notwithstanding that, are considered as "essential use" products, because of a lack of acceptable alternatives.

2. Risk mitigation measures

Non target will be put at risk also related to the quantity of bait needed. In this sense, products should be available in non-refillable bait stations, tamper resistant, which do not allow access. The authorised use could be limited for the outdoor use to professionals only, whilst the amateur use in a ready-to-use product may be restricted to indoor use. The most appropriate risk mitigation measures is closely linked also to the design and pack size as well as composition and formulation type. The inclusion of a bittering agent may reduce accidental ingestion, while inclusion of a blue dye renders the product unattractive. Interpretation of the term 'professional' and all the linked subcategories can cause possible diverging interpretations: harmonized definition of "trained" at the moment fulfill different or no requirements among EU. Instead of using words as "domestic" or "amatorial", an unique definition of "non professional" should be taken into account. Rodenticide products shall be labelled in accordance with preparations directive, however, it may not be sufficient for some special risks. Thus, in addition, all packaging of anticoagulant rodenticides need to show specific safety precautions in the form of additional harmonized standard phrases.

3 Conclusions

The safe use of rodenticide products is highly dependent on the application, formulation and weight of sold baits. 2nd generation anticoagulant substances might have to be reviewed again after Annex I inclusion as candidates for comparative assessment. Specific phrases for special risks and safety precautions should be defined, in addition to good practice guidelines and training for professional users.

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WE 164

Risk reduction in rodenticide use - does it work?

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Rodenticides, in particular the second generation anticoagulants (SGAR), are very potent toxins. In the EU they are regulated as biocides by the Biocidal Products Directive (BPD, 98/8/EC) and also as plant protection products by the Directive 91/414/EEC (PPPD). There are today five active substances included in Annex I of the BPD, i.e. approved for use in biocidal products (brodifacoum, bromadiolone, difenacoum, difethialone and flocumafen) and two of those (bromadiolone, difenacoum) are included in Annex I of the PPPD. Their strong effect is similar on target organisms as well as non-target organisms and the environmental risk assessments of these compounds conclude that the risk for primary and secondary poisoning is very high. Studies on wildlife have confirmed this and SGARs have been found in non-target animals such as other rodents, predatory birds and mammals. The SGARs are approved only because there is a strong need to control rodents like rats and mice and because the existing alternatives are not sufficient to carry out this task.

Associated to the approvals of these compounds are a number of risk reduction measures, e.g. use of secured bait stations, use in limited time periods, recovery of dead animals and leftover bait and also the restriction to professional use only. The extent to which these measures shall be used is not specifically regulated and their efficacy is generally not evaluated. An important reason for this is that it is very difficult to perform such evaluation and especially to be able to draw conclusions from any measure taken with regard to a specific (observed) reduction in risk, e.g. secondary poisoning.

Presently, product evaluations of rodenticide products are carried out in EU member states, and the implications of imposing risk reduction measures become of immediate interest. Examples of measures that are used are restriction to professional use only and the use of secured bait stations. Despite the obvious problems with evaluation of efficacy, requirement for such evaluation has been included in the approval for SGAR as active substance in plant protection products. Is this at all possible, and if so, how should it be accomplished and what studies will be needed? The presentation will discuss this problem, which is of central importance for future risk assessment of rodenticides and for driving the development of new methods having less environmental impact.

WE 165

Application of a human dimensions and landscape ecology framework to evaluate rodenticide use and human-wildlife interactions within ecosystems

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Research on Second Generation Anticoagulant Rodenticides (SGARs) often involves the toxicokinetics and -dynamics of products within organisms and observation of secondary non-target effects among wildlife. Fewer studies have focused on details about the human mechanisms and drivers of SGAR use. For example, product selection, use behaviors, target species, and interactions between human behaviors and characteristics of the broader landscape may influence human-wildlife conflict that results in rodenticide use. My objective is to use results of past and ongoing research to illustrate a conceptual framework for how rodenticide use may be used as a process for understanding both human-wildlife conflict, and human interactions with wildlife and nature within ecosystems. Human land use practices may contain attractive habitat characteristics that draw wildlife into proximity of residences, resulting in infestation and/or damage. Human reaction to wildlife presence, regardless of whether wildlife species are exotic or native, may result in behaviors that include rodenticide use both indoors and outdoors. Outdoor product use, or the result of animal consumption of products indoors followed by travel outside, can result in availability of raw or ingested products that may be consumed by wildlife. Humans also may seek to repair damage caused by perceived pests and in turn reinforce landscape characteristics attractive to wildlife. Therefore, application of a human dimensions and landscape framework not only allows for evaluation of human mechanisms of rodenticide use, but a greater understanding of human-wildlife interaction within landscapes. Such information can aid assist both product manufacturers and policymakers in designing products and application procedures that both meet consumer needs for pest control and minimizing impact of products on non-target species.

WE 166

Secondary exposure of Red Kites (*Milvus milvus*) to anticoagulant rodenticides

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Red Kites became extinct in Scotland in the late nineteenth century but a recent reintroduction programme has resulted in the re-establishment of a breeding population. Science and Advice for Scottish Agriculture (SASA) operates the Wildlife Incident Investigation Scheme (WIIS) in Scotland and routinely monitors raptor carcasses for anticoagulant rodenticide residues. Residue testing of tissues samples from more than 100 red kites over the last decade has found that around 80% of individuals contained measurable rodenticide residues and > 30% contained multiple residues. In addition, around 20% of the sample had total residue levels of > 0.2 mg/kg, a concentration that has been correlated with clinical evidence of rodenticide related mortality in several raptor species. In this sample, rodenticide exposure was attributed as the cause of death in

ca 20% of individuals, although not all were confirmed by post mortem and the data included some individuals that may have been deliberately poisoned. Although this is a limited dataset, patterns of red kite exposure were fairly consistent over time and were significantly greater than found in a range of other UK raptor species. Whilst, it has been hypothesised that the intensive monitoring involved in reintroduction programmes may increase the likelihood of carcass analysis and residue detection (Carter & Burn, 2000), it appears that the red kite's lifestyle and feeding ecology make it particularly vulnerable to rodenticide poisoning. SASA also conduct biennial surveys of rodenticide use on arable farms and there is a correlation between the relative quantity of active ingredients applied and their detection rates in tissues. These data suggest that further quantification of exposure and associated risk of rodenticides to red kites is warranted and that possible deficiencies in working practice and compliance among rodenticide users in areas where red kites are resident may need to be addressed.

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Anticoagulant rodenticides levels in Euroasian eagle owls (*Bubo Bubo*) from Southeastern Spain

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Anticoagulant rodenticides (AR) have not been routinely included in biomonitoring studies. However, this type of toxicants may pose a risk for birds, especially those that feed on rodents. In addition AR have shown a high frequency of detection in bird samples from UK and USA, including in animals without haemorrhages. In contrast, data regarding rodenticide levels in Spanish wildlife are restricted to cases of suspected poisoning. In Southeastern Spain, the Eurasian eagle owl can live close to agricultural areas where AR could be used. In order to evaluate AR exposure, liver samples were obtained during necropsy from 18 Eagle owls that arrived dead or died at the Wildlife Rehabilitation Centre "Santa Faz" (Alicante). Also, blood samples from 9 adult Eagle owls that were caught between 2008 and 2010 and 41 chicks born in the same period were analysed. Samples were extracted with acetonitrile to look for the 5 most frequently used AR in Spain and analysed with LC-MS/MS. AR were detected in 83% of the samples, being difenacoum, brodifacoum and bromadiolone the most abundant and frequent compounds (82%, 64% y 17% respectively). These most common compounds figure among the highest number of products available on the Spanish market which suggests a higher frequency of use. The lack of detection in blood could be due to the fast transport of AR to the liver, where they can persist up to a year. At necropsy, only one individual presented haemorrhages. Although this owl also presented the highest levels of rodenticides (total=332.78 ng/g), and levels between 100-200 ng/g ww of AR have been related to toxic effects in owls, the lesions are probable due to a shot, which caused its admission in the Rehabilitation Centre. Levels above 100 ng/g ww were found in two other owls that died due to electrocution and traumatism. It should be recalled that the impact of AR must extend well beyond those cases in which acute lethal haemorrhage is the proximal cause of death. Sublethal haemorrhages may alter locomotion or cause lethargy, predisposing animals to predation, accidental trauma or reduced food intake. Because 72% of these individuals presented levels above 10 ng/g ww, which are related to subcutaneous haemorrhage, we could suggest that these compounds could be involved in the cause of death. Acknowledgements: Generalitat Valenciana for providing the samples. Supported by the Spanish Government (CGL2004-5959/BOS, CGL-2008-4318/BOS), Seneca Foundation (08758/PI/08).

WE 168

Monitoring impacts of vertebrate pesticides in the UK: 1993 to 2007

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The Wildlife Incident Investigation Scheme (WIIS) is a pesticide post-registration monitoring scheme operated in the UK. Incidents in which pesticides are involved in non-target casualties are recorded, their circumstances investigated and, where possible, attributed to cause. This paper describes 1116 WIIS incidents that occurred between 1993 and 2007 and involved non-target casualties caused by exposure to vertebrate pesticides, including the anticoagulant rodenticides used for rodent control.

WE 169

Poisoning or exposure? Birds of prey and anticoagulant rodenticide residues from the Wildlife Incident Investigation Scheme in England

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The Wildlife Incident Investigation Scheme (WIIS) is a reactive surveillance scheme that monitors the suspected pesticide poisoning of wild animals, companion animals (pets) and beneficial insects. The suspected pesticides include plant protection products and biocides used to control vertebrate pests and similar schemes operate in England, Wales, Scotland and Northern Ireland. WIIS is partly funded by a levy on pesticide sales in the UK and it is co-ordinated by the Chemicals Regulation Directorate of the Health and Safety Executive (HSE), which is the lead authority on pesticide legislation. The Wildlife Incident Unit has maintained the historical records of WIIS data for over forty years from England and Wales and many of these investigations have included birds of prey. The feeding habits of some species of birds of prey, particularly red kites, mean that they are vulnerable to exposure and possible poisoning from anticoagulant rodenticides. Their visibility, size, conservation status and the re-introduction programmes for some species eg. red kite, also mean that they are more likely to be found, reported and accepted for investigation by WIIS. Over the years, due to the greater use of LC-MS/MS, there has also been increased testing, certainty and sensitivity of the analytical methodology. All these factors contribute to an increase in the number of possible lethal and sub-lethal anticoagulant rodenticide residues that have been reported by WIIS. Where poisoning with an anticoagulant rodenticide is detected, it may be difficult to trace all likely sources of exposure in the area of the incident despite the detailed field investigations that may be carried out as part of WIIS. Therefore, how the poisoning occurred may remain uncertain. However, if enough evidence can be gathered, and an infringement of pesticide or environmental legislation is established, appropriate enforcement action may be taken by the Police, HSE or the local authority. The results from WIIS are part of the pesticide regulatory process and they are reported quarterly on the internet. This poster will review the suspected anticoagulant rodenticide poisoning and residue data for birds of prey over several years, from 1995 to 2010.

WE 170

Resource use of the red fox (*Vulpes vulpes*), as revealed by stable isotopes, uncovers variation in exposure to anticoagulant rodenticides

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Anticoagulant rodenticides (ARs) are widely used to control commensal rodent species. However their use can lead to the exposure of non-target predatory species. Exposure varies widely between populations of predators but the reasons for the variation in occurrence is poorly understood. Diet is undoubtedly a contributing factor but few studies have shown a clear link between the occurrence of ARs and diet of a predator. We therefore use stable isotopes to infer resource use in a population of red foxes (*Vulpes vulpes*) in an attempt to explain variation in the occurrence of ARs. Foxes exposed to ARs fed at a higher trophic level than foxes with no residues, suggesting different resource use strategies between the two groups. It is likely that the differences in resource use are a reflection of the habitats that foxes were sampled from. This is because the prey species likely to have contain ARs residues (target and non-target small mammal species such as brown rat (*Rattus norvegicus*) and wood mouse (*Apodemus sylvaticus*)) from the same habitats also showed a difference in trophic position. This is the first study of its kind to show a direct relationship, without inference, of how an animal's resource use relates to exposure to ARs.

WE 171

Sustainable control of grassland small mammals: reducing the need for rodenticides and the risk for non-target species by a multi-scale approach

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Small mammals such as the European rabbits, the plateau pikas, the prairie dogs have been traditionally perceived as pests and targeted for control on a large scale despite their importance as key-stone species in their native ecosystem. This is also the case for the fossorial form of the water vole (*Arvicola terrestris*). This species can reach large population densities and causes heavy damage to grassland with subsequent economic losses for farmers (9,000 to 25,000 € for a 70 ha farm during an outbreak year in Franche-Comté, France). However, higher population densities of *A. terrestris* and also of *Microtus arvalis*, help maintaining a large and rich community of vole predators. Such non-target species (red kite, common buzzard, wild boar, red fox, etc.) are impacted, some of them heavily, by the unconditional use of rodenticides (e.g. bromadiolone). In order to minimize the use of rodenticides in controlling *A. terrestris* populations, a long term research program has been undertaken since the late 80s, aiming at identifying the key-parameters of such regional systems. Based on a systems approach, this collaborative research network involves academic researchers, farmer, game, conservationist organizations and governmental and local administrations together. On a regional scale (area of about 2500 km²), Delattre et al. (1992), Giraudoux et al. (1997), Fichet et al. (2000) provided evidence that *M. arvalis* and *A. terrestris* population dynamic patterns correlate with land composition. On a sectorial scale (area of about 25 km²), Delattre et al. (1996), Delattre et al. (1999) showed for *M. arvalis* that landscape heterogeneity dampen population fluctuations and may modulate prey/predator relationships; Duhamel et al. (2000) that *A. terrestris* outbreak epicentres occur in homogeneous grassland and Foltete et al. (2008) that hedgerow networks slow down the propagation of travelling waves. On a local scale (area of about 0.01 km²), Delattre et al. (2006), Morilhat et al. (2007, 2008) showed that *A. terrestris* population growth was enhanced by larger gallery networks of *Talpa europaea*, was slowed down by ploughing and cattle tramping and was modified by neighbouring landscape (10 - 100 ha). This led to a number of recommendations, now implemented for a more sustainable control of *A. terrestris* (Delattre and Giraudoux 2009) with substantial decrease in rodenticide utilization and lesser impact on non-target species.

WE 172

Determining critical liver concentrations of second generation anticoagulant rodenticides in Canadian predatory bird species

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Second-generation anticoagulant rodenticides (SGARs) are widely used to control rodent pests but exposure and poisonings occur in non-target species, such as birds of prey. Liver residues are often analyzed to detect exposure in birds found dead but their occurrence is now so widespread that their use to assess the toxicity of SGARs is problematic. We analyzed published data on hepatic rodenticide residues and associated symptoms of anticoagulant poisoning from 270 birds of prey using logistic regression to estimate the probability of toxicosis associated with different liver SGAR residues. We also evaluated exposure to SGARs on a national level in Canada by analyzing 196 livers from great horned owls (*Bubo virginianus*) and red-tailed hawks (*Buteo jamaicensis*) found dead at locations across the country. Calculated probability curves suggest significant species differences in sensitivity to SGARs and significant likelihood of toxicosis much below previously suggested concentrations of concern. For example Great horned owls showed a 10% probability of exhibiting toxicosis at 0.03 mg/kg liver wet weight. This is much below the proposed thresholds of 0.1 - 0.2 mg/kg (Newton et al. in litt.) or the 0.7 mg/kg proposed by the Rodenticide Registrants Task Force. If the latter value was accepted as a threshold, we estimate this would place 15 to 20% of all raptorial species at risk of acute toxicosis. Of the two species for which we had samples from across Canada, great horned owls are exposed to SGARs to a greater extent than red-tailed hawks and liver residue levels were also higher. Using our probability estimates of effect, we estimate that a minimum of 11% of the sampled great horned owl population was at risk of being directly killed by SGARs. It is likely the risk is now higher because most of the samples underpinning this analysis were from the 1990's. This is the first time the potential mortality impact of SGARs on a raptor population has been estimated.

WE 173

Bioaccumulation of bromadiolone and chlorophacinone in anticoagulant-resistant rat strains

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Introduction. Anticoagulants (AVK) are the only available compounds in the EU to control rat populations. Resistance to AVK is described and widespread across Europe. Our objective was to determine whether resistance was associated with an increased potential for bioaccumulation of AVK in the liver. We selected rats from three major resistant genetically identified strains across Europe: Y139C (Germany), Y139F (France) and L120Q (UK). Rats were housed in individual cages and fed chlorophacinone wheat-bait (50 mg/kg) daily for up to 14 days. Bromadiolone, a more potent 2nd generation AVK was distributed on days 1, 4, 9 and 13. Animals were assigned to groups for euthanasia either on day 1, 4 (all rats), 9 or 14 (resistant rats). AVK liver residues were determined by an internal, validated HPLC method with limits of detection of 0.02 µg/g. Non-parametric tests were used to compare results between groups.

Results. Food or bait consumption was fairly consistent across strains and only showed a lower amount ingested on day 1 (bait shyness). PT measurement showed that the resistant rats could tolerate higher amounts of both chlorophacinone and bromadiolone, since susceptible rats all displayed very high values (>120s) after only 4 days, while the resistant strains had either moderately increased PT values (Y139C and Y139F) or unaffected PT values after 4 to 9 days (L120Q). Bromadiolone induced fairly similar results.

Chlorophacinone accumulated from day 1 through Day 4 in all strains (max 160 µg/liver) and remained stable thereafter. There was no significant difference between strains. Intensive metabolism of chlorophacinone was also evidenced and similar (nature and proportion of metabolites) across strains (3 OH-metabolites identified). Only the survival time differed significantly (L120Q>Y139C=Y139F>susceptible). All bromadiolone results will be included at the time of the presentation.

Conclusions. Accumulation of chlorophacinone occurs from day 1 through 4 and an equilibrium is reached, suggesting rapid elimination (estimated half-life 4-5 days).

Resistant and susceptible rats accumulate chlorophacinone to the same extent and only differ in terms of survival times. Resistant rats may then be a threat for non-target species for prolonged periods of time (up to 14 days vs 3-4 days).

WE 174

New developments for the analysis of rodenticides

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The rodenticides are biocides used for the control of rodents. The mechanism of action most common is the inhibition of the biosynthesis of the coagulation factors dependent of the vitamin K action, altering the normal process of coagulation, which leads to the death like consequence of the internal bleedings that suffers the animal. They are pesticides of professional use and/or no-professional use. At present, products that contain difenacoum, bromadiolone, clorofacinone, or brodifacoum are commercialised, all they coumarinic derivatives. These substances require a rigorous analytical control since their concentrations have to be sufficient to guarantee their efficiency like rodenticides and, at the same time, cannot exceed some levels of security regulated. This demands the continuous realisation of controls of the products during and after their manufacture. However, the available analytical methods are single methods for each one of the active ingredients, involving so complexity of analysis that prevents the achievement, in a reasonable time, of indispensable analytical results for the control of quality of the products, and causing stopped of production of length excessively prolonged.

In this work, new methodologies of analysis multi-rodenticide are presented for the simultaneous determination of the active principles in the distinct commercial products.

The methods are based on recent extraction techniques, like Matrix Solid-Phase Dispersion, Ultrasound Assisted Extraction, Microwave-Assisted Solvent Extraction, and Accelerated Solvent. These techniques involve a low consumption of organic solvent and a minimum generation of residues and, therefore, are inside the field of the Green Chemistry. In addition, the new methods developed allow a significant reduction of the times and costs of analysis.

Acknowledgements

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WE 175

Proposal of the German federal environment agency (UBA) on specific risk mitigation measures for the use of anticoagulant rodenticides covered by the biocidal products directive 98/8/EC

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Anticoagulant rodenticides (AR) which are used in order to control rodents like rats, mice or voles in a non-agricultural application area are biocidal products within the scope of the Biocidal Products Directive 98/8/EC (BPD). The environmental risk assessment of the notified active substances used as anticoagulant rodenticides resulted in a high risk for primary and secondary poisoning. Nevertheless, these substances were approved and included in Annex I of the BPD as they were considered essential for reasons of public health and hygiene as no adequate alternatives were available.

Due to the identified risks it has been considered appropriate within the Annex I Inclusion Directives to require the application of specific risk mitigation measures (RMM) at product authorisation level. Such measures are aiming at limiting the risk of primary and secondary exposure of humans and non-target animals. Moreover, long-term effects on the environment have to be minimised particularly for second-generation anticoagulant rodenticides (SGAR) which have been identified to be potentially persistent, bioaccumulative and toxic (PBT) and in part very persistent and very bioaccumulative (vPvB).

In due consideration of these legal obligations and with regard to both the outcome of the environmental risk assessment and an adequate resistance management the UBA proposes to restrict the use of SGARs to certified and trained professionals only. As regards the area of use, it is considered expedient to approve both indoor as well as outdoor (e.g. around buildings, open areas etc.) applications of SGARs. With reference to first-generation anticoagulant rodenticides (FGAR), the UBA intends to approve their use also to non-certified users (amateurs and professionals without certificate to use rodenticides) for the control of mice. However, the use of FGARs by not certified users shall be restricted to in and around buildings. For all ARs detailed instructions which reflect the codes of good practice of rodent control should be stipulated and linked to their authorisation.

As the product authorisation of ARs has already started within the EU it becomes obvious that further harmonisation of the specific RMM among all Member States is needed.

EP03P1 - Environmental characterisation, fate/detection, exposure, effect and risk of nanomaterials

WE 176

Use of single particle (SP)-ICP-MS to investigate silver nanoparticle size and forms in wastewater systems

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Some of the most rapidly growing classes of nanoproducts are consumer materials that contain inorganic engineered nanoparticles (ENPs). The environmental prevalence of ENPs released from these products is expected to increase substantially in the future. Thus, it is imperative to develop techniques for determining key characteristics of ENPs in complex media such as wastewater and biological samples. Few current analytical techniques seem fully capable of determining the concentration, size, and composition of this new class of emerging contaminant. Single particle inductively coupled plasma-mass spectrometry (SP-ICP-MS), a novel application of ICPMS, provides number, size, and composition data for nanoparticles (> 20-30nm diam.) at environmentally relevant concentrations (< 100 ppt). Furthermore, distinction and quantification by SP-ICP-MS of dissolved versus nanoparticulate forms is a unique characteristic of the technique.

In this present study, we spiked synthetic wastewater with Ag NP (size range 10 to 100nm), Ag⁺, or a combination of the two at environmentally relevant (<200 ppt) concentrations. Here, we determined under what conditions NP may dissolve, aggregate, or form complexes, and if particles form with free Ag⁺ in solution. The synthetic wastewater components were systematically altered and/or omitted to better understand which constituents play a role in these processes. Specifically, the effects of chloride and sulfide were investigated on how they may change Ag NP or interact with dissolved Ag⁺. Using SP-ICP-MS, changes in NP size, through aggregation and complexation, was determined. The continued validation and application of this analytical technique provides a powerful tool for investigations of inorganic nanoparticulate behavior in both environmental and biological systems.

WE 177

Surface analysis of silver nanoparticles after being exposed to environmentally relevant conditions

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Silver nanoparticles (AgNPs) are currently being very widely used in industry, mainly because of their anti-bacterial properties, with applications in many areas (1). Once released into the environment, the mobility, bioavailability, and toxicity of AgNPs in any ecosystem are dominated by colloidal stability (2). AgNPs released in wastewater may be converted into Ag⁺ ions, complexed with ligands, agglomerated or still be present as nanoparticles (3). By using HR-STEM and electron energy-loss spectroscopy (EELS) we have assessed the surface changes of citrate stabilized AgNPs prepared in the lab after being exposed to conditions similar to those in the environment, using fulvic acid, conditions similar to fresh and seawater and the presence of light. Preliminary results show that particles in fulvic acid remain stable and unchanged for months, while particles exposed to light change in size and form.

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WE 178

Behaviour of Au NPs in the standardized ecotoxicity test media and natural waters

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To understand the risks of NMs to aquatic organisms, many researchers have performed toxicity tests for NMs using standardised ecotoxicity test media. However, the speciation of NMs in these standardised media may be very different from the speciation that occurs in the natural environment. Results from standardised ecotoxicity experiments could therefore have little relevance to toxicity in the natural environment. Therefore, in this study, the aggregation of negative charged Au NPs (average size: 30nm) was investigated over 48h in a range of standardised media, namely: artificial pond water (APW), OECD algal and daphnid M4 media, Gamborg's B-5 basal medium, artificial salt water (ASW) and US EPA test media covering a range of hardnesses. The effect of the presence of a test organism on aggregation was also assessed with following combinations: M4 media/Daphnia magna/48h, APW/Gammarus pulex/96h, and Gamborg's media/Lemna minor/24h. To explore the effects of natural organic matter on the aggregation of Au NPs, tests were also done in the presence of humic acids (HAs) at concentrations of 1mg/L and 5mg/L of HAs. Aggregation was also assessed in de-ionised water. To assess behaviour in the natural environment, studies were done on 23 natural water samples which ranged in pH from 3.79-7.90. The aggregation of Au NPs in all tests was followed using nanoparticle tracking analysis using a NanoSight 2.2 (NanoSight Ltd, Amesbury, UK). Two-Way analysis of variance (PASW 18; SPSS Inc.) was used to evaluate differences, the significance level was p<0.05.

No aggregation was seen in deionised water and EPA soft water. In the other media, the NPs were found to aggregate very quickly and the size of the aggregates had reached a maximum within 6h. For the Lemna and algae media, the particle size reached a maximum after 2h and 6h respectively after which time, the particle size decreased. Greatest aggregation was seen in APW and the EPA hard water. Addition of HAs reduced the aggregation of the particles in all media types. The addition of D. magna to M4 media and L. minor to Gamborg's media had no significant effect on mean particle size. The addition of G. pulex to APW significantly reduced the mean particle size of the Au NPs over 96h. Of the 23 natural water samples, 19 samples had at pH value between 3.79-7.40. In these samples, no aggregation of the Au NPs was observed (≈ 50nm). Four samples showed significant aggregation (≈ 100nm) at pH value between 7.68 and 7.90.

WE 179

Weathering of polycarbonate composites containing ¹⁴C radio-labelled carbon nanotubes

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Due to their outstanding mechanical, electrical and thermal material properties, carbon nanotubes (CNT) are among the most intensively studied nanoscale materials. Compounding with inorganic or organic materials aims at transferring physical properties of CNT to the matrix material. Meanwhile, first polymer-CNT composites have reached consumer markets, for example in sporting goods. We investigate the end of the lifecycle of a polymer composite containing radioactive (¹⁴C-labelled) multi-walled CNT (MWCNT) to trace their fate due to weathering. The ¹⁴C label allows the determination of possible CNT release after disposal of composites in the environment. Here, ¹⁴C-MWCNT containing polycarbonate composites were prepared by dispersing the CNT in chloroform by ultrasonication and, afterwards, mixing with solutions of polycarbonate. Solution-casted thin films were prepared and characterised. Accelerated ageing of the thin films was studied by irradiation with a sun-like Xe-arc lamp spectrum under dry conditions. The irradiation dose was varied to study the time scale of the polymer decomposition. Released debris was collected and quantified with respect to composite amount and ¹⁴C-MWCNT content in order to estimate what fraction of CNTs may possibly be released upon environmental disposal of CNT-containing polymer composites. Detailed analysis of morphological and chemical changes of the composite surface was performed on identically-prepared ¹²C-MWCNT containing polycarbonate samples, employing electron microscopy, infrared absorption spectroscopy, X-ray photoelectron spectroscopy and Raman spectroscopy.

WE 180

Environmental exposure modeling of engineered nanomaterials and comparison to their corresponding bulk/total material flows

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There is a clear necessity to assess the environmental risks of engineered nano-materials (ENMs) due to their rapidly increasing application in consumer products, which might lead to an environmental exposure to these ENMs. Using probabilistic material flow modeling, we have reported in 2009 the first environmental concentrations for different ENMs in various environmental compartments. The present study aims to present more comprehensive and up to date environmental concentrations of ENMs by considering both ENMs and also their corresponding bulk/total material flows (e.g. pigment-TiO₂, silver and zinc metal and carbon black). The analysis is conducted in two stages: (1) prediction of updated environmental concentration of five ENMs, namely nano-TiO₂, nano-ZnO, nano-Ag, Carbon Nanotubes (CNT) and Fullerene (C₆₀); (2) prediction of the environmental concentration of nano-TiO₂ derived from pigment TiO₂ and of total metals (zinc and silver) and of carbon black. Our modeling is based on newest available data for production volumes of ENMs, allocation of the production volume to product categories, particle release from products and flow coefficients within the environmental compartments. For environmental concentrations of the nano fraction of bulk TiO₂ a similar probabilistic material flow modeling is performed. The comparison to total silver, zinc and carbon black is based on information from the literature.

The resulting predicted environmental concentration (PEC) for the five ENMs and different environmental compartments (wastewater, surface water, sewage sludge, sediments, soils) are compared to the previous estimates from 2009, to nano-TiO₂ derived from pigment-TiO₂ and to total metals. These comparisons will enable a more realistic assessment of the risks of ENM to the environment by focusing not only on the "nano" issue but taking a more complete view and relating the ENM to already existing compounds.

WE 181

Characterization and quantification methods for assessing the interaction of silver nanoparticles and growth media of different aquatic model organisms

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The industrial expansion of manufactured silver nanoparticles (nAg) production will likely enhance their accumulation into environmental and biological matrices. nAg main application is in consumer products, applying its bactericidal potential. The fundamental form of silver, ionic (Ag⁺), is a highly reactive and persistent entity. Release of Ag⁺ from nAg will depend on both solution and nanoparticles composition. The main goal is to develop methodologies to characterize and quantify nAg and Ag⁺, in different aqueous growth culture media: algae (Woods Hole MBL), daphnid (ASTM hardwater) and cell-lines (D-MEM). FI FFF (Flow field flow fractionation), using online UV and ICP-AES (Inductively coupled plasma atomic emission spectroscopy) detection, provides separation and characterization of nAg. More conventional approaches, as equilibrium dialysis (ED), microfiltration and ultracentrifugation were used to quantify the total dissolved Ag⁺, using ICP-AES. Mixtures of citrate-stabilized nAg (40 & 100 nm) and AgNO₃ were added to culture media (50 ppb). Two timepoints (T0 & Tend) were used, following the respective OECD guideline of each culture organisms. Along the ED experiments, in all culture media a high percentage of Ag was lost in AgNO₃ and nAg. In ASTM with AgNO₃ (1 KDa), the external solution decreased from 49 ppb to 3.7 ppb, while the final Ag value in the ED cell was 11 ppb. Similar values were found in MBL and D-MEM, since acid extraction of the test containers showed substantial adsorption of Ag⁺. The hypothesis is that the ED process is slower than adsorption to the solution containers. Therefore, the final value in the dialysis bag represents equilibrium with an external Ag concentration that was intermediate between the T0 and Tend concentrations. Since the analytical approach was wrong, it suggests that all containers should be saturated with Ag⁺, prior to test, under similar conditions.

The FI FFF, coupled to UV analysis (two wavelengths) was used to analyze a nAg (2 ppm) mixture (40 & 100 nm) in FFF carrier solution. The fractogram show two distinct signals separated by 6 minutes. Dispersion of the nAg in the culture media shows changes in the fractogram owing to either aggregation or NP-membrane interactions in the FI FFF. On-line ICP-AES was used for determination of mass distribution. The comparison among all the quantification methods and all the characterization detectors and their implications on the results will be discussed.

WE 182

Development of a new method to extract and analyze fullerenes and fullerene-derivatives from soil using HPLC with UV and fluorescence

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Fullerenes are carbon-based nanoparticles considered as some of the most promising materials in emerging nanotechnology. Their current use vary from industrial to household applications and the expected increase in production is likely to imply a release of such materials into the environment. In particular, fullerenes have been suggested to enter the terrestrial ecosystem by direct release (e.g. leaching from fullerenes-containing materials) as well as deposition from the atmosphere and transport through aqueous media. However, their environmental fate and behavior are still largely unknown and standard procedures for their analysis in soil are missing. In the present study we investigated the extraction and analysis of several fullerenes (C60, C70) and exohedral-functionalized fullerene derivatives ([60]PCBM, [60]PCBB, [60]PCBM, [60]ThCBM, bis-[60]PCBM and [70]PCBM) from soil matrices. For the first time such a large variety of fullerenes dissolved in toluene and water were characterized with liquid chromatography and UV/fluorescence detection. Therefore, we were able to spike soil samples separately with fullerenes dissolved in toluene or water. Several methods of extraction (e.g. ASE, sonication, shaking in different combinations) were tested and compared for their efficiency in terms of recovery. UV detection is a powerful tool to detect and quantify fullerenes owing to its high linearity and low LoD. We were able to analyze soils down to fullerene contents of 1 µg kg⁻¹. This limit of quantification could be even decreased to 0.1 µg kg⁻¹ using mass spectrometry for detection. Our suggested extraction method for soils has to be further tested with soils differing in important properties controlling the fate of fullerenes, such as the contents of organic C, clay and Fe oxides. Furthermore, our approach to dissolve fullerenes in water will give studies on the environmental fate of fullerenes a large boost.

WE 183

How to evaluate the exposure to engineered nanoparticles in freshwater?

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The increase in production and use of engineered metal-oxide nanomaterials makes the exposure to nanoparticles (NPs) more likely in the natural environment. Current state of knowledge on fate, exposure and toxic effects of NPs makes it difficult to evaluate the environmental risk posed by the NPs. Several reports on nanotechnology underline the importance to further progress in research on fate and transport of NPs in the environment, as this field of research is still rather undeveloped. Therefore, current evaluation of the predicted environmental concentration (PEC) of NPs is mainly based on material flow analysis (MFA) where the NPs are treated as bulk material. In such studies the important particulate nature of the material has not been considered. A correct evaluation of the exposure, in principle needs to consider all fate processes in order to estimate the bioavailable fraction. Two processes that seem to be significant are the fate processes of aggregation (incl. sedimentation) and dissolution. Environmental scientists have recently encouraged modeling of NP fate in freshwater based on colloidal chemistry and it has also been recognized that abiotic factors such as ionic strength and pH could influence the colloidal behavior of NPs. Therefore, based on colloidal and water chemistry, the aim of this research is to develop a fate model evaluating the water suspended concentration of metal-oxide NPs (e.g. TiO₂) to which organisms are exposed. The framework is focused on two main processes that affect the environmental behavior of the metal-oxide NPs in freshwater: aggregation (incl. sedimentation) and dissolution. To model the agglomeration and dissolution in freshwater spherical NPs are assumed along with Brownian motion, fast aggregation, and the correlation of effects to the suspended NPs. The considered freshwater compartment is a box with a constant inflow of NPs where water temperature and fluid viscosity are constant. The framework combines colloidal science and water chemistry and takes into account abiotic factors that influence the dissolution and aggregation, which leads to gravitational sedimentation. The developed model will in the end be applied in Life Cycle Impact Assessment (LCIA) with the aim to improve the substance coverage of the LCIA method.

WE 184

Impact, behavior and fate of silver nanoparticles in aquatic environments

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Engineered nanoparticles and materials are increasingly used in a broad variety of consumer products; their economic importance has risen strongly over the last years.

Especially silver nanoparticles (Ag NP) are frequently added to products such as sportswear and cosmetics nowadays. In contrast, knowledge about characteristics and effects of nanoparticles in the environment is still insufficient.

The German Federal Ministry of Education and Research (BMBF) has launched the "Hightech-Strategie" programme "NanoNature" in 2008 to support and promote research projects aiming to understand the impacts of engineered nanoparticles on the environment in general and on organisms in particular. The BMBF-funded project "UMSICHT" is a research and development project by "NanoNature". The project focuses on the environmentally relevant effects of engineered silver nanomaterials.

The Ecotoxicological Laboratory of the German Federal Environment Agency (UBA) as one of 17 "UMSICHT"-project partners is currently investigating the biological effects of Ag NP on aquatic organisms. Main objective is to provide a solid data set on Ag NP in aquatic ecosystems which can be used for hazard estimation and risk assessment in chemicals policy.

Therefore, the effects of Ag NP has been tested in several species representing different trophic levels in aquatic ecosystems, ranging from luminous bacteria (*Vibrio fischeri*) and cyanobacteria (*Anabaena flos-aquae*) to green algae (*Desmodesmus subspicatus*) and higher macrophytes (*Lemna minor*), invertebrates (*Daphnia magna*) and vertebrates (*Danio rerio*). All tests were conducted in accordance with standardized OECD guidelines, OECD guideline drafts or DIN ISO standards for acute and chronically ecotoxicological testing. In addition it should be demonstrated if, where and in what quantity Ag NP is incorporated by the organisms over time. All results regarding Ag NP were compared to the effects of silver nitrate to distinguish the impact of Ag NP and silver ions.

Furthermore, all tests have been accompanied by several analytical methods such as dynamic light scattering and UV/VIS spectroscopy to describe the particle size distribution of Ag NP, optical microscopy to visualize Ag NP accumulation and incorporation and determination of Zeta potentials and silver contents (total silver and silver ions) in aqueous solutions to characterize Ag NP and its effects on aquatic organisms.

WE 185

Effect of copper ion on adsorption of chlorinated phenols and 1-naphthylamine to surface-modified carbon nanotubes

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Understanding adsorptive interactions between organic contaminants and carbon nanotubes is critical to both the environmental application of carbon nanotubes as special adsorbents and the assessment of the potential impact of carbon nanotubes on the fate and transport of organic contaminants in the environment.

To understand how transitional metal ions with strong complexing capabilities might affect the adsorption of hydroxyl- and amino-substituted aromatics to different functional groups of carbon nanotubes, the adsorption of organic compounds (1,2-dichlorobenzene, 2,4-dichlorophenol, pentachlorophenol, and 1-naphthylamine) to different surface modified carbon nanotubes (O-functionality-rich SWNTs and N-functionality-rich SWNTs) was evaluated.

Adsorption of 2,4-dichlorophenol, pentachlorophenol, and 1-naphthylamine to O-SWNT and N-SWNT can be significantly enhanced in the presence of Cu(II) (50mg/L), whereas adsorption of 1,2-dichlorobenzene is essentially unaffected. The most likely mechanism for the Cu-enhanced adsorption is that Cu(II) serves as a bridging agent between organic solutes and the functional groups on CNT surfaces. For the adsorption of 1-naphthylamine to N-SWNT, an additional mechanism might be possible; that is, the complexation of Cu(II) with the N-functional groups of N-SWNT can change these groups from strong Lewis bases to Lewis acids and thereby significantly enhancing adsorption via the mechanism of Lewis acid-base interactions. In addition, adsorption of Cu(II) can be significantly enhanced by the adsorption of organic cosolutes.

A significant implication of the present study is that the effects of heavy metals have to be taken into account when assessing the role of CNTs in the fate and transport of organic contaminants containing polar functional groups. Moreover, the mutually beneficial adsorption of organic molecules and Cu(II) might prove to be an extra advantage for CNT-based wastewater treatment technologies.

WE 186

Leaching of nanoparticles from paints under controlled test conditions

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Paints containing engineered nanoparticles (ENPs) have recently been introduced on the market thanks to their improved chemical-physical performances, such as for coating protection against UV light (nano-TiO₂), for antimicrobial activity (nano-Ag), as well as to improve the thixotropy properties of paints (nano-SiO₂). Recent studies on the release of ENPs from paints containing them when applied to exterior facades highlighted the urgent need to carry out more detailed investigations on such nanoproducts.

Within this scenario experiments concerning the ENPs release from paints and coatings under laboratory test conditions are strongly required. Standardized water immersion tests, such those proposed by OECD and CEN, have already been applied and evaluated for the estimation of biocides or chemicals emissions from treated surface into water, but modifications of such procedures in order to be applied on surfaces coated with paints containing ENPs have not been investigated yet.

The EU-funded FP7 NanoHouse project (Life Cycle of Nanoparticle-based Products used in House Coating; <http://www-nanohouse.cea.fr/>) aims at addressing the potential Environmental Health and Safety (EH&S) impact of coatings including ENPs according to the Life Cycle Thinking (LCT) approach. The goal of the activity described in this work was to develop an experimental protocol for determining the potential release of selected ENPs (TiO₂, SiO₂ and Ag) from various industrial nano-based paints, taking into account literature results, available standard methods (ISO, ASTM) and inputs from project partners. Weathered and un-weathered panels were immersed in water according to an official permanent immersion test (ISO 2812-2:2007), specifically tailored to our case study. Water conductivity, pH and T were concurrently monitored during the tests. Nano-based paints, pristine ENPs to be added to paints, coated panels and leaching liquors collected at different time intervals from immersion, were characterized by a set of different techniques. Preliminary results obtained by ICP-OES indicate presence of Ti and Si but not Ag in the collected leaching liquors.

WE 187

Stabilization of titanium dioxide nanoparticles in sea water by natural biopolymers

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The large-scale use of titanium dioxide nanoparticles (n-TiO₂) in consumer and industrial applications suggests potentially significant releases into the environment, raising environmental health and safety concerns. Potentially impacted ecosystems include estuarine and coastal biotopes/organisms. Previous results from ecotoxicological studies with n-TiO₂ dispersed in salt exposure media are difficult to interpret due to fast flocculation and sedimentation phenomena, affecting the concentration of suspended material. The goal of this study was to investigate the effect of alginate, a relevant natural polysaccharide biopolymer, in stabilising n-TiO₂ in artificial seawater solutions usually applied in ecotoxicity bioassays. The size distribution of n-TiO₂ dispersions was monitored by dynamic light scattering (DLS) measurements, while the sedimentation behaviour was investigated with a stability analyser under centrifugal forces. Under these conditions n-TiO₂ agglomerates were formed with average sizes < 270 nm for the entire period of observation, as measured by DLS. The size distribution remained constant after re-suspension, indicating that no agglomeration occurred after deposition. Preliminary ecotoxicity tests on two marine species, the crustacean *Artemia franciscana* and the micro-alga *Phaeodactylum tricornutum*, showed moreover that the presence of alginate in the exposure media did not negatively affect the tested organisms.

WE 188

Determination of functionalized engineered carbon nanoparticles in environmental samples by HPLC-UV/LTQ-ORBITRAP MS

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The use and production of nanomaterials is increasing and expanding with lightning fast speed during the last decade. C₆₀ fullerene is of special interest due to its widespread application in nanocarbon technology as starting point for a whole new set of functionalized engineered carbon nano particles. The global annual production is estimated to be around four to five tons. The occurrence, fate and ecotoxicology in aquatic environments are difficult to investigate. Although the bare fullerenes like C₆₀ and C₇₀ are highly apolar they can form very stable aggregates in water. The more functionalized groups are attached to a bare fullerene the more polar it will become. This also makes them more susceptible to leach into the aquatic environment. In 2008 no C₆₀ was detected in surface waters in The Netherlands above the LOQ of 5 ng/l. In 2010 the occurrence at the µg/l range was reported of both C₆₀, C₇₀ and C₆₀-methylfulleropyrrolidine in samples from sewage treatment plants in Spain.

We developed a highly specific analytical method for the determination of C₆₀, C₇₀ and 6 functionalized eCNPs. Good separation for all 8 compounds was achieved by optimizing the elution with normal-phase chromatography in combination with UV detection. Elution of all compounds took place within 30 minutes. The HPLC was interfaced with the LTQ-Orbitrap-MS by means of an electrospray interface and operated in negative ionisation mode. Detection of the eCNPs was performed with the accurate masses of the molecular ions and those of the fragmentation products. The 8 eCNP could be separated and determined at a level of 20 pg on-column.

The biggest challenge was to create a reproducible suspension of fullerene aggregates in water to spike environmental samples. This was performed by means of solvent exchange from a toluene standard solution under continuous sonication while keeping the experimental conditions as constant as possible. Different sample preparation techniques, like SPE and LLE were tested to extract the eCNPs from surface water samples.

The overall procedure provides a solid base for confirming the presence by means of the retention time, the detection with UV, the accurate mass using a window of 5 ppm, and finally the accurate mass fragmentation of the molecular-ion down to the bare fullerene. The use of full scan accurate mass creates an exciting possibility to retrospectively look for the presence of other functionalized fullerenes in environmental samples.

WE 189

Dissolution, aggregation and transport of ZnO nanoparticles in aqueous matrices containing dissolved organic matter

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The fate and toxicity of engineered nanoparticles (NPs) in natural waters are strongly related to their physicochemical behaviour. In particular, aggregation and sedimentation modulate the transport of NPs towards organisms. In the specific case of ZnO, one must also take into account the important dissolution process, which is highly dependent on pH. Some authors even attribute the toxicity of ZnO NPs to the ensuing relatively high free Zn(II) concentrations, rather than to the NP form. Apart from pH and salinity, dissolved organic matter (DOM) plays a key role in the stability of NP suspensions, because adsorption of organic matter, such as humic acids or fulvic acids, onto the NP surface can dramatically vary its surface charge.

The recent technique AGNES (Absence of Gradients and Nernstian Equilibrium Stripping) is specially suited for studying ZnO dissolution, given that it provides a direct and robust determination of the free Zn concentration. DGT (Diffusive Gradients in Thin Films) provides dynamic information on the fluxes arriving to an ion exchange resin. AGNES and DGT have been applied to dispersions of ZnO NPs from different sources to gain information on equilibrium and dynamic release of zinc ions from the particles under different environmental conditions (pH, DOM, size of NPs, etc.). The results were compared with thermodynamic and kinetic theoretical models. We conclude that both analytical techniques can contribute to the knowledge of the properties of ZnO NP dispersions, which are relevant for environmental impact and toxicity assessment.

The interaction of humic acid (HA) with the NPs was analysed by UV-vis absorption and Laser Doppler Electrophoresis. It is shown that DOM adsorbs readily on the NPs and inhibits further aggregation by stabilizing the agglomerates due to electrostatic repulsion. The sedimentation of the HA-coated NP aggregates was studied by UV-vis spectroscopy and Dynamic Light Scattering. The results compared satisfactorily with a transport model based on Brownian diffusion and Stokes sedimentation of fractal, polydisperse NP clusters, which allows the estimation of their effective density. The implications in the context of environmental fate of NPs and uptake fluxes for in vitro toxicity experiments are also addressed.

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WE 190

Engineered nanoparticle disposition and agglomeration in artificial biofluids

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It is known that different environmental matrices (e.g., salt water, freshwater with humic acids, soils) cause changes in nanoparticle (NP) agglomeration states. These environmental matrices contain similar components as biological fluids (e.g., salts, surfactants), so it is possible that similar disposition and agglomeration states will be seen within different biological fluids. The movement of NPs through biological organ systems (e.g., gastrointestinal tract, respiratory tract) will expose NPs to different environments, which will likely alter the agglomeration state of NPs. In this project, we examined the disposition of 1 mg/l coated silver (Ag) NPs in three artificial biological fluids, gastric, alveolar, and interstitial, to simulate what will happen in the stomach and lungs, respectively. Ethylenediaminetetraacetic acid (EDTA)-coated Ag NP (80 nm) settling in gastric fluid was measured spectrophotometrically over a 30 min period. Lower pH (e.g., 1-3) caused EDTA-Ag to settle quicker, whereas EDTA-Ag stayed more dispersed at higher pHs (e.g., 5-7). In alveolar fluid, both 20 and 80 nm citrate-Ag agglomerated, as determined by dynamic light scattering (DLS) analysis and field flow fractionation-inductively coupled plasma-mass spectrometry (FFF-ICP-MS), yet both NPs showed little settling over 30 min, likely due to the presence of surfactant (phosphatidylcholine). In contrast, artificial interstitial fluid caused increased citrate-Ag agglomeration and settling over the 30 min exposure. Furthermore, NP coating and particle size had a dramatic effect on NP settling in interstitial fluids. Citrate-Ag (20 and 80 nm) showed marked settling over 30 min, whereas only 80 nm PVP-Ag showed a similar settling pattern. In contrast, 20 nm PVP-Ag remained suspended over 30 min. These data demonstrate how artificial biofluids can be used to predict the agglomeration of NPs within the body, giving researchers clues as to NP behavior within different organ compartments and chemical fractions the body may actually be exposed to (e.g., dissolved ions, nano-sized particles, micron-sized particles). These data may help researchers predict the actual amount of NP that is bioavailable, based on the NP dissolution rate and agglomeration size.

WE 191

Mechanism of nanoparticle interference in biochemical assays

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Nanomaterials are becoming common in commercial products and the assessment of their safety is crucial. The evaluation of the toxicity of nanomaterials can be hindered by conflicting reports demonstrating differential degrees of toxicity with the same nanoparticles. We have shown that nanoparticles can interfere with biochemical assays commonly used to assess physiological or biochemical status of an organism. We show that protein assays, the catalase assay, alamar blue and MTS assays, and LDH assays are each affected. This interference can lead to erroneous reports of toxicity. We determine the mechanism by which nanoparticles can interfere with toxicity assays using silicon, cadmium selenide, silver, titanium dioxide, and zinc oxide nanoparticles, and single walled carbon nanotubes. We predicted that nanoparticles could affect assays by binding to proteins and dyes present in the assays, affect structure and activity of these molecules, change the fluorescence or absorbance characteristics of the molecules, and/or cause reduction of the dyes to their fluorescent forms. Using circular dichroism, non-denaturing gels, and dynamic light scattering, we determined that various nanoparticles could bind and change the structure and agglomeration state of the dyes (e.g. tetrazolium-based dyes, resorufin) and proteins (e.g. bovine serum albumin). Spectrophotometric measurements were used to establish the change in optical properties after incubation with nanoparticles. The ability of nanoparticles to reduce dyes was examined using X-ray photoelectron spectroscopy. This work will contribute to the understanding of the unique physicochemical effects of nanomaterials, and help nanotoxicologists control for nanoparticle interference in their biological assays, which could reduce conflicting toxicity reports.

WE 192

Quantification of a series of fullerenes nC₆₀ and related transformation products in water

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This study describes the development of a sensitive analytical method to quantify nC₆₀ in water, using accurate mass screening liquid chromatography-hybrid linear ion trap Orbitrap mass spectrometry. The formation of oxidation, reduction and photochemical transformation products of nC₆₀ was studied as well.

The developed method enabled to detect and quantify aqueous concentrations of the summed nC₆₀ and its transformation products as low as 5 ng/L. It was observed that nC₆₀ transformation products exceed quantities of the parent C₆₀. Despite the high sensitivity of the developed method, no nC₆₀ or transformation products were detected in an array of Dutch surface waters.

HPLC/UV/MS analysis was performed with an LC-DAD-LTQ-FT Orbitrap MS system. Both ESI and APCI were used for ionization. Aqueous samples were extracted using preconditioned C18 SPE columns, surmounted by filtration columns. Surface water samples were collected throughout the Netherlands.

The toluene/methanol eluents and ESI ionization method resulted in most sensitive determination of C₆₀ with liquid chromatography-hybrid linear ion trap Orbitrap mass

spectrometry of the eluents and ionization methods tested. Besides the parent compound C60, other ions related to fullerene are observed. The ions are identified as C60 transformation products, due to the characteristic isotopic pattern of carbon-based ions MS-spectra. The response of the C60 transformation products can exceed the parent C60. The transformation of parent nC60 by oxidation, reduction and photochemical reaction is well-known, but the exact nature of the transformation products is not well studied. The described approach to determine accurate mass with LC-DAD-LTQ-FT Orbitrap MS using ESI ionization, seems promising for a more in-depth study on various transformation processes under environmental circumstances and identification of the transformation products.

With the method described it is possible to detect nC60 and transformation products in water at concentrations as low as 5 ng/L. nC60 was not found in several different Dutch natural waters. This may be due to low emissions, or to high loss factors during effluent treatment or due to sedimentation and further transformation processes. Currently, this method of analysis is being adapted and applied to 13 different derivatives of fullerene. Results of this further study will be presented at the conference.

WE 193

Nano-QSAR modelling - efficient way of predicting the toxicity of metal oxide nanoparticles to human keratinocyte cell line

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Together with an increasing role of nanotechnology in our every-day-life, we can also expect increasing emissions and, consequently, increasing levels of nanoparticles (NPs) in the environmental compartments. However, there is an increasing number of contributions that report toxicity and/or ecotoxicity of selected NPs and highlight the potential risk related to the development of nanoengineering. Therefore, novel, fast and inexpensive procedures for identifying potentially hazardous NPs without necessity of extensive empirical and animal testing are needed. Toxicity of NPs can be predicted in alternative way by applying Quantitative Structure-Activity Relationships methods (QSAR). QSAR methods are based on the assumption that the variance in a given physico-chemical or biological (e.g. toxicity) property in a set of compounds (so-called endpoint) is determined by the variance in their molecular structures, encoded by so-called descriptors. Consequently, when the values of the endpoint are available only for a part of the group, it is possible to interpolate lacking data from an appropriate mathematical model.

The main purpose of the study was to illustrate the thesis above by developing a Nano-QSAR model that describes the relationship between the structure and toxicity of 22 nano-metal oxides to human keratinocyte (HaCaT) cell line. We have investigated the changes in cell viability and the process of generating reactive oxygen species by employing a human keratinocyte cell line as a model for dermal exposure. The experimental results expressed in term of LD50 values indicated that ZnO was the most toxic one in the set of the NPs tested. The LD50 value determined for ZnO was 27 µg/ml, while the values of LD50 for the majority of the other NPs (In₂O₃, La₂O₃, SnO₂) were higher than 250 µg/ml. Additionally, in order to find the best structural parameters, reflecting the essential properties of the studied nanomaterials (shape, porosity, surface area, the electronic states resulting from quantum effects, etc.) we proposed a set of (i) image descriptors (based on images taken from transmission electron microscopy) and (ii) quantum-mechanical descriptors (based on quantum-chemical calculations). The combined experimental-theoretical study allowed us to develop a Nano-QSAR model that reliably predicts the toxicity of all considered compounds. Such a model could be applied not only to NPs investigated in the current work, but also to unexplored related species.

WE 194

Ultratrace detection of engineered nanoparticles by single particle ICP-MS: promises and limitations

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The limitations of spICP-MS are weighed against the clear benefits of this technique for characterisation of environmentally relevant exposures to engineered nanoparticles (ENP) for the case of Ag and Au ENP using a sector field ICP-MS. The size determinations were within 10 % of nominal values, but the accuracy can be increased by optimizing nebulisation efficiency determination and developing more robust data treatment algorithms. Overlap between dissolved and particle signals constraints spICP-MS to determine sizes larger than 20 nm and 10 nm for Ag and Au ENP. Distinction of these signals was based on iterative outlier detection which was shown to require a threshold of at least 5 × the standard deviation to limit the false positive count to less than 1 % of the total count. Signal overlap increases at a too low dwell time (1 ms), whereas too high dwell time (10 ms) leads to an increase in multiple particle event frequency. The linearity of particle number concentration determination extends over two orders of magnitude at the optimal dwell time, in this case 5 ms, but this range may be extended to lower particle numbers by measuring more data points and to higher concentrations, by improving data treatment algorithms. However, lack of particle counting reference materials obstructs validation. The feasibility of spICP-MS detection of ENP in environmental samples was finally demonstrated by counting particles containing Ag, Ce and Ti in a wastewater treatment plant effluent. Particle concentrations corresponding to stochastically predicted concentrations were found.

WE 195

Behaviour and toxicity of silver nanoparticles to freshwater and estuarine microbial communities and their processes

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Little is currently understood about the fate and behaviour of engineered nanoparticles in aquatic systems and concerns have arisen over the potential risks they pose to living organisms and biological systems. Silver nanoparticles are used in a number of consumer products due to their antimicrobial properties, but on entering aquatic systems (e.g. via wastewater treatment plant outflow pipes) they have the potential to disrupt *in situ* microbial communities and their processes. The aims of this study were to physically and chemically characterise capped and uncapped silver nanoparticles, measure their toxicity to pure bacterial cultures, and investigate their effects on key aquatic microbial processes including nitrification and hydrocarbon biodegradation.

Nanoparticle characterisation revealed that uncapped nanosilver particles were larger in size than capped nanosilver particles, but both types of nanoparticle formed aggregates in natural estuarine water and seawater. Nanoparticle toxicity as measured by bacterial growth assays at three concentrations (0.5, 5 and 50 mg L⁻¹) revealed that capped silver nanoparticles completely inhibited the growth of *Escherichia coli* and *Bacillus subtilis* at 50 mg L⁻¹ whereas uncapped silver nanoparticles caused an extension in the lag phase in both bacteria at 50 mg L⁻¹ but did not completely inhibit growth at any concentration. TEM images of NP-bacterial cell interactions indicate that AgNP and Ag⁺ have the potential to cause cell membrane damage and may enter cells.

In freshwater sediments amended with crude oil in order to stimulate microbial hydrocarbon biodegradation, significant changes in microbial community structure were observed in the presence of 0.5 and 50 mg L⁻¹ capped and uncapped nanosilver, yet hydrocarbon degradation rates were unaffected by their presence. In freshwater sediments amended with ammonium to stimulate nitrification, ammonium oxidation rates were significantly reduced after 1 day, 7 days and 14 days in the presence of 50 mg L⁻¹ capped nanosilver, yet in estuarine sediments that underwent the same treatment, reduced ammonium oxidation rates caused by capped nanosilver (at 50 mg L⁻¹) began to recover after 14 days.

Results suggest that although nanosilver particles may have a detrimental effect on aquatic microbial communities and their processes, this will be dependant on the physical and chemical characteristics of the nanoparticle itself as well as the concentration it is present in.

WE 196

Monitoring silver close to a nanoparticle production company

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Silver emissions into the environment decreased in the nineties due to the disappearance of the traditional photoindustry, which was the main polluter source of silver.

However, in recent years, the application of silver is increasing again, including silver in the form of engineered nanoparticles (ENP). Whilst nanosilver is usually integrated in a robust matrix and its release is either controlled, highly diluted (nanosilver textiles), or prevented (computer modules), high point source silver emissions may occur at the production sites or during disposal of nanosilver-containing products. Atmospheric emissions are of particular concern due to the deeply inhalable fraction with corresponding potential long-term adverse effects to humans.

The aim of this study was to quantify silver deposition in the immediate surroundings of a nanosilver production company, (i) in terms of concentration in mosses (*Brachythecium rutabulum* and *Hypnum cupressiforme*) over three years, (ii) to measure wet and dry silver deposition on a monthly basis with the Bergerhoff method, assuming that eventually all silver particles in the air are deposited (dry deposition) or washed out (wet deposition), (iii) to analyze spatially explicit deposition patterns with increasing distance from the potential emission source, (iv) to compare morphological changes of the nanosilver from production to deposition, and (v) to compare the detected silver concentrations with known background concentrations in other Swiss regions and with historical data (150 sites). This step provides valuable information on potential anthropogenic silver sources.

Silver concentrations in the mosses were between 0.002 and 0.033 µg g⁻¹. The silver concentration at the sampling point >8km away from the production facility was below the quantification limit, indicating that the silver concentrations decrease with increasing distance from the production facility. We found lower silver concentrations in the opposing average wind direction than in the prevalent direction. The absolute deposition rate of airborne silver was between 0.05 µg d⁻¹ m⁻² and 0.44 µg d⁻¹ m⁻². In comparison to other regions in Switzerland, the deposition rates were slightly higher, but in the same order of magnitude. The same holds for the silver concentrations in the mosses. Concerning the morphology, electron microscopy showed silver in the form of nanosize silver chloride.

WE 197

The binding of phenanthrene to engineered metal nanoparticles

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The rapidly increasing development, production and use of engineered nanoparticles (ENP) likely lead to their entry into the environment. In addition to potential harmful effects of ENP, which have been found for different organisms, ENP are suspected to influence the transport, bioavailability and toxicity of a range of environmental contaminants that may adsorb to their surface. In this study the binding of the polycyclic aromatic hydrocarbon (PAH) phenanthrene to stabilized silver and gold ENP

was investigated using a novel mass balance based single-equilibrium approach. Only citrate coated gold ENP (AuNPCIT) were found to bind phenanthrene, with ENP concentrations to initiate a measurable binding required to be higher than 1 mg L⁻¹. No binding was observed for polyvinylpyrrolidone coated silver ENP (AgNPPVP) and, citrate coated silver ENP (AgNPCIT) even at high ENP concentrations (10 mg L⁻¹). This suggests that the properties of the core material have a major influence on the binding reactions. The binding coefficient, K_b, defined as the ratio between the concentration of phenanthrene associated to the AuNPCIT and that freely dissolved in the exposure medium, was 3.5 ± 1.5 . Temperature was not seen have a significantly influence on K_b within an environmentally relevant range (4° - 25° C), although K_b appeared to slightly increase at higher temperatures. The presence of methanol significantly reduced or prevented the formation of the AuNPCIT - phenanthrene complex. Results suggest that the binding is a low energy physio-sorption possibly related to direct π - π interactions between phenanthrene and AuNPCIT. The binding between metal ENP and phenanthrene was determined with a new method, preventing most of the common measuring artifacts. Gold metal nanoparticles were found interact with phenanthrene, making them a potential vector for contaminants. However, further research is needed to investigate the influence of environmental conditions and ENP concentrations.

WE 198

Stability of hematite nanoparticles in function of pH and Fulvic Acids concentration

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Fulvic Acids (FAs) are playing important roles in the reactivity and transport of colloids in aquatic systems. They are also suspected to play important roles in the surface charge transformation, stability and aggregation behavior of manufactured nanoparticles. The exact conditions under which aggregation or dispersion occurs will depend on the nanoparticle surface charge properties, FAs concentration as well on the solution conditions, such as pH and ionic strength. The systematic calculation of stability-aggregation phase diagrams is therefore a key aspect in the prediction of the environmental fate and behavior of released nanoparticles in aquatic systems. In this study, response to changes in pH and FAs concentration are examined in details by considering purified iron oxide nanoparticles (NPs) and the resulting surface charge transformations. By adjusting the pH, different nanoparticle surface charge scenarios are investigated corresponding to positively, neutral and negatively charged nanoparticle solutions. For each situation, the adsorption of negatively charged FAs at variable concentrations is considered by analyzing surface charge modifications and calculating the kinetics aggregation rates. Results show that, under the conditions used, and range of environmental relevant conditions, the nanoparticle aggregation process is promoted only when the nanoparticle positive surface charge (solution pH less than the charge neutralization point) is compensated by the adsorption of FAs. In all the other cases FAs adsorption and increase of FAs concentration are expected to promote not only the NPs stabilization but also the dissolution of NPs aggregates. NPs charge overcompensation is systematically achieved at high FAs concentration. In addition, our study suggest that very low concentrations of Fulvic acids > 0.05mg-L⁻¹ are sufficient to rapidly stabilize iron hydroxide NPs solutions at concentration < 5mg-L⁻¹, independently of the pH of the solution, and play important roles in the fast dispersion of NPs aggregates.

WE 199

Past and present dispersion state strongly affects the sorption behavior of carbon nanotubes

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CNTs have strong sorption affinity towards organic compounds. Understanding the influence of dispersion/aggregation on sorption is essential for evaluating CNT's potential environmental impact and their application as superior sorbent. However, there is currently very limited data on the sorption potential of partially/fully dispersed CNT systems. This can be mainly explained by limitations associated with the generally-applied batch sorption test setup that does not allow investigations on dispersed systems. We applied a passive sampling method to investigate the influence of dispersion state on the sorption behaviour of CNTs. The objectives were to investigate the effect of (i) CNT pre-treatments (sonication, shaking) and (ii) natural dispersants on the sorption characteristics of the CNT system. Single point sorption coefficients and sorption isotherms for pyrene were measured over a wide range of concentration and in the presence of HA (1-200 mg L⁻¹). CNT suspensions were extensively characterized in order to support mechanistic interpretations of the results.

Results indicated that dispersion can significantly affect the sorption behaviour of CNTs. On the one hand, sonication broke down CNT aggregates and greatly increased pyrene sorption (sorption increased by 1.4 orders of magnitude). Sorption surfaces newly exposed during sonication remained available to pyrene molecules even if re-aggregation occurred, supporting an irreversible effect of sonication. On the other hand, the presence of HA decreased the sorption of pyrene. At the highest HA concentration investigated (200 mg L⁻¹), sorption was still 1.6 orders of magnitude stronger than sorption of pyrene to HA itself. This indicates that specific interactions between pyrene and CNTs are still occurring, in spite of a HA coating of the CNTs' surface. A greater suppression of sorption by HA occurred when combined with a sonication pre-treatment.

Sorption isotherm fitting indicated that the maximum sorption capacity, affinity and heterogeneity of CNTs surface were all affected by sonication and the presence of HA. The present results contribute to an improved understanding of the sorption behaviour of CNTs in both natural and waste water systems.

WE 200

Fate of isotopically labelled ZnO nanoparticles in sediment and biochemical and behavioural responses of two marine invertebrates Nereis diversicolor and Scrobicularia plana

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Though it is reported that nanometals, which are among the most rapidly expanding materials, can cause toxicity to organisms, their fate in the environment and nanotoxicity in marine organisms are not well understood. The use of the stable isotope labelling approach enables tracing of the fate of nanoparticles (NPs) in sediments and also investigation of bio-uptake in two estuarine intra-sedimentary invertebrates *Scrobicularia plana* and *Nereis diversicolor*. We selected exposure to 3mg.kg⁻¹ sediment ZnO NPs since this level is a realistic prediction of the environmental concentration in sediments. 67ZnO NPs (DLS: 21-34 nm, positively charged: 31.3 mV) suspensions were synthesized in diethylene glycol (DEG). We explored the fate of 67ZnO NPs in sediment, 67Zn bioaccumulation and the biochemical (biomarkers of defense and damage) and behavioural (burrowing kinetics and feeding rates) biomarkers in both species to 67ZnO NPs. and DEG on its own during a 16d laboratory exposure. After exposure, 67Zn concentrations in sediment showed higher levels in the upper section (1cm: 2.59 mg.kg⁻¹) decreasing progressively (2cm: 1.63 mg.kg⁻¹, 3cm: 0.90 mg.kg⁻¹, 4cm: 0.67 mg.kg⁻¹) to a minimum value at the bottom (5cm: 0.31 mg.kg⁻¹). 67Zn bioaccumulation was observed in both organisms exposed to 67ZnO NPs in DEG but no major inter-species differences were found (5.4 ± 2.2 µg.g⁻¹ w.w in clams and 3.7 ± 1.5 µg.g⁻¹ w.w in ragworms). At the biochemical level, 67ZnO NPs exposure induced increased glutathione-S-transferase (GST) activity in worms and catalase (CAT) activity in clams revealing that animals endured oxidative stress. In clams, increased lactate dehydrogenase activity and caspase-3 like induction showed metabolic disturbances and apoptosis process, respectively. However, exposure to DEG on its own, increased metallothionein-like protein levels in clams and CAT activity in ragworms were observed compared with controls. Burrowing behaviour as well as feeding rate were significantly impaired in both species exposed to 67ZnO NPs. However exposure to DEG on its own, burrowing behaviour impairments were also shown in both species whereas feeding rate was impaired only in bivalves. These behavioural impairments were not accompanied by an inhibition of acetylcholine esterase activity. Since behavioural and biochemical biomarker responses were observed in animals exposed either to 67ZnO NPs in DEG and DEG on its own, there is no clear evidence for a nanoparticle effect.

WE 201

Biotransformation of carbon-based nanomaterials by Horseradish Peroxidase

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Due to the steady increase in the use and mass production of carbon-based nanomaterials (CBN), such as fullerenes and carbon nanotubes (CNTs), the presence of these materials in the environment is an eventuality. However, there is very little information regarding the fate and transport of CBNs, and almost no information regarding the possible biotransformation pathways that CBNs might undergo once released into the environment. Without this information assessing the possible human and ecological health effects of CBNs remains a challenge for the scientific community. To this end, we are developing analytical methods for the proper characterization, identification, and quantification of selected fullerenes and CNTs and their possible biotransformation products. Here we present the results from an initial set of experiments where different types of CBNs in aqueous suspensions were subjected to enzymatic oxidation with horseradish peroxidase. No substantial differences were observed in SEM images of the CBN suspensions tested before and after the proposed enzymatic degradation. Nevertheless, changes were observed in the agglomerate size, the particulate and dissolved organic carbon concentrations, and the CO₂ concentration in the headspace of the experimental vials during the same treatment. However, this far we are not able to assess the degree of degradation, as the current proposed methodology is not sensitive enough. The use of more sensitive quantitative analysis, e.g. 14C labeling of CBNs and HPLC-MS of the CBN suspension after degradation, will provide more insightful and more conclusive information.

WE 202

The influence of exposure mode in the hazard identification of metallic nanoparticles: the case of TiO₂ and Daphnia magna

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Contrasting results from ecotoxicological studies performed with nanoparticles (NPs) highlight the need for a consensus on how to administer NPs to aquatic organism in ecotoxicity tests. Recent findings suggest that ecotoxicity values are dependent on the method of treatments of NPs in solution and the set-up of experiment. With the aim of developing a standardized protocol for ecotoxicological testing of NPs, we evaluated whether aquatic ecotoxicity of n-TiO₂ depends on exposure mode. A suite of acute toxicity tests on *Daphnia magna*, extending the exposure length to 96h, with n-TiO₂ were performed (OECD 202). The n-TiO₂ (XRD size: 15 nm; DLS size: Zave = 183 ± 7 , Polydispersion = 0.3; crystalline phase : mostly anatase) are manufactured by laser pyrolysis at the ENEA Research Centre of Frascati. The exposure mode has been evaluated focusing on: the treatments of the sample, the volume of sample tested (50ml and 2ml) and the length of exposure (until 96h). Two experiment set-up have been performed. In the first one four treatment of the sample were performed, and the organisms were exposed to 50ml of substance. In the second experiment set-up the *D.magna* was exposed

to 2ml. Also the evolution over time (until 96 h) of particle was measured by DLS (Dynamic Light Scattering). No adverse effect in the set-up 1 was observed. By contrast, a value of EC50 of 20 mg/L at 96h in set-up 2 was detected. We observed that the exposure duration affects the test result: EC50 values could not be determined until 96h, and the slope of the dose-response curve became steeper over time. The analysis of the evolution of the size of NPs with time (96h) in the media showed that TiO2 tends to form aggregates starting from 24h. This increased NPs sedimentation and decreased the amount of NPs in suspension at which the organisms are exposed. Consequently the volume tested might be a significant parameter to the exposure mode. As the test results show, no adverse effect with a volume of 50ml has been noted (set-up 1). By contrast, adverse effect with lower volume (set-up 2) was found. Ecotoxicity of n-TiO2 to *D.magna* was influenced by the mode of exposure. For insoluble metal oxide NPs (as TiO2) the volume is a relevant parameter that control the exposure mode to *D.magna*. Also, exposure length of 96h seems to be relevant in deriving ecotoxicity values, but further researches are needed to investigate whether this holds true for other metallic NPs and other organisms

WE 203

Effects of gold nanoparticles to green algae *Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii*

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Gold nanoparticles are used in various fields, especially biological area such as biosensor, carrier for drug and gene, and labeling. We assessed the toxicity of gold nanoparticles (AuNPs; 10 nm) to green algae *Pseudokirchneriella subcapitata* and *Chlamydomonas reinhardtii*. They are widely present in freshwater ecosystem. Both test species were exposed to five different concentrations of AuNPs in OECD algal media, and exposure duration was 72 hours. The growth rate of *Pseudokirchneriella subcapitata* was inhibited at 0.5 mg/L of AuNPs. *Chlamydomonas reinhardtii* was significantly influenced by 10 mg/L of AuNPs. *Pseudokirchneriella subcapitata* was more sensitive than *Chlamydomonas reinhardtii*. We observed that gold nanoparticles negatively affect the freshwater green algae. *This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2011- 0015985).*

WE 204

Effects of aged Carbo-Iron composite nanoparticles on green algae and zebrafish embryos in combination with the environmental contaminant tetrachloroethylen

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Composite particles of colloidal activated carbon and zero-valent iron are under development to be used as in situ reagent for remediating contaminated groundwater (Carbo-Iron®). Activated carbon acts as a sorbent to bind contaminants such as e.g. tetrachloroethylene (PCE) and the iron in the activated carbon structure subsequently dechlorinates the contaminants while it is oxidized itself. Carbo-Iron particles are designed to remain in the environment after application in the aged form (iron oxide/hydroxide on activated carbon). Therefore, we accompanied the particle development with ecotoxicological studies to identify the hazardous potential of the catalyst after the reaction towards the aquatic model organisms *Scenedesmus vacuolatus* (green algae) and *Danio rerio* (zebrafish). We found that aged Carbo-Iron particles (that are no reducing agents anymore) are not toxic to zebrafish embryos up to 100 mg/L upon 48 h of exposure. Furthermore, we found growth inhibition in the green algae *scenedesmus vacuolatus* which we could show to be due to a shading effect. That means that toxicity is due to light absorption which is similar when algae are exposed to decreasing light intensities without particles.

PCE is an often occurring volatile groundwater contaminant exerting toxicity in many organisms. A combination of aged Carbo-Iron particles with PCE reduced its toxicity in zebrafish embryos assumed to be due to adsorption of PCE to the activated carbon and therewith a reduced bioavailability. In algae we expect a joint effect resulting from PCE toxicity and reduced growth as a response to shading due to light absorption by the Carbo-Iron particles. These investigations are still in progress. Our study provides data for the assessment of the application and regulation of Carbo-Iron particles for in situ remediation and contributes to the development of reference methods in testing nanomaterials. We additionally provide data useful for the ecotoxicological evaluation of the environmental contaminant PCE.

WE 205

Silver nanoparticles: different toxicity mechanisms in *Vibrio fischeri* and *Pseudokirchneriella subcapitata* using different kinds of nanoparticles?

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In recent years the market for nanotechnology-based consumer products has been rising. Silver nanoparticles (nAg) are widely used in several consumer products and they are mainly designed to release silver ions (Ag⁺), the source of antibacterial activity. Unconventional features result from nAg small size and large surface area per unit mass, eventually generating significant differences between the physicochemical properties of bulk and nanoparticulate forms of Ag. Ionic silver (Ag⁺) is one of the most toxic forms of metals in the aquatic environment. The release of Ag⁺ from nAg will depend on both solution and nanoparticle characteristics, including the composition of nanoparticles surface. The main goal of this work is to assemble and evaluate the toxic effects of citrate-stabilized nAg solution (100 nm) and non-stabilized nAg solution (100 nm) and AgNO₃, to bacteria (*Vibrio fischeri*) and algae (*Pseudokirchneriella subcapitata*). To determine the bioluminescence inhibition rate, *V. fischeri* was incubated at 15°C and exposed to both kinds nAg and AgNO₃, for 5 and 15 minutes, according to Microtox® test procedures. Algae were exposed to nAg or AgNO₃ in MBL medium and allowed to grow at 25°C, for 72h. The growth inhibition rate was determined according to OECD Guideline 201. At the same time, DLS (Dynamic Light Scattering) was used to measure particle size distributions and the zeta potential of nAg. The DLS results demonstrate that non-stabilized nAg solution (100 mg/L) had a stable zeta potential (-35 mV) for 72h. The *V.fischeri* and *P.subcapitata* results show that there is an influence of the surface composition in the toxicity of nAg to these aquatic organisms. A comparison of the nanoparticles was made to Ag⁺ toxicity. The implications of manipulation of nAg surface composition on the toxicity assessment and its ecological relevance will be discussed.

WE 206

A comparative study on the influence of physicochemical properties to uptake and elimination in *Daphnia magna*

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Monitoring the distribution and subsequent effects of nanoparticle (NP) contaminants in aquatic ecosystems will be pivotal to developing regulations that minimize the environmental footprint. To answer the question of distribution, our research was designed to characterize the influence of particle size, shape and surface chemistry on uptake and elimination of gold NPs in a primary consumer. Uptake and elimination rate constants were derived empirically for citrate coated spheres (20 and 30 nm), and poly(acrylic acid) and poly(allylamine hydrochloride) coated rods (18 x 58 nm) using *Daphnia magna* (a pelagic filter-feeding crustacean) as a model organism. Differences in uptake rate constants was most pronounced when the surface coating was varied, with positive coatings exhibiting the highest uptake rate constants of all the treatments. Size and shape, however, did not appear to impact uptake. Elimination by *D. magna* followed the two-compartment model for each particle configuration predicting that the slower compartment will control the concentration of nanospheres in the organisms after *t* days of exposure. Significant differences between elimination rate constants were observed for particles varying in size but not for particles with different shapes or surface chemistry. Further study of the gut-lumen interface revealed that particles are in contact with the microvilli and in some cases adsorbed to the surface, which could represent the slow exchange compartment. Images, however, could not confirm accumulation of particles outside of the gut tract, though this does not preclude the possibility that particles are localized in other compartments.

WE 207

Effects of TiO₂ nanoparticles on a freshwater bacterial community - influence of DOC and UV radiation

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Nanotechnology is a rapidly growing industry with the extensive applications reaching from the technical-, medical and research sector over to a wide range of consumer products. Especially the widespread use in consumer products will lead to their release into the environment, particularly into the aquatic environment, as a sink for many anthropogenic contaminants. The nanoparticles special chemical and physical properties are associated with potential toxicological effects and there is still considerable uncertainty concerning their hazard for humans and the environment. In this study the effects of titanium dioxide nanoparticles (TiO₂NP) on natural bacterial communities of three Swedish lakes were assessed, taking environmental parameters, such as dissolved organic carbon (DOC) content and UV intensity into account. The hydrodynamic parameter of the TiO₂NP was approximately 75 nm, with a zeta potential of -60 mV. The nanoparticles were found to be stable in MilliQ water and high DOC lake water; however agglomeration processes were taking place in lake waters with lower DOC concentrations. Significant reductions in the bacterial abundance were found at 100 µg L⁻¹ in higher DOC lakes, and 1000 µg L⁻¹ in the low DOC lake, reflecting the influence of the particles agglomeration state on their toxic potential. However, differences in UV intensity did not influence the observed effects. The absence of induction of reactive oxygen species formation further corroborates the finding that the toxicity is not derived from the phototoxic potential of TiO₂NP. Influences of TiO₂NP on the bacterial community composition are currently under investigation. The presence of TiO₂NP was found to have a strong impact on natural lake water bacterial communities, with the toxic potential of the nanoparticles being significantly enhanced in the presence of stabilizing DOC.

WE 208

Effects of silver nanoparticles on freshwater microbial communities

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Nanoparticles (NPs) display special chemical properties because of their size, shape, composition and electronic structure. These properties lend NPs their functionality, but may also lead to toxic effects. Due to their widespread use in consumer products an exposure of the aquatic environment to NPs is anticipated and already proven in first analytical surveys. Especially metal/metaloxide NPs are widely used, of which silver nanoparticles (AgNPs) have gained considerable attention due to their broad microbiocidal properties. This implies a specific hazard for exposed aquatic primary producers (algae) and bacteria.

In a case study with AgNPs, the toxicity to natural freshwater microbial communities was determined. The microbial communities were exposed to AgNPs of different sizes (10, 20, 40 and 50 nm) as well as different coatings (non-coated, citrate coated) in concentrations from 0.1 - 5000 nmol/L except for one silver nanoparticle type (50 nm, uncoated) which was tested in a range from 100 - 10 000 nmol/L. The selected silver nanoparticles were all purchased from commercial producers and distributors (Amepox, British Biocell, NanoTrade, Tedpella). To distinguish between particle related effects and effects caused by free silver ions silver nitrate was tested as a reference in corresponding total silver concentrations. The effects on the algal parts of the community was studied through pigment profile analysis with HPLC. The bacterial community composition was analysed by metabolic profiling on so called ecogplates[TRADEMARK] containing different carbon sources with a redox dye responding to respiration with colour change. The metabolic activity i.e. the colour change was detected as absorbance and was measured over time in several intervals for a total time period of 96 hours. Inhibition of growth detected as decrease in total biomass was seen in both bacterial and algal communities. All experiments were backed up by analytical measurements, measuring the total silver content, dissolved Ag⁺ (ultrafiltration) and particle size distribution (Nanosight).

WE 209

Different sensitivity cyanobacteria and protist by silver nanoparticle

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Of the 1015 Nanotechnology-based consumer products available on the market in August 2009, the products containing silver nanoparticle (SNP) are the largest (25%) and fastest growing group. This growth of SNP used products is due to its extraordinary usefulness as a broad-spectrum antimicrobial agent. Despite the growing market of silver nanoparticle, little is known about the aquatic environmental effects of widespread use of SNP contained consumer products. Recently reports have showed that SNP is detected in natural environment. Many reports have revealed highly toxic to aquatic organisms, and the United States Environmental Protection Agency has set water quality regulation values for silver in salt and fresh water at 1.9 and 3.4 ppb, respectively. Toxicity and exposure data for SNP, however is little for prediction environmental risk.

Cyanobacteria, algae, protist, and bacteria consist of microbial food web. When the microbial food web is destroyed, aquatic environmental ecosystem should be damaged leading to become seriously environmental problem. Previous studies showed Gram-negative bacteria are more sensitive than Gram-positive bacteria. Algae exposed silver nanoparticles has been reported many articles showed inhibitory was difference every genus. Cyanobacteria and protist however is little report. Selective inhibitory effect is important for thinking environmental risk.

In this study, we try to reveal inhibitory and selective potential of silver nanoparticle on the growth of a cyanobacteria, *Microcystis aeruginosa* and grazing the cyanobacteria protist, *Monas guttula* under laboratory condition. And grazing cyanobacteria activity of *Monas guttula* was analyzed under several concentration of SNP.

At 1.0 mg/L of SNP, *Monas guttula* was perfectly inhibited the growth. At the concentration 10 µg/L, the inhibitory effect on *Monas guttula* growth showed small, but grazing cyanobacteria activity did not show inhibitory effect. SNP at 10 µg/L and 1.0 mg/L inhibited around 40% and almost 100%, respectively growth of a cyanobacteria, *Microcystis aeruginosa* NIES 102. This result is more sensitive than *Microcystis aeruginosa* UTEX 2388 which was inhibited over 80% growth at 1.0 mg/L and 40% growth at 10 µg/L.

Therefore, it was considered that selectively inhibitory effect depend on strain level as well as genus level. And growth inhibition and grazing activity inhibition separately are thought is better for predict environmental risk.

WE 210

Influence of metal oxide nanoparticles on the toxicity of selected pharmaceuticals evaluated in the Spirotox assay

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Spirostomum ambiguum is a very convenient protozoan for ecotoxicological studies due to its large size, ease of culturing and high sensitivity to a broad spectrum of toxicants including metal ions and pharmaceuticals. Moreover, it can be maintained for several days in inorganic medium at pH from 5.5 to 8.0 and at low and high levels of dissolved oxygen.

The goal of the study was the evaluation the toxicity of selected pharmaceuticals in the presence of bulk and nano sized metal oxides TiO₂ and CuO. The concentration of tested pharmaceuticals was monitored with HPLC-DAD. Morphological deformations and subcellular changes were observed under EPI-fluorescence microscope.

Titanium dioxide was not toxic to the protozoan. The toxicity of copper oxide depended on the pH of the medium. Both bulk and nano particles were ingested by the *S. ambiguum* and they were observed in food vacuoles. The suspension of metal oxides changed the toxicity of tested pharmaceuticals, but it is not clear if it was caused by the adsorption of the compounds on the particles or by catalytic activity of the metal oxides.

WE 211

Ecotoxicity of engineered nanoparticles on a test battery of aquatic organism

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The ecotoxicity of different diameters of engineered silica nanoparticles and polystyrene nanoparticles on a test battery of aquatic organisms representing four trophic levels is investigated in order to better understand how these nanoparticles can adversely affect the environment. Given the increase in the production of engineered nanoparticles, and thus, likely exposure to the environment, assessing the benefits and risks of nanoparticles requires a better understanding of their ecotoxicity in the environment; therefore, preliminary studies of this nature are extremely important for the assessment of their risks.

The ecotoxicity tests used are validated and standardized short-term methods for estimating the acute and chronic toxicity of chemical toxicants to bacteria, algae, invertebrates and fish.

Acute studies are complemented with standard and relevant chronic studies following OECD guidelines, as long-term effects are the most likely in an environmental exposure scenario. Furthermore, using fluorescent labelling and confocal microscopy, their uptake and depuration is studied to better elucidate the final fate of engineered nanoparticles in aquatic organisms.

The ecotoxicological work is also complemented with cellular studies, thus drawing linkages between in vitro studies and simple organism studies, as well as between approaches developed for cytotoxicity to ecotoxicology.

WE 212

Toxicity of the nanocomposite Carbo-Iron to *Danio rerio* and *Hyalella azteca*

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In the project Fe-NANOSIT, iron-based nanocomposite materials are developed for remediation of contaminated groundwater and wastewater. The iron nanocomposite Carbo-Iron (CIC) has been developed for in situ treatment of aquifers polluted with halogenated organics. Potential effects of aged CIC (ACIC) on the aquatic environment are investigated using acute and chronic toxicity tests with organisms of different trophic levels.

So far, effects of ACIC were determined with the acute toxicity test and the embryo toxicity test using the zebrafish *Danio rerio* according to OECD 203 (1992) and OECD draft (2006), respectively. Furthermore, acute and chronic tests with the amphipod *Hyalella azteca* were performed on the basis of US EPA (2000). Guidelines were adapted where required, e.g. by stabilizing ACIC suspensions with the dispersing agent carboxymethyl cellulose. During exposure, particle size was measured in test media with dynamic light scattering. Transmission electron microscopy was used to investigate uptake of particles into test organisms.

First results indicate a moderate toxicity of ACIC. In the acute toxicity test with *D. rerio*, no mortality was observed at ACIC concentrations between 4.3 and 100 mg/L during 96 h of exposure. In the fish embryo toxicity test, no significant lethal effects occurred up to concentrations of 100 mg/L. However, at ACIC levels between 10 and 50 mg/L the percentage of precocious hatch and growth of mycelia on the chorion was increased compared to control. Variation between replicates was high, but in repeated tests effects were observed in the same concentration ranges. In the 10-day acute test with *H. azteca*, biomass was significantly reduced at 100 mg/L compared to control. In the 42-day chronic test with *H. azteca*, biomass and number of offspring were significantly lower at 100 mg/L and 50 and 100 mg/L, respectively.

During the first three days of exposure, particle diameters were between 200 and 500 nm in all test systems used. After 14 days in the chronic test with *H. azteca*, determination of particle size was not possible due to low particle concentration, indicating sedimentation of ACIC.

TEM images of exposed *H. azteca* show uptake of ACIC into the gut but not into cells. For zebrafish embryos, ACIC agglomerates were detected on the chorion but not in the perivitelline space or the embryo.

Currently, an early life stage test is performed with *D. rerio* and effects of ACIC will be investigated in the sludge respiration test.

WE 213

Toxicity and accumulation potential of fullerenes (nC60) in crustacean *Daphnia magna* in four freshwaters

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Increasing usage of carbon nanoparticles (NPs), such as fullerenes (C₆₀), in commercial products enhances the risk that they are released into the environment. Some applications, such as cosmetics, provide a direct route to aquatic environments. Water treatment systems do not fully remove NPs. Moreover, fullerenes have been detected in wastewater effluents. When released to natural waters, fullerenes may interact with dissolved natural organic matter (DNOM), these interactions may alter the fullerenes' toxic effects and bioaccumulation potential as well as their stability in the aqueous phase. This study measures the toxicity and accumulation of water suspended fullerenes (nC₆₀) in four natural waters on crustacean *Daphnia magna*. Amounts of fullerenes accumulated by *D. magna* differed between freshwaters. Nevertheless, acute toxicity was relatively low in all test waters. Fullerene concentrations substantially decreased during the test in every treatment. This decrease in exposure prevented estimating acute toxicity parameters.

WE 214

Effects of titanium dioxide nanoparticles on the immune system of *Dreissena polymorpha*

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The purpose of this study was to investigate the effects of an *in vivo* exposure to titanium dioxide nanoparticles (TiO₂ NP) on the immune system of *Dreissena polymorpha*. Mussels were exposed to TiO₂ NP (1, 5 and 25 mg/L) during 24 hours in the dark. Viability, phagocytosis activity and mitogen activated protein kinase (MAPK) activation level were determined on hemocytes extracted from exposed mussels. Our results showed that exposition of *Dreissena polymorpha* to TiO₂ NP can affect immune response. Indeed, we observed a significant reduction of phagocytosis activity at the lowest concentration (38.4 % lower than control). Furthermore, we observed that this reduction is less important at higher exposition concentrations (19.8 and 14.3 % lower than control respectively at 5 and 25 mg/L). Exposition to 1, 5 and 25 mg/L of TiO₂ NP increased the MAPK phosphorylation levels which are respectively 1.5, 1.2 and 1.3 fold higher than control. Concerning p38 activation study, no difference has been observed at the lowest concentration (1 mg/L). However, an exposition to 5 and 25 mg/L of TiO₂ NP induced increases of p38 activation which are respectively 1.3 and 1.7 fold higher than control. Given these results, we can suggest that immune system of *Dreissena polymorpha* could be used as a bio-indicator not only for nanoparticles pollution in continental aquatic environment; but also for other pollutants.

WE 215

Ecotoxicological effects of the nanoparticles NiO (100nm and 10-20nm), Fe₂O₃ and TiO₂ on aquatic organisms

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The rapid development of nanotechnology and the increasing use of nanomaterials (NM) have been raising concerns about their fate and potential effects in the environment, since some of them could be used for remediation purposes. Despite emerging literature addressing the biological effects of NMs to aquatic organisms the existing information is still scarce and contradictory. Therefore, aimed in selecting NMs for the treatment of organic effluents, we assessed the potential toxicity of four NMs NiO (100nm and 10-20nm), Fe₂O₃ (d=40-130nm, c=250-600nm) and TiO₂ (<25nm), on a battery of assays using aquatic organisms from different trophic levels: the bacterium *Vibrio fischeri* (Microtox®) the microalgae *Pseudokirchneriella subcapitata* (growth inhibition test), the aquatic macrophyte *Lemna minor* (growth inhibition test), the cladoceran *Daphnia magna* (acute and chronic test) and two additional marine organisms, rotifer *Brachionus plicatilis* (RoToxKit) and crustacean *Artemia salina* (ArtoxKit). Suspensions of each NM, prepared on the different test mediums and at different concentrations tested, were characterized by Dynamic Light Scattering (DLS). For the assays with marine species (*V. fischeri* included) no toxicity was observed for any of the compounds tested. In what concerns the growth inhibition test with *P. subcapitata* no significant inhibitory effects were observed for Fe₂O₃, contrary to the outcome for the other NMs, being both nano-NiO the most toxic, with EC50 values of 8.24 mg L⁻¹ for NiO 100nm and 15.15 mg L⁻¹ for NiO 10-20nm. Regarding the results with *L. minor* no significant effects were observed for TiO₂ and Fe₂O₃, whilst for NiO 100nm and 10-20nm the LOEC values were 5.24 and 8.19 mg L⁻¹, respectively. In the acute test with *D. magna* the EC50 ranged from 9.74 mg L⁻¹ (24h) and 14.59 mg L⁻¹ (48h) for both nano-NiO of 100nm and 10-20nm. In the 21-day reproduction test the LOEC values determined for the same NM ranged from 0.045 to 0.14 mg L⁻¹. Regarding, nano-TiO₂ no acute toxic effects were observed but in the chronic test a LOEC value of 20 mg L⁻¹ was recorded. According to all the results the nano-NiO (100nm) was the most toxic for the aquatic species tested. From the results of this study we can establish a decreasing order of toxicity NiO(100)>NiO(10-20)>TiO₂>Fe₂O₃ for the tested NMs and the most sensitive organism was *D. magna*. According to the results, Fe₂O₃ NM seems to be the one with less risks to the receiving aquatic systems.

WE 216

Effects of nano CuO on aquatic decomposers: from community to cellular responses

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Intensive use of metal nanoparticles increases the chance of their release into freshwaters that may pose risk to biota and associated ecological processes. In streams, microbes play a key role in detritus foodwebs transferring carbon and energy from plant litter to invertebrate shredders. Here, we investigated the effects of nano CuO (< 50 nm, nanopowder, Sigma) on aquatic detritus foodwebs by examining i) leaf-litter decomposition by bacterial and fungal communities, ii) cellular damage and physiological responses of fungal populations collected from non-polluted and metal-polluted streams, and iii) survival, growth and leaf consumption by an invertebrate shredder. Results were compared with those obtained with ionic copper.

Stream-dwelling microbial communities were obtained by immersion of leaves in a non-polluted stream (Portugal). Microbial communities were exposed in microcosms to nano CuO (≤ 500 mg L⁻¹) and Cu²⁺ (≤ 30 mg L⁻¹). Leaf decomposition decreased with increasing concentrations of nano and ionic copper. Both copper forms reduced biomass of bacteria and fungi, and fungal reproduction. Cu²⁺ had stronger effects than nano CuO. Exposure to Cu²⁺ and nano CuO led to a decrease in fungal diversity and to shifts in species dominance.

Increased concentrations of nano CuO (≤ 100 mg L⁻¹) stimulated extracellular laccase activity by fungi. Populations from non-polluted streams were more affected by nano CuO than those from polluted streams, as shown by a stronger inhibition of biomass production, higher Cu adsorption, higher levels of reactive oxygen species and DNA strand breaks.

Acute lethality tests suggested low toxicity of nano CuO to the shredder *Allogamus lignifer*. However, sublethal concentrations of nano CuO (≤ 75 mg L⁻¹) strongly reduced leaf consumption and invertebrate growth under aqueous and dietary exposure. Concentration of leached Cu²⁺ in the stream water increased with increasing nano CuO concentration. Exposure to 75 mg L⁻¹ of nano CuO via water or food led to higher Cu adsorption and accumulation in larvae. Moreover, leached Cu²⁺ appeared to have a role in inducing toxicity of nano CuO.

WE 217

Effects of gold nanoparticles on planktonic crustacea (*Daphnia magna*, *Moina macrocopa* and *Simocephalus mixtus*)

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Gold nanoparticles have been used in a wide area due to the unique properties. This study evaluated the effects of gold nanoparticles (10 nm) on three cladoceran species (*Daphnia magna*, *Moina macrocopa* and *Simocephalus mixtus*). According to OECD TG No.202, test was conducted to observe immobilization and mortality for 48h, but exposure period for *S. mixtus* was reduced to 24h due to food stress of *S. mixtus*. In acute toxicity studies for *D. magna*, 24-h EC50, 24-h LC50 and 48-h L(E)C50 values were 0.24, 0.19, and 0.20 mg L⁻¹ respectively. In acute toxicity studies for *M. macrocopa*, 48-h EC50 and 48-h LC50 values were 0.12 and 0.15 mg L⁻¹. The 24-h EC50 and 24-h LC50 values were 0.22 and 1.12 mg L⁻¹ for *S. mixtus*. We observed surface adsorption of gold nanoparticles, and the accumulation of gold nanoparticles was also confirmed in gut. This study suggested aquatic toxicity data of gold nanoparticles for three cladoceran species and further studies are needed to investigate the ecotoxicity of gold nanoparticles to various aquatic species. *This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2011- 0015985).*

WE 218

Do C60-fullerene nanoparticles enhance the uptake and toxicity of pesticides towards *D. magna*?

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The role of engineered nanoparticles as vectors for environmental contaminants is so far an almost overlooked aspect of nanoparticle toxicity and practically no knowledge exists about toxicity of nanoparticle co-transported pesticides. The aim of this project is to clarify whether the presence of C60-fullerenes in aqueous suspension (nC60) will enhance the bioavailability, and hence the toxicity, of organophosphate insecticides with different lipophilicity towards the filter feeding crustacean *Daphnia magna*. The hypothesis is that pesticide sorption to C60-fullerenes will increase with increasing lipophilicity of the pesticides. As a consequence uptake will increase more than expected from simple log Kow based BCF calculation in the presence of C60-fullerenes due to the accumulation of pesticide loaded particles in the gut of *D. magna*. If the organophosphorous insecticide is released in the gut of the daphnia, toxicity is also likely to increase in the presence of C60-fullerenes. If it is irreversibly bound, however, toxicity is expected to depend on water concentrations only. The experiments were performed using C14 marked dimethoate, methyl-parathion and chlorpyrifos with a log Kow of 0.7, 3 and 4.7, respectively. Studies of pesticide uptake with and without 3 mg nC60 L⁻¹ were performed, and *D. magna* mobility was monitored after 24h together with pesticide quantification (14C analysis by liquid scintillation counting) in the water, daphnia and nC60 fraction in order to establish mass balances for the experiments. Preliminary results show limited adsorption of organophosphate insecticides to nC60. The influence of pesticide lipophilicity on sorption to nC60 and facilitated uptake in *D. magna* will be illustrated and discussed.

WE 219

Chronic ecotoxicity of ceria nanoparticles on freshwater micro-invertebrates

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Nanoparticles are more and more incorporated in commercial products. It is consequently inevitable that a large amount of NPs will be discharged into environment, underlining the need to evaluate the ecotoxicological effects of NPs. Although there is some evidence for adverse effects on aquatic organisms, there is still a lack of consistency in nanoeotoxicity investigations likely due to differences in the physico-chemical parameters of the tested NPs or in the way the NP suspensions were dispersed. Furthermore, most of the available data are still related to acute toxicity after exposure at concentrations exceeding those predicted to occur in the environment. In that context, our work aimed at assessing the chronic effect on freshwater micro-invertebrates exposed to sublethal concentrations of different ceria NPs in suspension (ie. a 25 nm and a 10 nm primary particle size NPs). The selection of ceria NPs was motivated by their growing commercial use in number of applications such as outdoor paints or as fuels catalyst for which release to aquatic compartment is therefore expected. Besides, ceria NPs are also one of the selected NPs for priority testing by the OECD. Chronic ecotoxicity was investigated on *C. dubia* following the standardized methods (ISO 20665).

After 8 days of exposure, a dose-dependent inhibition of growth and reproduction of daphnids were reported for both ceria NP tested (10 nm and 25 nm). Interestingly, the 10 nm nCeO₂ induced higher toxicity than the 25 nm nCeO₂. We reported EC₅₀ values from 5.7 to 15.7 mg/L depending on the method used to disperse the 25 nm ceria suspensions, whereas EC₅₀ from 1.2 to 1.6 mg/L were recorded after exposure to the 10 nm ceria suspensions. This result highlights that the primary particle size is an important parameter when focusing on nCeO₂ ecotoxicity. The influence of the dispersive methods on the ecotoxicity towards micro-invertebrates will also be discussed. In addition to these results, histological and ultrastructural investigations of target cells in *C. dubia* (i.e. midgut epithelium) were performed by TEM. The results obtained have clearly shown that the ceria NPs were present in the digestive tract lumen of *C. dubia*. Disappearance of the apical microvillous of intestinal cells and reduction of rough endoplasmic reticulum network were observed. These ultrastructural damages are sufficient to disturb cellular metabolisms such as nutrient absorption, protein synthesis or phospholipid assemblage.

WE 220

Combined effects of UV radiation and ZnO nanoparticles to *Daphnia magna* and *Danio rerio* embryos

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Zinc oxide nanoparticles (ZnO-NP) are among the most studied nanoparticles regarding toxicity to aquatic invertebrates mainly because of its large-scale production and usage which ultimately leads to the entrance of ZnO-NP in the aquatic environment. Different sources of stressors, such as chemical discharges as well as natural environmental stressors like high UV radiation levels are factors that have been described as harmful to aquatic organisms. Because ZnO-NP has already been shown to be toxic to a few model species and considering that one of its major applications is as UV filter, the aim of this study was to investigate the joint effects of UV radiation and ZnO-NP to *Daphnia magna* and *Danio rerio* embryos.

In brief, preliminary single dose-response curves of *D. magna* and *D. rerio* to ZnO-NP and UV radiation were taken into consideration to design the combined exposures. The organisms were exposed simultaneously to UV radiation and ZnO-NP for short time periods followed by a continued exposure to ZnO-NP. The endpoints analyzed for *D. magna* were survival (OECD 202), feeding inhibition and reproduction (OECD 211) whereas for *D. rerio* the embryonic development was daily checked for any abnormalities and survival. Data from single and combined exposures were pooled on the MixTox model, and assuming that UV radiation and ZnO-NP have dissimilar modes of action on both species, the Independent Action model was used and possible deviations to synergism/antagonism, dose-ratio and dose-level evaluated.

WE 221

Influence of hardness, humic acids and temperature on the lethal toxicity of gold nanoparticles to *Daphnia magna*

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The potential of nanoparticles (NP) to cause adverse effects on biota has been widely reported. However, most works were carried out under standard laboratory conditions and using standard test media. Although this approach is important to compare experimental results, it has poor ecological relevance, as it is expected that, in the environment, NP will occur under different and varying conditions of several abiotic factors, which will influence their fate and toxicity. Therefore, it is important to understand how such factors will change their bioavailability and toxicity. Accordingly, the present work aimed to evaluate the influence of three abiotic factors (temperature-T, hardness-HAR, and humic acids-HA) on the lethal toxicity of gold nanorods (Au-NP) to the cladoceran *Daphnia magna*. To attain this goal, neonates of *D. magna* were exposed, for 48h, to five concentrations of Au-NP (3.8 to 7.9 µg/L) plus a control (ASTM medium), under three different levels of HAR (180, 90 and 45 mg/L-1 CaCO₃; hard-standard media, moderately hard, and soft, respectively), HA (1, 3 and 5 mg/L; low, medium and high, respectively) and T (15, 20 and 25°C; low, medium-standard conditions, and high; respectively). At the end of exposure, immobilised organisms were counted at each test vessel. For each tested treatment, NP suspensions were characterized, using light scattering: zeta potential (electrophoretic mobility based on Doppler effect), hydrodynamic diameter (Dynamic Light Scattering). Temperature did not cause changes in the measured parameters of NP-Au suspensions. But, a decrease in HAR caused a decrease in hydrodynamic diameter and an increase in zeta potential corresponding to a change in surface charge of the suspended NP-Au (data for HA is still being analysed and will be shown in the poster). Regarding observed toxicity, an increase in T caused an increase in toxicity of Au-NP, as reflected by the computed LC_{50,48h}: 3.2, 4.0, and 9.1 µg/L of NP-Au, respectively for 25, 20, and 15°C. An increase in HAR did not cause a significant change in lethal toxicity of NP-Au. Finally, increasing concentrations of HA provoked a decrease in lethal toxicity: 6.1, 6.0, 9.1 µg/L NP-Au, respectively for 1, 3, and 5 mg/L of HA. The results obtained showed the high influence that some abiotic factors may have on the toxicity of NP-Au, highlighting the need to fully understand these processes in order to avoid the underestimation of risk that NP may pose to the environment.

WE 222

Ecotoxic evaluation of zero-valent iron nanomaterials in the aquatic environment

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New environmental technologies emerge with the development of nanotechnology. The use of zero-valent iron nanomaterials (NZVI) is promising to remediate ground water contaminated with a variety of common environmental pollutants, including chlorinated organic solvents, organochlorine pesticides, and polychlorinated biphenyls (PCBs). The reduction of organic contaminants is a progress successfully applied in the passive barrier remediation technique with micro scale zero-valent iron. A benefit of nanomaterials is the possibility to directly inject them into the contamination source by in situ remediation technologies. The project NAPASAN funded by the German Federal Ministry of Education and Research aims to develop zero-valent iron nanomaterials that are cost efficient and have good transport qualities in the groundwater aquifer. A key aspect of new technologies is their safe and beneficial application in the field. In this project, the technology is evaluated to ensure the suitability of NZVI for safe remediation. The unintended release as well as potential entrance of this iron nanomaterial into groundwater reservoirs might lead to the partitioning of iron transformation products to surface waters. Reference nano as well as bulk iron materials were therefore subjected to standardized acute aquatic and mechanistic ecotoxicity tests: the *Daphnia magna* acute immobilization test, algae growth inhibition test, Ames fluctuation test with cytotoxicity evaluation, and the fish embryo toxicity test. Dispersions were prepared by means of ultrasonication with a microtip. Afterwards, the particles were characterized by means of dynamic light scattering and TEM imaging. By now, little toxicity of tested iron species was observed. From the present results, it can thus be derived that iron species formed during transport of NZVI to and in the aerobic aquatic environment do not present a risk for its inhabiting biota. However, the influence of the formation of complexes with iron and/or its oxides and salts on the bioavailability of pollutants remains unknown and will be investigated at the moment using radiolabelled organic compounds.

WE 223

Long-term effects of nanosilver - a multigeneration experiment with different *Daphnia* species

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Nanosilver is increasingly applied in a variety of consumer products. Due to its antibacterial properties the application ranges from wound dressings, textiles, paints, personal care and cleaning products to food storage containers. Currently, no data exists for environmental concentrations, but the incremental production of nanosilver inevitably leads to an increased environmental release. Chronic exposure data for aquatic invertebrates, however, are currently lacking.

The aim of this study is to determine long-term effects of nanosilver in a multigeneration experiment with three commonly found *Daphnia* species: *D. magna*, *D. pulex* and *D. galeata*. Toxicity tests were conducted with nanosilver (NM-300 silver; particle size 15 nm) from RAS GmbH provided by the OECD sponsorship programme. The material is coated with PVP and is delivered in dispersion. Dispersion stability was characterised by dynamic light scattering and transmission electron microscopy. Due to the distribution of particles in the water phase, daphnids as filter feeders and key species of aquatic ecosystems are considered as suitable organisms for the assessment of nanosilver toxicity. In the multigeneration experiment the effects of nanosilver on growth, reproduction, and viability were assessed with particular emphasis on trans-generational effects. To evaluate whether toxicity is related to nanoparticle properties or to the effects of released silver ions, the effects were compared with silver nitrate. Results show different reactions to nanosilver depending on species and exposure time. Acute exposure of *D. magna* results in an EC₅₀ value of 121 µg/L whereas *D. pulex* and *D. galeata* show higher susceptibilities (EC₅₀ values of 8.95 and 13.9 µg/L). Long-term studies with *D. magna* and *D. galeata* show negative effects on reproduction with an EC₁₀ of 1.13 and 1.37 µg/L, respectively. In addition, the exposure of consecutive generations of *D. magna* results in a higher susceptibility of the daphnids. Although *D. pulex* shows the strongest susceptibility after acute exposure, it was the least sensitive species under long-term exposure to nanosilver. In summary, the results indicate an increasing toxicity of nanosilver with exposure duration. Considering the varying effective concentrations caused by elongated exposure time and different daphnids tested, long-term studies should be given a high priority to make an adequate evaluation of the potential environmental risks of nanomaterials.

WE 224

The formation and toxicity of silver nanoparticles generated in the presence of natural organic material

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Silver nanoparticles are one of the most widely used engineered nanomaterials (NMs) and their wide spread use in consumer products has led to them becoming a potentially important factor in environmental risk assessment (ERA) and monitoring. Naturally occurring nanoparticles have always existed in the environment without documented adverse effects. Despite this, the risks associated with engineered nanoparticles (ENPs), in the environment are becoming a significant concern. As silver is often found in the waste effluents from hospitals and medical facilities there is a risk that aquatic habitats receiving such effluents and containing natural organic materials (NOM) could provide suitable conditions for the generation of silver nanoparticles in situ. In this study the generation of silver nanoparticles by reduction in the presence of environmentally relevant levels of NOM is described under various relevant conditions (temperature, pH etc.). This study aimed to develop the methodology to generate NOM functionalized silver nanoparticles and investigate the toxicity of particles generated under differing conditions. Silver nanoparticles were synthesised by the reduction of silver nitrate in the presence of two different NOMs, Suwannee River and Nordic Lake NOM. In addition the production of silver nanoparticles employing sewage effluent containing NOM was also conducted. A range of varying NOM concentrations and AgNO₃ were employed to investigate the effects of varying amounts on nanoparticle size distribution and potential toxicity. A thorough characterisation of all silver nanoparticles was conducted employing Dynamic Light Scattering (DLS) for size measurements and zeta

potential, X-ray absorption spectroscopy (XAS) and Transmission Electron Microscopy (TEM). The generated nanoparticle toxicity was then assessed employing standardized ecotoxicity acute and chronic tests for *Daphnia magna*.

WE 225

The acute test with *Daphnia magna* underestimates environmental risks of iron oxide nanoparticles

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Nanoparticles are more and more used in a wide variety of applications. One of these is the use of zero-valent iron (ZVI) for remediation of contaminated groundwater. ZVI is highly redox-reactive. In a nanoparticle dimension the surface-volume-ratio is enormously increased. So far, there is a serious lack of knowledge about risks or impacts after the use of these particles. When released to the environment, the ZVI particles are oxidized. Therefore, this study concentrated on iron oxide nanoparticles (IONP).

The IONP, consisting of Fe_3O_4 , were coated with Polyvinylpyrrolidone (PVP) against agglomeration. The coated particles' diameter ranged between 100 and 200 nm (measured via DLS). Acute and reproduction tests with the waterflea *Daphnia magna* were performed according to OECD guidelines 202 and 211 in Elendt M7 medium. The acute test was prolonged to 96 h and performed in 24-multiwell plates with 10 replicates and 1 neonate per replicate. Nominal concentrations ranging from 1 to 100 mg Iron/L were tested.

The prolonged acute test showed a slight increase in toxicity after 96 h in the highest test concentration of 100 mg/L, which was not significant compared to the control. The reproduction test showed various effects of the IONP. High mortality occurred in test concentrations over 50 mg/L after 5 to 8 days, showing that acute tests might not be appropriate for testing nanomaterials. In lower concentrations reproduction was significantly inhibited by the IONP. This might result out of direct toxicity (e.g. oxidative stress) or indirect effects. E.g. an increase in agglomeration of algae for feeding could be observed, which resulted in blocking the filtering apparatuses of the daphnids. This might have inhibited the young daphnids in their development. In a further investigation, the uptake and potential bioaccumulation of the IONP was tested. A quantitative accumulation was measured with a photometric assay, while the qualitative uptake was investigated via histology and Pearls' staining.

The study clearly shows potential risks of IONP when released to the environment. Therefore, the use of ZVI and other iron nanoparticles has to be considered thoroughly. Risk regulators for remediation purposes should take into account the limitations of acute tests for assessing environmental hazards.

WE 226

Stability of citrate, PEG-SH and PVP Silver nanoparticles in toxicology media

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Many toxicology studies of silver nanoparticles (AgNPs) to a range of organisms have been performed since the discharge potential of AgNPs to the environment is to be expected. However there has not been any consent result yet. Mechanism underlying their toxic effect has also not been fully understood because of information lacking on properties of AgNPs both in pristine suspension and especially in toxicology media used for exposure study. In fact, the media used for exposure study may affect the nature and dose of toxicant. In this study, monodisperse citrate, PEG and PVP coated AgNPs with a core size of approximately 10 nm were synthesised and characterised both in stock suspension as well as in standard OECD media used for *Daphnia* sp. acute and chronic tests; bolt's basal medium and fish embryo toxicity assay media. Surface plasmon resonance, surface charge, size, aggregation and shape of AgNPs in those media were monitored and used as indication of stability of AgNPs in toxicology media.

WE 227

Is there a risk of iron (oxide) nanoparticles used for remediation? A case study on combinatory effects in *Daphnia magna*

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Iron and iron oxide nanoparticles (INP) are increasingly used for the in situ remediation of contaminated groundwater and soil. Made of zero-valent iron (ZVI), these INP have a high redox potential and are able to reduce many organic compounds to less dangerous metabolites very effectively. Furthermore, they can bind heavy metal ions. In contrast to conventional remediation techniques, the application of INP is extremely cost-effective. Therefore, these particles will be applied on many contaminated sites in the future. So far, an environmental risk assessment has not been made. The possible risks could be direct effects (e.g. oxidative stress via iron) as well as indirect effects (INP as a co-transporter for contaminants into organisms).

ZVI is quickly processed to iron oxides. Therefore, IONP were used in this study. Being less reactive, IONP are also easier to handle. The IONP (Fe_3O_4) were tested with the waterflea *Daphnia magna*. The IONP were functionalized with Polyvinylpyrrolidone (PVP) to stabilize them and to prevent them from agglomerating in the medium (Elendt M7). To investigate direct and indirect effects, single and combinatory acute tests with IONP and different potential target contaminants (Cadmium, Copper, Glyphosate, Resorcinol) in concentrations up to 100 mg iron/L were made. Furthermore, the influence of residues from the synthesis was investigated. Normally, the IONP were separated from the synthesis solvent (Diethyleneglycol DEG). The acute test was performed according to the OECD guideline 202 with some adaptations for testing nanomaterials. The test was prolonged to 96 h hours and performed in 24-multiwell plates.

The pure IONP had no significant immobilizing effect on the Daphnids within 96 h. The combinatory tests revealed different interactions. The toxicity of Cadmium and Copper was significantly reduced. There was no effect on Resorcinol, while the toxicity of Glyphosate after 24 and 48 h was reduced, but after 96 h the combination increased toxicity. Residues of the synthesis enforced the toxicity of IONP dramatically. The combination of DEG and IONP coming directly out of the synthesis increased the toxicity of DEG 6 times compared to pure DEG.

The results show that IONP are suitable for remediation applications. At the current state of knowledge it cannot be excluded, that INP and IONP might increase the co-transport of toxic substances into organisms. Besides a "green synthesis", a thoughtful application of INP is recommended.

WE 228

The influence of humic acid on the toxicity of double walled carbon nanotubes to Algae, *Daphnia* and fish

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Double walled carbon nanotubes (DWNTs) are regarded as an excellent compromise between the single walled carbon nanotubes and multiwalled carbon nanotubes due to their potential applications in; electronic devices and composites for water treatment. The anticipated increasing applications of DWNTs will lead to their ultimate release into the aquatic ecosystems through wastewater discharges, accidental spills, and during use phase as water treatment devices. However, presently data on the toxicological effects of DWNTs in the aquatic ecosystems is largely lacking, and in this study, we seek to elucidate their effects to aquatic organisms at different trophic levels. Natural organic matter (NOM) fractions like humic acid are among the major influencing abiotic factors for the toxicity of various toxicants in the aquatic ecosystems. Therefore, in this paper, data on the influence of humic acids on the toxicity of DWNTs in the aquatic ecosystems is presented.

Here, acute toxicity tests for evaluating the effects of DWNTs in aquatic systems were; the algal growth inhibition test, water flea (*daphnia*) acute toxicity test, and the fish acute toxicity test. The effective concentration (EC_{50}) of DWNTs on algae, *daphnia* and fish were found to be 11.2 mg/L, 4.49 mg/L, and 374 mg/L respectively, in the absence of humic acid. Addition of humic acid in the exposure media (3.64 mg/L) yielded a decrease in the toxicity of DWNTs towards algae with an EC_{50} of 29.6 mg/L. However, for the *daphnia* and fish, the toxicity increased insignificantly. The findings demonstrate the effect of NOM on the toxicity of DWNTs to biological life forms in different trophic levels in the aquatic ecosystems.

WE 229

Cumulative damage of tungsten carbide nanoparticles with cobalt doping in *Daphnia magna*

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The effects of tungsten carbide nanoparticles without (WC) and with cobalt doping (WC-Co) on *Daphnia magna* mobility were compared after 24, 48, 72 and 96 h of exposure. After 24h, almost no individuals were immobilized by WC. In contrast, 50% of the individuals were immobilized by 0.89 mM WC-Co. After 96h, the median concentration that immobilizes 50% of the individuals (EC_{50}) was 2.75 mM WC and 0.11 mM WC-Co. To investigate the influence of cobalt on the WC-Co toxicity, the effects of the cobalt content in WC-Co and in cobalt chloride ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$) were compared. A similar effect was observed after 24h, but the EC_{50} after 96h was two times higher for $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ than for WC-Co. The decrease of EC_{50} with increasing exposure time was described by a concentration-time-effect model, which combines the rule of Haber and the Hill model. The slope of the curve that describes this decrease was similar for WC and for WC-Co, and was two times higher for the cobalt in $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ than in WC-Co. The same model was used to extrapolate the EC_{50} at infinite or the asymptotic EC_{50} , which does not change with exposure time. This EC_{50} was 4.8 and 12 times lower than the EC_{50} after 24h for the cobalt in $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and in WC-Co, respectively. These results lead to the suggestion that the cobalt component of the WC-Co influences the toxicity level, while the WC component influences the cumulative damage.

WE 230

Bioaccumulation and internal distribution of silver nanoparticles and ionic silver in *Pseudokirchneriella subcapitata* and *Daphnia magna*

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The increasing production and consumption of products with antimicrobial effect which are based on silver nanoparticles (AgNPs) has caused the release of AgNPs and derived free ions into the environment, most likely to the aquatic compartment. This represents a discrete and additional source of contamination from that derived from non-nano scale processes.

Many recent studies have investigated the toxicity and effects of AgNPs (differing in size, coating material and surfactants), on sentinel aquatic organisms such as *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Danio rerio*. This study aimed to address the bioaccumulation pattern of silver nanoparticles and silver nitrate by green algae and *daphnia*, focusing on uptake rates, dissolution of silver nanoparticles in the test media, and internal distribution of silver in *Daphnia* and algae cells. Algae at the mid-exponential growth phase were spiked with silver nitrate or silver nanoparticles. The uptake phase was monitored at 8 hour intervals and lasted for 48 hrs. After 48 hrs of uptake,

the remaining algae was centrifuged and re-suspended in MBL medium for depuration phase, which lasted for another 48hrs with sample taken after 24 and 48 hrs. For the *Daphnia* bioaccumulation assay, 7 day-old *Daphnia magna* were exposed to two concentrations of AgNO₃ and AgNPs for 96 hrs (24 hrs uptake followed by 72 hrs depuration). Bioaccumulation patterns were similar for both algae and *daphnia* with increasing internal concentration of silver at higher medium concentrations. Algae reached a plateau of internal concentration after 20 hrs exposed to 20 µg/L of silver nitrate and AgNPs. *Daphnia magna* exposed to 1 µg/L of either silver nitrate and nanoparticles had a higher body burden of silver. After 48 hrs of depuration, the concentration of silver in *Daphnia magna* was insignificant when compared to control animals, indicating that complete elimination of silver occurred within the 48 hrs following exposure. Internalization was also one of the parameters used in this study giving patterns for accumulation inside organisms, which were dependent from the exposure used. These results indicate a first insight of bioaccumulation of nanoparticles in comparison with metal ions in algae and *daphnia*, and will be useful to assess the metal trophic transfer within an aquatic trophic chain.

WE 231

Effects of ZnO nanoparticles in *Daphnia magna*: size dependent effects and counterparts

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Nanotechnology has increasingly grown worldwide in the recent years and it's clearly becoming crucial to understand the potential impacts of nanomaterials in the environment.

Zinc oxide nanoparticles (ZnO-NP) are metal oxide nanoparticles known to have a broad spectrum of applications in personal products, coatings and paints. The last fate of these compounds is likely to be the aquatic environment. The aim of this study was to evaluate the effects to *Daphnia magna* of different sized ZnO-NP and also compare them with the effects of bulk and ionic counterparts (ZnO non-nano scale and zinc chloride (ZnCl₂), respectively). The toxic effects of these substances were studied on survival, feeding activity and offspring production.

In this work, differences on particle size showed to influence the toxicity of ZnO to *Daphnia magna*. In addition it was also observed that ZnCl₂ presented higher toxicity when compared to the ZnO particles.

The overall outcome from this study highlights the importance of evaluating nanoparticle toxic effects, considering different sizes and counterparts on relevant endpoints besides survival, such as feeding inhibition and reproduction responses.

WE 232

Do titanium dioxide nanoparticles (nTiO₂) induce adverse effects in the released offspring of *Daphnia magna*?

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Titanium dioxide nanoparticles (nTiO₂) are due to their chemical (e.g. photocatalytic) properties widely and increasingly applied. Hence, nTiO₂ may finally end up in the surface water bodies where they may pose a risk for aquatic ecosystems. Most of so far conducted toxicity studies with nTiO₂ investigated the acute and chronic toxicity for *Daphnia magna*. Interestingly, those studies differ in their outcome by up to two orders of magnitude, which may be driven by varying particle characteristics, although this information is not always provided. Therefore, the present study assessed the chronic and acute toxicity of two different nTiO₂ products (A100 and P25) on *D. magna*, accompanied by a thorough particle characterization. For this purpose, both the conventional semistatic test design (OECD 211) and an especially developed flow-through system were used. The latter is capable of avoiding larger agglomerates of nTiO₂, and hence an "nTiO₂-bottom-layer", which was recently suggested to be the main pathway of nTiO₂ toxicity for leaf shredding amphipods in darkness. The semistatic test design displayed larger agglomerates of A100 (~330nm) revealing with approximately 10% a significantly reduced cumulative mean offspring and size of adult daphnids at nominal nTiO₂ concentrations of 0.60 mg/L. Smaller agglomerates of A100 and P25 (~150nm) did not reveal any statistically significant effect on these endpoints, neither using the flow-through system nor the conventional semistatic test design. Furthermore, we additionally conducted a series of 96 h acute toxicity tests using juveniles released during the fifth brood by adults exposed to 0.00, 0.02, and 2.00 mg/L nTiO₂ in the flow through system, displaying that even 0.02 mg/L A100 increased the test organisms sensitivity significantly. As environmental concentrations were predicted to be only a factor of 13 below the reported effect concentration of A100 in this study, adverse implications in aquatic ecosystems cannot be reliably excluded, even more by considering the existing diversity of nTiO₂ products. Therefore, further ecotoxicological investigations by the means of thorough particle characterizations are recommended in order to build up an adequate database allowing a confident risk assessment regarding nTiO₂.

WE 233

Comparing the cytotoxicity of high aspect ratio nanomaterials: does nanomaterial architecture contribute to toxicity?

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Nanotechnology is a transformative discipline, stimulating technological and biomedical innovations that have fueled this field's expansion. The wide-spread production, use, and disposal of this technology is resulting in their release into the environment; as such, it is crucial to investigate the potential biological interactions of NMs and their associated toxicity. An important consideration in the toxicological evaluation of NMs is their cytotoxicity. This is important because many intended applications of NMs are biomedical in scope and cytotoxicity is a critical factor to consider when evaluating their potential.

Research suggests that the physico-chemical properties of NMs govern their toxicity; specifically, size, shape, solubility, charge, and surface functionalizations.

I use helical rosette nanotubes (RNTs), carbon nanotubes (CNTs), and nanocrystalline celluloses (NCCs) to investigate the cytotoxicity of NMs. These materials share a similar tubular architecture and can be synthesized with a variety of functionalizations. This enables me to investigate how architecture and physico-chemical properties can influence the biological interactions of NMs.

A mammalian cell line, RBL 2H3, was exposed to NMs, each with a variety of functionalizations, for 6, 12 and 24 hours. Viability was assessed using propidium iodide, a fluorescent dye that binds to DNA in dead or dying cells, coupled with flow cytometric analysis. We investigate if NM exposure affects the metabolic activity of cells using the MTS assay. To gain insight on how these materials are interacting with cells, we use confocal microscopy to visualize FITC-functionalized RNTs. Cells are stained with both a membrane dye (RH414) and an intracellular marker (DAPI) to determine if uptake can be observed in cells incubated with these materials for 6, 12 or 24 hours. Lastly, we investigate if exposure to CNTs, RNTs and NCCs are compromising membrane integrity using DiBAC (bis-oxonol) dye.

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WE 236

Poly-εpsilon-caprolactone nanocapsules loaded triazine herbicides: safer formulations for herbicide applications

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Triazine herbicides are the most important class of agricultural chemical ever developed. Because of their extensive use in agriculture, they are often found as a pollutant in rural aquatic environments. In order to reduce their toxicity in the environment, the use of polymeric nanoparticles as carrier systems can be a good alternative to increase their effectiveness and minimize their environmental impacts. The objective of the present study was to characterize and investigate the genotoxicity of poly-ε-caprolactone (PCL) nanocapsules loaded three triazine herbicides (simazine, atrazine and ametryn). The nanocapsules were characterized by the measurements of encapsulation efficiency (EE, %), mean diameter, polydispersion index and their zeta potential of the nanocapsules. Genotoxicity of the formulations were analyzed using cytogenetic, micronucleus, comet and Allium cepa assays, as well as molecular analysis. The association efficiencies of the herbicides in the nanocapsules were higher than 84 %. Assessment of stability (considering particle diameter, zeta potential, polydispersity) was conducted over a period of 270 days, and the results showed that the particles were stable in solution. In vitro release kinetics experiments showed that there was a reduction in the release profile of the herbicides in relation with free herbicides. Genotoxicity analysis showed that the cell response was improved when the pesticides were encapsulated in PCL nanocapsules, compared with the free pesticides and that there was a decrease in the damage index of chromosome alterations, after pesticide exposure, when pesticides were encapsulated in PCL nanocapsules. The use of PCL nanocapsules as carrier systems for triazines herbicides is therefore safer for the environment applications and ensuring sustainable development. Acknowledgments: Fapesp, CNPq and Fundunesp.

WE 237

Effect of light condition and particle size on toxicity of nano-TiO₂ in *Daphnia magna*

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TiO₂ can be unintentionally discharged to the environment through the point source and non-point source. Due to its photocatalytic property, conventional laboratory toxicity test that was performed under fluorescent light might underestimate actual toxic effects of TiO₂ in the environment. However, very little is known about the phototoxicity of TiO₂ in aquatic organisms such as *Daphnia magna*. The aim of this study was to evaluate effect of light condition and particle size of TiO₂ on *D. magna* in TiO₂ toxicity test. Acute toxicity test was performed on *D. magna* using pyro-TiO₂^{P25-70} (< 25nm) and TiO₂^{P25-70} (> 1,000nm) under different particle size and light conditions such as fluorescent light, UV-B light, and solar light. In addition, antioxidant enzyme levels such as catalase, superoxide dismutase, and glutathione-S-transferase and lipid peroxidation levels were analyzed to understand the mechanism of phototoxicity. Test concentrations were chosen 0, 0.31, 0.93, 2.78, 8.33, and 25 mg/L for acute toxicity test and 0, 0.034, 0.103, and 0.310 mg/L in antioxidant enzyme test. *D. magna* was more susceptible to TiO₂ particles with > 1,000 nm in size under UV-B and solar light condition than fluorescent condition. The CAT increased at the highest concentration of TiO₂^{P25-70} under the solar light condition but GST, SOD and TBARS increased at lowest concentration of TiO₂^{P25-70}. The present study demonstrates that light conditions could alter toxicity of TiO₂ nanoparticles in *D. magna* and smaller sized particles are not always more toxic than bigger sized particles.

WE 238

How to measure toxicity of nanoparticles to generate data useful for QSAR modellers?

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Nanotechnology is currently one of the most promising areas of science. Regarding that the number and amounts of synthesised nanomaterials will increase exponentially, it is reasonable to expect that comprehensive risk assessment based only on empirical testing for all novel candidates will very soon become impossible. Therefore, development of computational methods complementary to experiments is very important.

Having properties that characterise the structure, along with systematic empirical data on toxicity/ecotoxicity, it is possible to establish mathematical models quantitatively describing relationships between the structure and activity. Quantitative Structure-Activity Relationship (QSAR) models, widely used in medicinal chemistry, cosmetics and environmental science can also be applied in nanotechnology to predict toxicity of empirically untested nanomaterials. Such approaches will significantly reduce costs and the time associated with the risk assessment for nanomaterials and lead to the designing of safer nanomaterials.

All QSAR modelling activities are based on available experimentally derived toxicity data. It is important that, within a given data set, all values are consistent, of high quality and measured according to a standardised protocol. Unfortunately, the number of such data is limited and seldom measured with a standardised protocol. This study was aimed at investigating these data sources and aspects of variability relating to toxicity data for Nano-QSAR. To this end the existing sources of experimental data that can potentially be utilised for developing models have been collated. In addition a simple algorithm was developed to evaluate these data and select data sets for modelling.

The analysis indicates that there are a number of different measuring/testing protocols, performed by different laboratories, which may result in significant variance in data, sometimes greater than the real variability in the nanoparticles' properties. This may result in the low applicability of those data for the development of Nano-QSAR. Simply speaking, there is insufficient communication between the experimental and theoretical (computational) research groups working on risk assessment of nanomaterials. The main findings of the evaluation exercise are summarised and further recommendations for experimental groups regarding the quality of empirical data useful for Nano-QSAR modelling purposes are presented.

WE 239

Usage of natural biocompatible intermediators for magnetic nanocomposites development

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This study is aimed to create the efficient sorbents viz. resistant nanocomposites on the basis of magnetite or ferrites and natural high-molecular polymers as biogenic coal-derived humic acids (HA) and beet-derived pectic acids (PA). The concept of using a natural polymers as a potential strategy for synthesis of nanocomposites could be based on its biodegradable and biocompatible nature and flexible structural networks allowing the device to be designed to a specific shape. The magnetic nanocomposites with particles of iron oxides (v-Fe₂O₃, Fe₃O₄) were produced both by a chemical precipitation method in situ and mechanochemical dispersion. The advantage of such an approach is that polymer-conjugated nanoparticles of iron oxides have been developed with the application of up-to-date approaches of "green chemistry", which require optimization of chemical process by the principle of minimal wastes and maximal safety as well as intermediators and of target products of the production. In addition, these nanocomposites have shown the characteristics not only as specific characteristics of magnetic materials but as well high-efficacy sorbents (2). In other words, they are the controlled magnetoactive nanocomposite materials.

The nanocomposites obtained were characterized by FTIR, XRD, Mossbauer, and SEM studies. Results of FTIR analysis indicated that ligand exchange between carboxyl/hydroxyl functional groups of humic and pectic acids respectively and iron oxide surfaces was the dominant interaction mechanism. According to X-ray diffractometry studies, the major phase formed during a chemical coprecipitation in the presence of polymers in situ is a magnetite Fe₃O₄. The average crystal size of the nanoparticles calculated from the diffraction peak half-widths according to Scherrer's equation for the Fe₃O₄ nanoparticles and nanocomposites obtained depends on ratio of precursors of composites and varies in range of 10-100 nm. These results agreed with the Mossbauer data and electron microscopy studies.

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WE 240

Nanomaterials in plant protection products: current state, foreseen applications and research priorities

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The recent advances in nanotechnology have led to a corresponding increase in the use of nanomaterials (NM) in a wide range of products. There is already a body of literature that examines the mechanisms of unwanted emission and toxicity of these materials in the environment. An ever growing world population is in demand for higher agricultural yields and thus, more effective agricultural practices are needed. Hence, there is an emerging trend for using NM to optimize agricultural practice. This could lead to an intentional, and much greater, release of NM to the environment than it has been estimated for other NM applications. Expectations regarding positive aspects of agricultural applications are high: Reduced use and impact of pesticides and nutrients in the environment, reduced costs and improved efficiency of crop production, as well as eased remediation and facilitation of soil management. Here, we would like to align the increasingly common but often non-reflected perception of nanomaterial use and benefit for crop protection with actual scientific facts and figures. We will give an integrated overview of the historic development of the "nano-plant protection"-idea, different nano-properties and materials that are envisioned to improve agricultural formulations, and of the current state in the Nano-Agro-Business. To identify research gaps and highlight future research needs, we will also compare the frequency distribution of different NM foreseen for agricultural applications with that in agro-environmental and -toxicological research. The resulting recommendations will be completed by the identification of missing elements of essential NM characterization, that often seem to be overlooked in toxicity studies relevant for agriculture, such as purity of the materials or characterization in the experimental media. Preliminary results already show, that - although heavily discussed in public media and grey literature - the development of nano-plant protection is still at a nascent stage. However, the topic is increasingly attractive to industry. Future development of this field is hard to predict, thus we consider a proactive approach to a risk assessment of these materials necessary. This will be especially useful to aid the development of environmentally safe nano-plant protection products.

ET04P - Bioavailability and bioaccumulation - impact of environmental, biological and ecological variation

WE 241

Development of a feed preparation protocol for the production of fortified experimental diets for fish metabolism and dietary accumulation tests

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In the future, metabolism studies on (freshwater) fish will be a regulatory requirement for the authorisation of plant protection products (PPP) when PPP are used in crops fed to farmed fish. In fish metabolism studies standard fish food of suitable composition should be used as applied in commercial fish farming. A spiking procedure must be applied which guarantees the safe use of the radiolabelled test item. A preferred spiking procedure, as long as the substance is soluble in triglycerides, is to dissolve the test item in a small amount of fish oil or vegetable oil before mixing with fish food. Alternatively, the feed can be spiked using a suitable organic solvent. For moderately lipophilic plant protection products (log K_{ow} < 5) appropriate measures are required to prevent the leaching of the test substance from the surface of the pellets prior to ingestion by the fish. In contrast to the oil-spiking procedure, solvent spiked pellets can be further coated after fortification to reduce leaching losses.

The revised OECD TG 305 for bioaccumulation studies on fish proposes for certain groups of substances a dietary exposure test, where this is considered more suitable than an aqueous exposure test. For surfactants a dietary accumulation test might be more feasible than a bioconcentration test with aqueous exposure. However, due to their amphiphilic nature significant leaching losses of the test substances from the surface of spiked pellets might occur.

A feed preparation protocol including a solvent spiking procedure followed by pellet coating was developed ensuring the production of stable and homogeneously fortified test diets. The homogeneity of test item in spiked pellets of different size enriched with a pesticide of low lipophilicity or different perfluorinated compounds was confirmed. The stability of the coated pellets was investigated in leaching studies. The results show that an alginate coating of larger feed pellets (4-6mm) can significantly reduce leaching losses without affecting the homogeneity of the test item and should thus be recommended for fish metabolism studies. Coating of spiked experimental diets for dietary accumulation tests is not recommended. Due to the small size of pellets (0.8mm) alginate coating is leading to an aggregation of the small feed particles. Therefore, care must be taken that such diets are immediately ingested by the experimental animals to avoid significant leaching losses.

WE 242

Determining the assimilation efficiencies of particulate metals by Zebra Mussel : interest and limits of the pulse chase-feeding method using stable isotopes

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The pulse chase feeding method, associated with stable metal isotopes is gaining interest to assess the assimilation efficiency of metal associated to particles to various aquatic organisms (Croteau et al, 2009). The methodology is very efficient to understand dietborne metal bioaccumulation by organism because a short period (30 min) of exposition of labelled food is realised (pulse) followed by a longer period (48h) of depuration (chase). During chase, non-labelled food is provided to organisms and the excretion of particles by mussels is measured. This methodology enables us to follow the transfer of contaminants from contaminated particles to organisms and the excretion as well (by collecting the faeces). The measurement of stable isotopes is performed by ICP-MS (Inductively coupled plasma mass spectrometry).

Since the natural abundance of the stable isotopes used is very low, they are easily detected in labelled samples. Hence, this method limits the risk that the metal concentration measured in the matrices are from an initial contamination and enables to work in laboratory at low concentrations that are representative of the environmental conditions.

However, the realisation of pulse-chase feeding experiments remains complex. The experiment is time and money consuming. The collection of small amounts of faeces in microcosms where organisms are fed is practically difficult and the interpretation of ICP-MS outputs to estimate the net amount of stable isotopes in the samples is not straightforward.

More problematic is the assumption of a mass balance of stable isotopes between the ingested algae in the one hand, and the mussel and faeces in the other hand.

We present several attempts to assess the assimilation efficiency of several metals to zebra mussel, *Dreissena polymorpha*, using the pulse chase-feeding method. Metal isotopes were ^{53}Cr , ^{63}Ni , ^{65}Cu , ^{67}Zn and ^{106}Cd . Several types of mineral and organic particles were tested. In most cases, assimilation efficiencies could be estimated from the experimental data with a great uncertainty. However, the metal mass balance could not be balanced, mainly due to water/particles exchanges that cannot be neglected. We propose to discuss the feasibility and the possible artefacts of this method.

WE 243

Bioaccumulation studies in the closed laboratory aquatic microcosm AquaHab®

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AquaHab® is a small aquatic microcosm on laboratory scale for the prospective risk assessment of chemicals. The 7,5 L water tank contains different groups of organisms (fish, snails, amphipods, plants, bacteria etc.). Test conditions are highly standardized and test results are highly reproducible due to an automated measuring and regulation unit (regulating temperature and $[\text{O}_2]$ concentration in water) as well as isolation from external environmental conditions.

In addition to the possibility of studying effects on different levels of biological organization (from subcellular endpoints to system endpoints) in species of different trophic levels, the system is highly suited for reproducible exposure analysis of chemicals, as past studies with tributyltin (TBT), Irgarol 1051® and ionic liquids have demonstrated. The bioaccumulation and transformation of chemicals in the different groups of organisms can be studied.

Different trophic levels of organisms implement different mechanisms for uptake, transformation and excretion of chemicals by organisms into the exposure assessment, as given in the real environment. Samples from water and biomass could also be taken during test runs, which enables spatio-temporal analysis. A further major advantage is the possibility to study bioaccumulation under competition of different groups of organisms for the chemical.

The integration of sediment as an important environmental compartment is planned as the next step of development.

Standardized test conditions enable studying of the influence of different single variables on bioaccumulation.

For example,

- the influence of environmental variation on bioaccumulation could be studied by testing same species with same biomasses under different environmental conditions (sediment composition, supply of nutrients, temperature and pH-value of water, illumination cycles, etc.)
- the influence of biological variation on bioaccumulation could be studied by testing different test species, different biomasses, different application forms of the chemical (e.g. via water or partly via food) at other constant conditions
- the influence of ecological variation on bioaccumulation could be studied e.g. by changes in the food web, changes in the composition of species (e.g. predator-prey relationship).

Results of these basic studies with the above mentioned chemicals (e.g. TBT) will be presented on the poster, as well as an outlook to future studies.

WE 244

Methods for testing the ecotoxicity of ionizing compounds at multiple pH using *Daphnia magna*

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Many of the emerging environmental contaminants that have recently come into focus have one thing in common: they are ionizing compounds. An evaluation of a random sample of the over 140000 chemicals pre-registered for REACH revealed that 27% were acids 14% were bases 8% were zwitterionics. Risk assessment of these ionizing compounds represents a challenge due to the different behaviour of the neutral and ionized molecules. Neutral molecules are generally more lipophilic, and will be taken up by organisms to a greater extent than ions. Ions are subject to electrical attraction or repulsion, and the uptake of ionizing compounds is further complicated by processes such as the ion trap and the differential effects of high cellular ionic strength on the activity of neutral and ionized molecules.

The ratio of neutral and ionized molecules is dependent on the pKa of the compound, but also on the pH of the exposure solution. Acids are more neutral (and thus probably more toxic) at pH levels below their pK_a, while bases are more neutral (and thus probably more toxic) at pH levels above their pK_a. A recent review recommended that bases be tested for toxicity at pH levels above their pK_a, acids at pH levels below their pK_a, and zwitterionics at the isoelectrical point. This recommendation calls for modification of ecotoxicological test procedures to allow tests at specific pH levels.

Based on a literature review of the methods previously used to buffer solutions in ecotoxicological tests, we selected eight buffers for further investigation, namely TRIS, MES, HEPES, MOPS, CAPS, CHES, phosphate and citric acid. The buffers were tested for toxic effects using the 48 acute immobilization test with *Daphnia magna* at various pH levels. The buffering efficiency was determined by registration of pH drift. Based on the experimental data, buffers and working concentrations can be recommended for at various pH levels with a pH drift of no more than ± 0.2 pH units, and a buffer toxicity of at least a factor three below EC10.

The methods developed in this study enable toxicity tests with *Daphnia magna* in the pH range of 6 - 9.5 for 24 hours exposure and in the range of pH 6 - 8 for 48 hours exposure. TRIS, MES, MOPS and HEPES buffers were applicable for both 24 and 48 hour exposure, while phosphate and CHES buffers were only applicable for 24 hour exposure times. CAPS and MES buffers were not applicable at any exposure times.

WE 245

A benchmark approach to determine absorption efficiencies in fish

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We present a novel method for measuring fish gut absorption efficiencies of organic chemicals using a benchmarking approach. Fish were given feed contaminated by test substances having log Kow-values ranging from 4 to 10, including several benchmarking chemicals. The benchmarking chemicals, three PCBs and decabromodiphenylethane, were selected to cover a range of molecular weights, and absorption efficiencies from high to negligible. A system was set-up with four fish tanks containing 40L of aerated water, and six fish weighing about 25 g each. The fish were given a single dose from feed contaminated with seven test substances and the four benchmarking chemicals simultaneously. Faeces were collected from the bottom of the tank every morning and afternoon for 7 days by siphoning. The slurry was filtered through a glass-fiber filter, dried, weighed, extracted, cleaned-up, and analyzed by GC/LRMS. After 7 days, fish were slaughtered, and one homogenate was made from each fish tank. Aliquots of the homogenate were extracted, cleaned-up, and analyzed by GC/LRMS. Mass balance was satisfactory for most of the substances, ranging from 40-110% recovered from the contaminated diet. The results indicate that variability in determining absorption efficiencies can be reduced by using the benchmarking approach, and allows us to close the mass balance without collecting all of the faeces

WE 246

Effect of natural organic matter on the bioavailability of hydrophobic organic substances in fish bioconcentration studies according to OECD TG 305

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The identification of PBT/vPvB-substances is of high importance in the regulatory chemical safety assessment. Bioconcentration factors (BCFs) are usually determined in fish bioconcentration studies according to OECD TG 305. This guideline does not define specific analytical techniques to determine analyte concentrations in the water phase. However, commonly applied is liquid liquid extraction (LLE) which yields total analyte concentrations. In contrast, solid-phase microextraction (SPME) is capable to additionally analyze the freely dissolved analyte concentrations. This is relevant due to the binding of hydrophobic organic compounds (HOCs) to organic matter (OM) present in the test system. OM, quantified by the total organic carbon (TOC), has an influence on the bioavailability of HOCs, because only the freely dissolved fraction of a test substance is capable to cross an organisms membrane. The natural organic matter (particulate and dissolved) occurring in test systems according to OECD TG 305 mainly consists of fish feed residues and fish faeces.

Sorption of more than ten HOCs (log K_{ow} 5.4 - 8.3) from different substance classes to OM was systematically investigated with water containing defined concentrations of TOC and dissolved organic carbon (DOC). Matrices were fish feed, filter residue of test vessels from OECD TG 305 studies (containing feed residues and faeces), and Aldrich-humic acid as reference. Samples were extracted by LLE and SPME and measured with GC/MS and GC/ECD.

Results from SPME extractions yield partition coefficients (K_g, K_{OC}, and K_{DOC}) and similarly predict the bioavailability of the test substances at defined concentrations of organic carbon in the test system. Extraction by LLE resulted in total analyte concentrations, independent from TOC/DOC. The bioavailable fraction of a test substance was significantly reduced due to sorption to OM already at concentrations below 2 mg L⁻¹ TOC. However, the amount of sorption was varying, depending on composition of OM, and probably depending on the molecular structure of the test substance (independent from its log K_{ow} value).

Results show that TOC concentrations should be kept as low as possible in BCF tests on HOCs to avoid the underestimation of the true uptake when total aqueous concentrations are measured. SPME may provide important information regarding the difference between total and freely dissolved analyte concentrations.

WE 247

Analysis of the distribution of Benzo(a)pyrene in a murine liver cell line

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Benzo(a)pyrene (BaP) is produced as a result of incomplete combustion of organic matter such as wood, cigarettes and gasoline (US EPA). According to several studies, BaP is toxic and enhances the risk of several forms of cancer. This is primarily due to the formation of a wide spectrum of BaP-metabolites generated by CYP1A enzymes. Several of those metabolites, especially the ones forming DNA-adducts, elicit mutations and show carcinogenic potential. Although there are many studies about the toxicity of BaP, its detailed mechanisms of action are not completely understood. Established cell lines are used to gain a better mechanistic understanding of toxicity. This understanding, however, requires consideration of kinetic processes of BaP-cell interactions. Thus, this research project focuses on determining the spatio-temporal pattern of BaP distribution

in the Hepa1c7 cell line. The aim is to quantify the uptake and distribution of BaP into the cell-, media- and the plastic fraction of the exposure scenario over time. According to a time- and concentration dependent analysis of the CYP1A1 enzymatic activity (concentration range: 0.5 nM to 5 μ M BaP; time points: 2, 4, 12 & 24 hours [h]), at least 12h of BaP exposure are required for the enzyme to be fully active. Further, a method was established to determine the cell internal concentration by using ¹⁴C-labeled-BaP at 5 μ M BaP and the same exposure times. The samples were measured by liquid scintillation counting for the analysis of total radioactivity and by high performance liquid chromatography (HPLC) and radio-HPLC to detect the presence of metabolites. The results of the analysis revealed absence of detectable metabolites at 2 and 4h, which agrees with measured CYP1A1 enzymatic activity. After 2h of exposure, 5.0-6.5% of the nominal BaP (75 nmols) was found in the cells, 76-96% remained in the media, while 3.6-4.7% was adsorbed to the plastic. After 4h, the BaP uptake into the cells increased (with cell numbers staying constant). BaP in the medium fraction was reduced, while in the plastic fraction stayed constant for both time points, indicating that the BaP absorbed to the plastic was not readily bioavailable. The cell internal concentration after 2 and 4h was calculated as 0.5-0.7 and 0.8-1.1 pg/cell, respectively. Preliminary results show formation of metabolites at 12 and 24h. Further adjustments are currently being performed to optimize the determination of BaP metabolites.

WE 248

Multi-species comparison of the mechanism of biotransformation of MeO-BDEs to OH-BDEs in fish

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Polybrominated diphenyl ethers (PBDEs) and their methoxylated- (MeO-) and hydroxylated- (OH-) analogs are ubiquitously distributed in the environment worldwide. The OH-BDEs are more potent than PBDEs for many endpoints and can be produced from the transformation of MeO-BDEs. The objectives of the current study were to 1) identify the enzyme(s) that catalyze the biotransformation of 6-MeO-BDE-47 to 6-OH-BDE-47 in livers from rainbow trout, and 2) compare transformation of 6-MeO-BDE-47 to 6-OH-BDE-47 among rainbow trout, white sturgeon and goldfish. Cytochrome P450 1A (CYP1A) enzymes did not catalyze the transformation reaction. However, transformation was inhibited by the CYP inhibitors clotrimazole and 1-benzylimidazole but not gestodene. Therefore, the reaction is likely catalyzed by CYP2 enzymes. When transformation was compared among species, concentrations of 6-OH-BDE-47 were 3.4 and 9.1 times greater in microsomes from trout compared to goldfish and sturgeon, respectively. Concentrations of 6-OH-BDE-47 in microsomes from goldfish were 2.7 times greater than in sturgeon. The initial rate of transformation in microsomes from trout was 2.9 and 6.2 times greater than that of goldfish and sturgeon, respectively, while the initial rate in goldfish was 3.1 times greater than in sturgeon. Overall, differences in CYP mediated transformation of MeO-BDEs to OH-BDEs could influence concentrations of OH-BDEs in different species of fish.

WE 249

In vitro fish metabolism using rainbow trout liver S9 fractions to evaluate the bioaccumulation potential of fragrance ingredients

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The EU REACH regulation requires information on bioaccumulation in aquatic species for any chemical with log K_{ow} > 3 if it is manufactured or imported in quantities of 100 t/y or more. In general, this means the determination of a fish bioconcentration factor (BCF). Although bioaccumulation is not a specified endpoint < 100 t/y, surrogate information (e.g. log Kow) is relevant for PBT assessment and *in vivo* BCF data may be needed. *In vivo* BCF determination (OECD TG 305) requires the use of large numbers of animals with costly and labour intensive procedures. REACH Annex XI encourages the use of alternative information before a new vertebrate test is conducted.

In many bioconcentration models, the BCF is mainly based on hydrophobicity (i.e. log Kow). However, absorption, distribution, metabolism and excretion (ADME) processes do affect the bioconcentration of a substance in fish. *In vitro* systems have been proposed as alternative methods that may substitute for *in vivo* BCF determination with measurements of fish metabolic potential being used to refine BCF computer model estimates. The goal of our study was to determine the *in vitro* metabolism of the most common chemical classes in the fragrance industry such as esters, alcohols and ketones using rainbow trout (*Oncorhynchus mykiss*) liver S9 fractions to assess their bioaccumulation potential. In addition to the determination of the enzymatic turnover rates, we investigated the impact of individual cofactors (NADPH, UDPGA, PAPS, GSH) on the metabolic stability of the test substances to provide insights into the potential metabolic routes involved.

We observed for the majority of molecules tested moderate to rapid enzymatic turnover by trout liver S9 fractions. Slow turnover was found with Piconia. The ester Serenolide was transformed rapidly in absence of any cofactors and the corresponding alcohol identified as a product of a putative esterase cleavage. Cofactor dependence indicated the involvement of Phase I enzymes (NADPH dependence) for the ketones or Phase II enzymes (UDPGA dependence) for primary alcohols. These data were confirmed by the identification of hydroxylated metabolites by GC-MS and of glucuronide conjugates by LC-MS. Estimated BCF values, refined BCF values using the *in vitro* S9 metabolism data and if available *in vivo* measured BCF values were compared.

WE 250

Compound lipophilicity as a descriptor to predict metabolic affinity (Km) in mammals

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In bioaccumulation models, biotransformation is one of the processes decreasing the concentration of chemicals in an organism. The enzymatic action of metabolism involves two processes. Firstly, the chemical needs to reach the enzyme and bind with it; secondly, a catalytic reaction must take place. The binding of the chemical and its successive catalysis are described by two enzymatic parameters: the Michaelis constant (K_m) and the maximum rate of the reaction (V_{max}), respectively. Measured K_m and V_{max} data are lacking for many chemicals and species.

The aim of this study was to estimate the relationships between binding affinity, represented by $1/K_m$ (1/ μ M), and lipophilicity, expressed by the octanol-water partitioning coefficient (K_{ow}), for *in vitro* oxidations catalysed by alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), flavin-containing monooxygenase (FMO), and cytochrome P450 (CYP). The focus was on finding generic patterns of metabolism across enzymes. The knowledge of the underlying biochemical mechanisms is useful to further develop models for whole-body biotransformation.

For the regressions developed, $1/K_m$ always increased with the K_{ow} , as expected from the partitioning theory. The binding to FMO was not well correlated to the compound Log K_{ow} , possibly because of its different reaction mechanism involving a nucleophilic attack. If strong interactions such as covalent or ion bonds are important, distribution of chemicals is expected to be weakly related to their K_{ow} . Good correlations were found for the substrates of ADH, ALDH, and CYP, after excluding specific classes of outliers. These correlations suggested that the enzyme affinity of the chemicals is driven by weak, in particular hydrophobic, interactions with these enzymes. The slopes of these regressions did not statistically deviate from the typical slope (ranging from 0.46 and 0.70) that correlates protein-water distribution (Log K_{pw}) and Log K_{ow} .

WE 251

Membrane affinity of munitions compounds and environmental breakdown products

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Lipophilic chemicals have the potential for high membrane affinity and increased bioaccumulation across lipid membranes. Traditional physicochemical methods use the octanol:water partition coefficient (K_{ow}) as the standard for predicting chemical uptake and bioaccumulation. However, octanol does not accurately reflect the phospholipids and proteins found in cellular lipid bilayer membranes. Newly developed methods utilizing phosphatidylcholine is a better surrogate than octanol for cellular membranes because of the natural presence of phosphatidylcholine in cell membranes, improved membrane fluidity, and improved range of complex interactions between the chemicals and membrane molecules. Thus, this new technique was utilized to evaluate the bioavailability of legacy nitroaromatic munitions (2,4,6-trinitrotoluene [TNT], hexahydro-1,3,5-trinitro-1,3,5-triazine [RDX], 2,4-dinitrotoluene [2,4-DNT]), as well as newly developed munitions (2,4-dinitroanisole [DNAN], 5-nitro-1,2,4-triazol-3-one [NTO]). Solid surface lipid membrane platforms (phosphatidylcholine-coated porous silica beads in phosphate buffered saline; Transil® Sovicell, Leipzig, Germany) were incubated with 1 mg/l munitions according to the manufacturer's instructions. Samples were centrifuged and the supernatant was removed for chemical analyses. The chemical membrane affinity (also referred to as lipid-water partition coefficient [K_{lp}]) was calculated according to the manufacturer's instructions. Results demonstrated that most K_{lp} values were within one order of magnitude higher than traditional K_{ow} values. Additional results will demonstrate the membrane affinity of these compounds in artificial biological fluids (e.g., gastric, intestinal) to see if there are any changes in bioavailability due to organ-specific environments. These data will assist scientists and risk assessors better determine the potential of chemical bioavailability.

WE 252

BCF base-line model: further concept and application developments

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The study describes further developments of the BCF base-line model focused on expanding its training set and applicability domain, re-formulation of mitigating factors and re-evaluation of model parameters. Software implementation of the model allows prediction of BCF based on chemical structure and observed or calculated input parameters. The original formulation of the model investigated the effect that metabolism, ionization and molecular size have on the 'B' behavior of compounds. The expansion of the training set led to a revision of the original mathematical formalism, inclusion of water solubility as a new chemical dependent mitigating factor and analysis of the ionization term. More general definitions of the ionization factor based on pKa and the ratio of log P and log D are suggested and their performance is analyzed. The new mathematical formulation of the BCF base-line model and its enlarged training set contribute to more accurate evaluation of BCF reducing factors and a broader applicability domain of the model. Inclusion of a mechanistically justified ionization term allows a more accurate description of the partitioning behavior of both neutral and ionized species.

WE 253

Assessment of bioaccumulation potential under REACH combining classification rules derived from physicochemical and structural information

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Determination of the bioaccumulation potential of a substance is part of its environmental hazard assessment. The European chemicals regulation, REACH, relies on the bioconcentration factor (BCF) to estimate bioaccumulation. According to the legislation, potentially accumulative substances (B) are distinguished from non-accumulative substances (non-B) by a BCF threshold of 2000. REACH encourages the use and combination of different methods and models to assess environmental hazards of industrial chemicals. The emphasis REACH puts on estimated chemical properties shall reduce experimental testing, thus lowering monetary costs and saving animal lives. The present study aims to classify substances as either B or non-B based on classification rules generated by conditional inference trees using physicochemical properties or generated by association rules extracted from frequent sets using structural information.

A database consisting of 844 distinct organic chemicals was created. In addition to the CAS number and the canonical SMILES, it contains information on nine descriptors. The various descriptors were estimated with Epi Suite and Open Babel. The entries of the database were cleaned for erroneous CAS, SMILES and BCF. Additionally a set of 307 chemical substructures defining the organic chemical space was used to generate a set of substructures present in each chemical. The database contains 163 B chemicals and 681 non-B chemicals. The analysis of the descriptors revealed that all of them are relevant for bioaccumulation in terms of added information versus added noise. However, there are different levels of importance among them. For the non-B rules derived from the trees it seems that degradation and metabolism of the compound are driving forces and that for B rules the interaction of the compound with the lipid membranes is more important. The analysis of the structural fragments and the derived association rules revealed that it is easier and more reliable to classify a chemical for being non-B than it is to classify it for being B. The present work contributes to a better understanding of mechanisms and processes leading to low or high BCFs. Classification rules which include either up to seven physicochemical descriptors or one or more of 107 structural fragments discriminate B and non-B chemicals.

WE 254

Transfer of perfluorooctane sulfonate (PFOS) from water to a marine benthic fish with the presence of sediment

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Uptake and depuration kinetics of PFOS from water in a marine benthic fish were investigated with and without sediment, and potential impact of the sediment on the kinetics was evaluated.

Two-year-old marbled soles (*Pseudopleuronectes yokohamae*) (avg. 46 g-wet, n = 89) were held in a flow-through system of filtered seawater (avg. water quality: 17.3 °C; 7.5 mg-O₂/L). There were control, water (W), bottom-sediment (BS), and suspended-sediment (SS) treatments (T) (control and WT: no sediment; BST: spiked BS; SST: no BS). Only the WT received spiked seawater. Spiked field-collected BS had been laid in the BST at 1-2-cm thick and was not renewed during the experiment. SS was also present in the BST due to the activity of the fish. The SST received effluent from the BST. An 84-d depuration period followed a 28-d exposure period (only exposure period for SST). Sole, water, and sediment were sampled regularly and analyzed for PFOS. The fish body surface, gills, and inside of gut were washed to remove particles. Uptake and depuration of PFOS were analyzed by using a mass-balance first-order kinetic model assuming fish whole body as one compartment. Dissolved PFOS was assumed to be the only source of uptake. The rate constants were estimated by nonlinear fitting.

Dissolved and particulate PFOS concentrations in the WT in the exposure period were relatively constant and averaged 74 and 18 ng/L, respectively. Those in the BST or SST were comparable and peaked on day 1 or 3 at 50-100 ng/L and then decreased. PFOS concentration in the BST sediment decreased during the 28 days from 110 to 15 ng/g-dry. PFOS concentrations in the WT fish increased during the exposure period and then decreased. Those in the BST fish peaked at day 14 and then decreased, whereas those in the SST fish were highest at days 14 and 28. PFOS concentrations were negligible in the water in the depuration period or in the control fish. Depuration rate constant was estimated at 0.023 [d] (95% confidence interval 0.019-0.026). Uptake rate constant from the dissolved phase was estimated at 22 [L/(kg-wet d)] (20-25), 30 (24-40), and 46 (39-54) for the WT, SST, and BST, respectively. Several factors that may affect the estimates of rate constant, i.e., remaining sediment particles in fish, dissolved oxygen, ingestion of sediment particles, did not explain the magnitude of the increase in the uptake rate constant with the presence of sediment. The cause of this increase needs further investigation.

WE 255

The bioavailability of different pyrogenic PAHs in field contaminated sediments

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Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous lipophilic contaminants that accumulate and persist in sediments and thus can be taken up by sediment dwelling organisms. The sources of PAHs vary from petrogenic to pyrogenic, and the source is an important estimator for bioavailability; the bioaccumulation of petrogenic PAHs has been found to be higher than those of pyrogenic PAHs. In addition, the characteristics, especially organic carbon and black carbon content, of the sediment affect the bioavailability of PAHs. In this study the role of PAH origin and sediment carbon content in bioavailability was assessed by exposing sediment dwelling oligochaete *Lumbricus variegatus* to four sediments contaminated by either creosote oil or combustion based pyrogenic PAHs. The chemical methods (polyoxymethylene stripes and Tenax extraction) were used for estimating the bioavailability. Kinetic bioaccumulation of PAHs and feeding behavior of *L. variegatus* were determined. Preliminary results show that the sediments contaminated with creosote oil had lower organic and black carbon content and thus the bioavailability was expected to be higher than sediments contaminated with other pyrogenic PAHs source. The slightly higher Log K_{oc} values of creosote oil based PAHs indicated that the bioavailability of creosote oil based PAHs could be lower than PAHs from other pyrogenic source. The rapidly desorbing fraction, which is considered to be the bioavailable fraction, was very small in all sediments indicating that the bioaccumulation potential should be limited. However, *L. variegatus* accumulated PAHs and accumulation was higher from creosote oil contaminated sediments than sediments of pyrogenic origin PAHs. Due to the very tight absorption of pyrogenic PAHs to black carbon during combustion the pyrogenic PAHs probably are less available than PAHs of creosote oil. This may explain the higher accumulation of creosote oil based PAHs. Part of the bioaccumulation results may be explained by the higher feeding activity of *L. variegatus* in creosote oil contaminated sediments.

WE 256

Uptake, accumulation and detoxication of Microcystin-LR in shrimps (*Palaemonetes argentinus*)

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Microcystins (MCs) are cyclic heptapeptide produced by cyanobacteria. There are over 100 different MCs congeners reported up to now, having different toxicity. MC-LR is by far the most studied and ubiquitous toxin, with known hepatotoxic effects, inhibition of protein-phosphatases 1 and 2-A, which promotes cancer and other diseases. MC-LR has been found in water bodies worldwide. Still today, little is known on how this toxin is transferred through the food web from water or cyanobacteria. Our main goal was evaluating the uptake of soluble MC-LR by small shrimps (*P. argentinus*) that share the same habitat with cyanobacteria. Additionally, we evaluated the detoxication period after initial uptake as well as the activity of several enzymes involved in detoxication: Glutathion-S-Transferase or antioxidant defence system Catalase, Glutathion Reductase and Glutathion Peroxidase. So, we look to evaluate both bioaccumulation and detoxication of MC-LR in shrimps as the first step in the transference of MCs within the aquatic food web, considering that shrimps are preyed by fish, which are then captured by other fish or used as food by humans. Analysis of MC-LR in water and shrimps was done by HPLC-PDA-ESI-MS (either triple quadrupole or Q-TOF). Shrimps were exposed to soluble MC-LR (50 µg L⁻¹) during 3 days and then translated to new tanks without MCs for additional three days. Crustaceans presented bioaccumulation after 3 days exposure (0.74 ± 0.15 µg MC-LR g⁻¹ shrimp). The amount of MC-LR was reduced to 0.18 ± 0.01 µg MC-LR g⁻¹ shrimp after three days detoxication (75% MC-LR removal during the detoxication period). The activity of studied enzymes remained almost unchanged during the exposure period but Glutathion-S-Transferase and Glutathion Reductase were induced (activated) during the detoxication period. These preliminary results suggest that MC-LR can be bioaccumulated and eliminated in shrimps. On the other hand both detoxication and antioxidant enzymes seem to be repressed during the exposure but stimulated after exposure, enabling detoxication. Our current results suggest that the uptake, bioaccumulation and detoxication process is complex and demonstrate that MC-LR is bioaccumulated by shrimps upon exposure to environmental relevant amounts of this toxin. Furthermore, detoxication enzymes seem to be inhibited during the exposure period. However, detoxication enzymes are activated as soon as the exposure stops, enabling 75% removal after three days.

WE 257

Antioxidative defence of *Lymantria dispar* from populations differently adapted to chemical stress

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Anthropogenic activities such as mining, fossil fuel, combustion, smelting and agricultural processes have increased the levels of heavy metals in the soil and air up to the levels that change metabolism and foliage chemical composition. Leaves are food for phytophagous folivorous insects such as *Lymantria dispar* L. Adaptations of insects to new leaf chemical composition include alterations in antioxidative and detoxification strategy, which needs allocation of energetic resources that alters life history traits of insect (growth, reproduction, mortality etc.) and their population number. There is scientific information that during heavy metal stress increases foliar phenol contents. The *L. dispar* caterpillars used in this experiment were originating from two populations, which were differently adapted to chemical stress (high amount of phenolic compounds in food and control). The responses of 4th instar *Lymantria dispar* L. to artificial diet with quercetin as a test phenol compound (2 % w/w) were monitored at the level of antioxidative defence in the midgut tissue: the activities of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione reductase (GR) and glutathione content (GSH). Regardless of population origin, activity of SOD was higher in the caterpillars if fed diet with quercetin than in a control group. In average SOD, CAT and GST activities were higher in the caterpillars from phenol adapted population than in unadapted population. An artificial diet with quercetin led to an increase of GST, GR activity and GSH in both populations. After quercetin treatment, mortality was higher in unadapted population of *Lymantria dispar*.

WE 258

Modeling the potential biomagnification of organic pollutants in aquatic food webs of the new Three Gorges Reservoir ecosystem at the Yangtze River

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The creation of the Three Gorges Reservoir at the Yangtze River caused the largely flooding of former urban, industrial and agricultural areas. Consequently, substantial amounts of organic and inorganic pollutants were released into the reservoir. Beyond contaminants and nutrients enter the reservoir by runoff from adjacent agricultural areas as well as from sewage of industry, aquacultures and households. Periodical changes in water level cause flooding events and thereby a relocation of contaminated water, particulate matter and sediment onto agriculturally used areas along the reservoirs shore.

The main aim of the presented research project is a deeper understanding of the processes that determine the bioaccumulation of organic pollutants, i.e. mainly pesticides, along aquatic food chains under the newly developing conditions of a huge reservoir. The project is part of the Yangtze-Hydro environmental program. Since the analysis of organic traces is very labor and cost intensive, we use deterministic modeling approaches for the description of the potential accumulation and biomagnification. We describe the consecutive calibration steps of the candidate model AQUATOX developed for the US Environmental Protection Agency.

As a starting point, pre-calibrated simulations were adapted to 'Yangtze specific' conditions ('regionalization') and the model output was analyzed in depth. The results of sensitivity and uncertainty analyses are presented in order to describe the influence of various variables. The changes of the complex food web structure are analyzed by multivariate statistics. We show modeled internal concentrations of important food fishes under various scenarios of pollution loadings. Our research is firstly focused on the influence of the nutrient regime on food web structures and thus shows the profound influence of nutrients on biomagnification processes, as well as local environmental conditions. The model predicts the internal concentrations of the model substance in fishes according to their food web position, which are expected to be highest in top predator fishes, e.g. basses.

Further steps towards regionalized models require data on site-specific parameter values. For this reason, the cooperation with Chinese monitoring institutions was intensified to establish a constant data exchange.

WE 259

Trophic transfer dynamics of polychlorinated biphenyls (PCBs) and cyclic volatile methylsiloxanes (cVMS) in a temperate freshwater lake

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The bioaccumulation potential, or 'B', of a compound is one of the criteria used to classify substances as being PBT (persistent, bioaccumulative, and toxic) materials in the environment. Bioaccumulative substances are defined as substances that biomagnify within a food web. A compound is said to biomagnify when lipid-normalized concentrations of accumulated chemical residues in biological organisms increase with increasing trophic position. Based on this definition, the most relevant criterion for assessing a compound's 'B' potential is the trophic magnification factor (TMF), and the most conclusive evidence to demonstrate that a chemical substance biomagnifies is a TMF > 1. Previous research aimed to determine the 'B' potential of several cyclic volatile methylsiloxanes (cVMS) was conducted in Lake Pepin, Minnesota, USA and concluded that biomagnification of cVMS materials was not occurring. Rather, cVMS materials had TMF values that were < 1.0 (range 0.1 to 0.3), indicating that concentrations of cVMS materials decreased with increasing trophic level and that trophic dilution was occurring across the aquatic food web. The objective of the present study is to verify that Lake Pepin is appropriate for conducting TMF studies by evaluating the trophic magnification behavior of polychlorinated biphenyls (PCBs), which are considered legacy 'B' contaminants and whose environmental behavior has been extensively studied. A subset of moderate to heavily chlorinated PCB congeners were quantified in biota at various trophic levels and TMFs were calculated. As expected, TMFs for the selected PCB congeners were greater than 1, ranging from 1.5 to 5.1. These results suggest that Lake Pepin is appropriate for TMF studies and that the TMFs of < 1.0 for cVMS compounds in the same aquatic food web appear to be valid. Moreover, it has been suggested that TMFs may be improved in instances where there is limited environmental data by using a standardizing benchmark to calibrate trophic level assignments. Using PCB 180 data from the present study to calibrate trophic level assignments in the aquatic food web of Lake Pepin, the TMFs of the cVMS materials were lower than the originally reported values. Based on this data, Lake Pepin appears to be an appropriate system for evaluating TMFs of environmental contaminants, wherein select PCB congeners are undergoing biomagnification and cVMS compounds appear to be trophically diluting across the aquatic food web.

WE 260

Polychlorinated Biphenyl and organochlorine pesticide levels in the amniotic fluid of women from Cukurova Region, Turkey

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In this study, organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) levels were determined in 200 women amniotic fluid samples from Cukurova Region. The concentration of OCPs (hexachlorohexane (HCH), hexachlorobenzene (HCB), DDT and various metabolites) and different PCB congeners (28, 52, 101, 118, 138, 153, 180) were detected by gas chromatography with ECD detection. Both agricultural and industrial products are common in our region. In this study the levels of analyzed compound were as follows: HCB 6.58 ± 4.68, ΣBHC 21.63 ± 14.24, ΣDDT 12.49 ± 7.5 and ΣPCBs 74.02 ± 123.5 ng/ml. No statistically significant correlation was found between the age and gestational age and the levels of OCPs and PCBs. While 5% of the pregnant women were detected with no organochlorine compound 79% was found to have more than one organochlorine compound. This study is of great importance in terms of identifying for the first time the exposure of fetal to organochlorine compounds.

WE 261

Salmon farming in the Chilean Patagonia, a source or receptor of POPs?

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POPs pollution has been described all over the world in different matrices; however the data availability is always lower in the southern hemisphere. Since few years we were interested into understand the levels and fate of selected POPs in the Patagonia in southern Chile, an area under increasing anthropogenic pressure during the last 20 years by tourism and aquaculture activities. We used different approaches to address the problem, measuring POPs both in abiotic and biotic matrices, lake sediments, soils, passive air samplers were used and more recently we moved towards understanding the biotransport of POPs by migrating salmon, that are non native from such ecosystems. Results provided by such approach led us to understand that POPs pollution is relatively recent in the area with PCBs starting to increase in sediments by 90's and PBDEs more recently. On the biological component, aquaculture (salmon fish farming) contributes also with escaping salmon that may deliver contaminants to rivers and lakes. <However, salmon farms may be also acting as receptors of the POPs pollution by atmospheric deposition processes. We have made a preliminary calculation that reveals that the biological pathway may represent a significant source of pollution arriving to such ecosystems in contrast with the atmospheric deposition processes. Supported by FONDECYT 1110719

WE 262

Persistent organic pollutants in juvenile Magellanic penguins (*Spheniscus magellanicus*) found on the coast of the states of São Paulo and Rio de Janeiro, Brazil

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The Magellanic penguin, *Spheniscus magellanicus*, is the most abundant of the penguins that live in temperate regions of the western South Atlantic. Its breeding season runs from October to March, when it feeds off the coast of Argentina and southern Chile. In its pelagic phase, the species migrates north and winters on the continental shelf off Uruguay and Brazil. Juvenile individuals are occasionally found dead on the beaches of southern and southeastern Brazil. Living specimens are found debilitated (i.e. hypothermic, cachectic and apathetic) and sent to rehabilitation centers. The present study demonstrates the occurrence of persistent organic pollutants (POPs) in Magellanic penguins found debilitated on the beaches of the states of Rio de Janeiro and São Paulo, Brazil, in 2008. Thirty-seven juveniles were analyzed. Due to the body state, only the liver was analyzed, as the birds were found virtually without adipose tissue. The following POPs concentrations were found: Σ PCBs (9.9 to 1984 ng g⁻¹), Σ DDTs (2.3 to 275 ng g⁻¹), Σ HCHs (1.0 to 100 ng g⁻¹) and HCB (2.4 to 108 ng g⁻¹). Among the PCBs, there was a predominance of hexachlorobiphenyls (138 and 153) and heptachlorobiphenyls (180 and 187). Among the organochlorinated pesticides, DDT predominated, mainly in the p,p'-DDE form. The maximum concentrations of POPs found in the specimens of *S. magellanicus* reached levels as high as 102 to 103 ng g⁻¹ (wet weight) above those found in *Pygoscelis adeliae* and *Pygoscelis papua* from Antarctica. Ecological factors (e.g. accumulation and biomagnification of pollutants through the food chain) and/or physiological/biological aspects (e.g., mobilization of lipids-pollutants in cachectic individuals) may be associated with the occurrence of POPs in the individuals analyzed. These data will contribute towards a better distribution and tendencies understanding of these pollutants in the western South Atlantic.

WE 263

Bioaccumulation of Xenobiotics in *Daphnia* diapausing eggs

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During the last century, most European lakes went through a phase of eutrophication. The installation of sewage treatment plants made the recovery of many lakes to their original trophic state possible. Urban and industrial sewage, erosional runoff, and leaching from agricultural areas resulted in the increase of phosphorus in natural waters affecting species of higher trophic level, such as zooplankton and fish.

Among the most important planktonic grazers in pelagic food webs are species of the genus *Daphnia*. *Daphnia* normally reproduce clonally but they switch to sexual reproduction when environmental conditions are not ideal. *Daphnia* resting eggs (ephippia) can sink to the bottom of the lake and remain there until conditions become favorable or be transported by wind or waterfowl to other water bodies and thus colonize new habitats. In deeper parts of lakes, these diapausing eggs do not get hatching stimulus. They remain in the sediment, providing an unbiased archive of past populations.

Chemical pollutants found in most ecosystems can be major selective forces and therefore may alter ecosystem functions. They are regarded as the second greatest threat to aquatic and amphibious species, just after habitat loss. Sediments contaminated with mutagenic substances are known to pose a hazard to indigenous biota including adverse

effects such as DNA adducts, DNA strand breaks and chromosomal aberrations and cancer. Chemicals in the sediment may be bioaccumulated in diapausing eggs and influence their fitness..

Lake Greifen sediments were characterized and the temporal resolution of different contaminants were obtained. After the characterization of the lake, a group of chemicals were selected to study their bioaccumulation and effect on *Daphnia* diapausing eggs. The chemicals were selected based on their physical and chemical properties as well as their occurrence in the lake.

The bioconcentration factors (BCF) of six different chemicals were calculated. The results show that *ephippia* can take up contaminants from the pore water in the sediment and that the BCF for the neutral compounds correlate with the log Kow values. In addition, the obtained BCF values were compared with values obtained via a QSAR based on log Kow for bioconcentration of organic chemicals in *Daphnia*, algae, and mussels. The estimated BCF are always higher than the measured ones indicating that *Daphnia* is not a good model for the bioconcentration in *ephippia*.

WE 264

Drastic decrease in the organochlorine blood concentrations in nestlings of Goshawk (*Accipiter gentilis*)

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Organochlorine pesticides have been identified as one of the main groups of environmental contaminants as a result of human agricultural activities. These compounds are bioaccumulated and biomagnified in the living beings located in the upper of the trophic chains, and they are able to alter the population dynamics of different species. Some studies have related their exposure with impairment of reproductive, endocrine, immune and nervous functions in organisms.

Blood levels in nestlings indicate the degree of short-term exposure determined by the presence of these compounds in the prey that parents bring them to the nest.

The studied area encompassed a mountainous area of Mediterranean forest (Murcia Region, Spain) that is surrounded by agricultural lands (main crops are grapes, almonds, olives, and cereals) and is distant from any urban, industrial, or mining areas. In this area, the goshawk population is sedentary and it has suffered population declines in Murcia Region during the last two decades.

During 2000-2003, all compounds were detected in some samples, being cyclodienes the most frequently detected (100%) in blood. Moreover, these compounds were also the pesticides with the highest blood concentrations. Since 2007, the detection of these pesticides in blood has decreased drastically. DDT and DDE have been detected in concentrations that probably were associated to the agricultural use of dicofol. On the other hand, lindane was not detected in any sample, possibly due to the prohibition in the European Union in 2000 (Decision, 2000/801/EC). In conclusion, we believe that the decrease of the organochlorine blood levels in nestlings of Goshawk have been consequence of the changes in agricultural practices in the growing area and the implementation of legislation on the prohibition of the use of pesticides since 2000.

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WE 265

Evaluation of three bivalves species as bioindicators of persistent organic pollutants in a Mediterranean coastal lagoon

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Bivalves are widely accepted as bio-indicators of chemical contamination in surface water by the International Conventions, such as Barcelona Convention and the Oslo and Paris Convention (OSPAR). Bivalves filter great seawater volumes, incorporating dissolved hydrophobic pollutants and accumulating them, being a good bioindicator of these pollutants in seawater. The concentrations measured in the tissue are a function of bioavailable hydrophobic pollutant levels in water. In fact mussels are commonly used in the national and international monitoring programs due to their wide geographical distribution, sessile character and capacity to filter high seawater volumes concentrating contaminants in their tissues. Although mussels are widely distributed, they are not present in many areas such as in the Mar Menor Lagoon, where other bivalve species must be used as bioindicators. Mar Menor is a hypersaline (42-47 psu) coastal lagoon located in the Cartagena Field area at the South East of Spain. This semi-arid area is subject to intensive agriculture, seasonal tourism, recreational activities and sporadic torrential rainfall regime. For these reasons, in this study we have characterized the distribution of polycyclic aromatic hydrocarbons, polychlorinated biphenyls and organochlorinated pesticides (OCPs) in three bivalve species. Concretely the bioaccumulation has been determined in cockle, oyster and noble pen shell. The specific aims of this study were to characterize the bioaccumulation of organic pollutants in three bivalve species from Mar Menor Lagoon, to evaluate the seasonal variation of persistent organic pollutants (POPs) levels in bivalves and to identify the bivalve specie most adequate as bioindicator of POPs in Mediterranean coastal areas where mussels were not present. The variability of POPs concentrations in the lagoon was mainly consequence of the sampling area (different hydrodynamic regime and pollution sources), and in less extension of seasonal variations. The bioaccumulation of PAHs is similar for cockle, oyster and *P. nobilis*, being higher close to ports and wastewater effluents. However, the bioaccumulation of DDXs and PCBs in oyster and *P. nobilis* is similar in all areas, being higher than detected in cockle. A monitoring programme using both cockle and oyster could be adequate to get representative samples for yearly (cockle) and biyearly (oyster) pollution periods.

WE 266

Environmental monitoring through analysis of linear alkylbenzene in bivalve molluscs as an bioindicator of exposure to sewage in coastal area, Santos, Brazil

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The contamination of coastal areas by human activities has been intensified in recent decades. In general, the introduction of contaminants occurs such as domestic and industrial wastewaters, boats, rivers, atmospheric deposition, agriculture and aquaculture activities intensive in some coastal areas. The discharge of domestic sewage is a major source of pollution in coastal regions. In Brazil it is estimated that only 20% of the population are connected to a wastewater treatment system, often being discarded into the environment without treatment.

The introduction of sewage into aquatic ecosystems can be monitored by different sentinel organisms such as oysters and mussels. One of the organic chemical markers used in many studies are the linear alkylbenzenes (LABs). The main use of LABs is the formation of linear alkylbenzene sulfonated (LAS), used in the formulation of detergents.

The aim of this study was the analysis of linear alkylbenzenes in bivalve molluscs (mussels - *Mytella falcata* and *Mytella guyanensis*) in a highly urbanized coastal region (Santos, Brazil) as a way to monitor the introduction of sewage in the marine environment. Santos is located in Brazilian southeastern coast, where there are the most

important industrial complex of the country and the biggest port of Latin America that have an important contribution of waste disposal can also be found in this region. Eleven samples were extracted with a mixture of hexane / dichloromethane 50% (v/v) and purified on a column of silica and alumina. Identifications and quantifications were made by gas chromatography with mass spectrometry detector (GC/MS).

The LABs concentrations ranged from 110 to 3339 ng g⁻¹ (dry weight), which are comparable to other studies that evaluated the densely urbanized around the world the contribution of sewage.

In relation to individual LABs, samples were more enriched with high input of C13-LAB isomers followed by C12, C11 and C10-LAB. Since the C13-LAB isomers are more hydrophobic than their homologues, they can be associated to the particulate material, and thus be available for the molluscs.

The results of LABs in the bivalve analysed showed they are good bioindicator of sewage input in coastal areas.

WE 267

Development of a field-based technique to assess soil pollution through measurement of contaminant bioavailability to earthworms

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It is recognised that pollutant uptake by earthworms can be used to interpret results of bioassays. Furthermore, uptake of soil pollutants by laboratory-bred, synchronised earthworm cohorts could be used to account for physico-chemical variation of soils, helping to address uncertainties linked to the use of Soil Screening Values. Therefore, measuring uptake of pollutants by earthworms could be used in both lower and higher tier assessments within regulatory frameworks for ecological risk assessment.

Moreover, extrapolation of results from laboratory bioassays to field conditions has been widely questioned. This ongoing research aims to develop a mark-release-recapture technique to expose naïve earthworms to polluted soil *in situ*.

The reported study focussed on 2 earthworm species, *Lumbricus rubellus* and *Octolasion cyaneum* (an epigeic and endogeic species respectively). Field-based experiments using tagged naïve earthworms have suggested that:

- Earthworms cannot be retrieved without a form of containment.

- PVC tubes (0.2m diam., 0.3m length) inserted to a depth of 0.1m, are suitable to prevent movement of earthworms (79% and 84% retrieval for epigeic and endogeic earthworms respectively). However, such tubes may be disturbed by the public.

- PVC tubes (0.2m diam, 0.1m, length) flush with the ground, are suitable to prevent subterranean movement of earthworms in unpolluted conditions, but may not prevent over surface movement (79% and 0% retrieval for endogeic and epigeic earthworms respectively). Ongoing experiments are investigating restricting surface movement by covering such tubes with a 0.5mm mesh.

To investigate pollutant avoidance, individuals of the selected earthworm species were introduced into gradients of polluted soil in the laboratory. Results suggested an avoidance response to pollution. 55% of *L. rubellus* and 90% of *O. cyaneum* introduced in polluted soil were recovered in less polluted soil after one week and, 70% and 90% of *L. rubellus* and *O. cyaneum* introduced into unpolluted soil remained there. These results emphasise the need for containment of earthworms (in the field) and the potential use of an avoidance response in wider ecotoxicological experiments. Chemical analyses investigating a potential link between soil heterogeneity and uptake of pollutants by earthworms are ongoing. In addition, laboratory-based experiments are being conducted to investigate earthworm movement in response to pollutants using 2D terraria.

WE 268

Foliar metal uptake: mechanisms and pathways involved for various plant species

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Nowadays, numerous fine and ultra-fine metallic particulate matters (PM) are emitted in the environment by industrial activities such as lead recycling facilities. These PM emissions increase in the atmosphere with the use of more effective filters in facilities (Zhang et al., 2005) and could become a real source of contamination for ecosystems and especially for plants (Schreck et al., 2011). Actually, Uzu et al. (2010) bring to light foliar Pb uptake in lettuce exposed to factory atmospheric fallouts. Then, this study investigated metal (Pb, Cd, Sn, Cu, etc.) absorption by atmosphere-shoot transfer in various contrasted plants (lettuces, cabbages, parsley and rye-grass) in order to better understand foliar pathways in relation with plant morphology. Plants were exposed one month in the courtyard of a secondary lead smelter under atmospheric contamination. A geotextile membrane avoided root transfers. Eight plants of each species were harvested every 2 weeks. Their shoots and roots were cut, washed according to human or animal exposure scenario and dried. Total metal concentrations were determined by ICP-OES. Several complementary techniques (micro-X-ray fluorescence, scanning electron microscopy coupled with energy dispersive X-ray microanalysis, time-of-flight secondary ion mass spectrometry, RAMAN and EXAFS) were used to investigate the localization and speciation of metals in leaves at different scales in the case of lead. Results showed that significant differences in metal uptake are induced by plant species and washing procedure with consequences on animal and human health. After one month of exposition, Pb concentrations raised: 122 ± 5.5 mg kg⁻¹ of dry weight (DW) for lettuce shoots, 299 ± 33 mg kg⁻¹ DW of parsley shoots, and 700 ± 28 mg kg⁻¹ DW for rye-grass shoots (not washed). Microscopic observations had shown a Pb accumulation in leaves and more specifically in necroses zones and in the base of the central vein due to rainfall. Some particles were observed near and at the edge of the stomata. Some compounds were internalized in their primary form (PbSO₄) underneath an organic membrane. Biogeochemical transformations occur on the leaf surface with the formation of lead secondary species (PbCO₃ and organic Pb). Metal internalization through the cuticle or penetration through stomata openings are proposed as two major mechanisms involved in foliar metal uptake.

WE 269

Bioaccumulation of trace elements by the earthworm *Eisenia fetida* exposed to mine contaminated soils: implications for bioavailability studies

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Although generally accepted that environmental risk assessment based on the total concentration of a contaminant can overestimate risks, and that organisms respond only to the fraction of the contaminant that is biologically available, the concept of bioavailability is not easily defined and it is even more tentatively measured. Bioavailability may be assessed in two complementary ways: (i) by chemical methods (e.g., extraction methods), which determine a specific available fraction of a well-defined class of contaminants; and (ii) by biological methods, which expose organisms to soil to monitor effects. The aim of this study was to link the results obtained by chemical methods, used to assess chemical (bio)availability, with the results for bioaccumulation of trace elements observed in organisms exposed to contaminated soils. Soils from São Domingos Mine, an abandoned mine located in the Portuguese sector of the Iberian Pyrite Belt, were used in the experiments. They were characterized considering general physicochemical characterization and pseudo-total and bioavailable trace element concentrations obtained by chemical extractions. Two different single step extractions were used: a mobile fraction (extracted by 0.01 M CaCl₂, pH 5.7, without buffer), sometimes referred as the effective bioavailable metal fraction, and a mobilisable fraction (extracted by a solution of 0.5 M NH₄CH₃COO, 0.5 M CH₃COOH and 0.02 M EDTA, pH 4.7), considered as a potentially bioavailable fraction. The bioaccumulation of trace elements (As, Cu, Pb and Zn) by the earthworm *Eisenia fetida* exposed to mine contaminated soils was assessed after 28 days of exposure to the contaminated soils. Results obtained in the study are discussed in order to provide an insight into the main issue of the investigation.

WE 270

Interspecific differences in bioaccumulation of heavy metals in three freshwater bivalves

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Bivalve molluscs can provide a powerful measure of the bioavailability of metals in aquatic ecosystems. However, interspecific and intraspecific differences, e.g. in habitat requirements, feeding preferences, size and filtration rates, have the potential to bias results and limit their utility.

We studied three sympatric freshwater bivalve species with different feeding behaviours and habitat requirements; the zebra mussel (*Dreissena polymorpha*), the Painter's mussel (*Unio pictorum*) and the Asian clam (*Corbicula fluminea*). Three populations of each species (Rivers Waveney, Thames and New Bedford River, United Kingdom) were compared for their copper, nickel and zinc tissue concentrations. Sediment and water concentrations of the three metals were also collected.

Metal tissue concentrations displayed a striking pattern with *C. fluminea* having the highest copper concentrations, *U. pictorum* the highest zinc and *D. polymorpha* by far the highest nickel levels. Differences in metal tissue concentrations between species far outweighed differences between populations from the three rivers. These differences might be attributed to the species-specific filtration rates, to related uptake from the dissolved and particulate metal phases, to assimilation from the sediment, and to elimination rates.

Our results strongly suggest that metal values for tissues from different bivalve species cannot be compared and that large-scale studies should utilise widespread and easily identified species. In addition, *D. polymorpha*, *C. fluminea* and *U. pictorum* seem to be exposed to metals in different compartments of the freshwater environment, which should be taken into account in management decisions for monitoring and remediation of metal pollution.

WE 271

Biodynamic model to predict bioavailable fraction of metals in sediment: the sediment ingesting aquatic oligochaete *Lumbriculus variegatus*

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Water Framework and Daughters Directives state that a risk assessment approach should be used to set Quality Standards (QS) for different environmental compartments, including sediments. The derivation of sediment QSs is particularly relevant for substances which, because of their chemical properties, accumulate in sediment and could pose a risk through direct toxicity to benthic invertebrates, or to benthic predators through the food chain (secondary poisoning). If QS are exceeded in sediments, it is recommended to trigger a more detailed assessment that accounts for bioavailability. The availability of sediment-bound metals to benthic invertebrates is element specific and depends on both the physico-chemical characteristics of the sediment (i.e. the presence of binding ligands which restrict the mobility of metals) and the organisms' feeding behavior and ecology. Since body residue, as a net result of all factors controlling bioaccumulation, is an explicit expression of a chemical's bioavailability, understanding the abiotic factors which determine metal uptake in benthic invertebrates would allow to forecast metal bioavailability in sediment.

In this study we elaborated bioaccumulation data for As, Ni, Pb, Cd, Cu, and Zn in the sediment-dwelling oligochaete *Lumbriculus variegatus* according to the Biodynamic Model to assess the factors affecting the bioaccumulation (and hence bioavailability) of these elements in sediments.

Results highlighted that, for *L. variegatus*, there is not a generic route of uptake (diet- vs water-borne metal) for all the considered elements. Ni is accumulated mainly from pore water, whereas sediment ingestion is the main uptake route for As, Cd, Cu, and Zn. Pb body burden is related to the freely dissolved concentration in pore water as well as to the diet uptake. When diet is the main uptake route, simple regression models relate the bioaccumulable fraction of some elements with organic carbon in sediment.

The study discusses how the developed regression models can influence: the use of sediment toxicity data to derive quality standards, the assessment of the metal bioavailable fraction in sediment as well as the application of food-web models to derive QS_{sediment} from QS_{bioa} and vice versa.

WE 272

The effect of soil pH on copper toxicokinetics in the springtail *Folsomia candida*

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Physical-chemical soil properties are determining metal availability in soil, and therefore have great influence on the uptake and the effects of metals in soil organisms.

Although many attempts have been made to develop relationships between metal toxicity and soil properties, it still remains unclear how such relationships may cope with the fact that toxicity may change with time. Toxicokinetics studies therefore are more suitable for describing the relationship between internal metal concentrations and exposure concentration of metals in relationship to soil properties. This study aimed at investigating the effect of soil pH on the uptake and elimination kinetics of the essential metal copper in the springtail *Folsomia candida*. Animals were exposed to three copper concentrations (0, 25, and 100 µg Cu g⁻¹ dry soil) in LUFA 2.2 soil, using three pH levels (4.5, 5.5, and 6.5). Internal copper concentrations were measured by sampling the animals at day 0, 0.25, 1, 2, 4, 7, 10, and 14 for the uptake phase and at the same time periods after transfer to clean soil (elimination phase). Copper concentrations in water and 0.01 M CaCl₂ extracts, in pore water and in soil were measured at the beginning and at the end of the exposures. Applying a first order one compartment kinetics model, data were analyzed and kinetics parameters estimated. At all three soil pH levels and both exposure concentrations, internal copper concentrations in the collembolans reached steady-state levels within 14 days exposure of animals. Uptake rates (k₁) seemed hardly affected by soil pH, while k₂ values (elimination rate) ranged between 0.008 and 0.2 day⁻¹ and were higher (faster elimination) at higher soil pH. Relating kinetics parameters to soil characteristics showed that pH was affecting uptake rates and internal copper concentrations especially when relating kinetics parameters to water and CaCl₂ extractable copper concentrations. Uptake rates expressed on the basis of Cu concentrations in pore water were best described taking into account both pH and dissolved organic carbon levels. Elimination rates were not correlated with total or extractable copper concentrations and were independent of soil properties.

WE 273

Evaluation of the bioavailability of copper in sediment using an aquatic plant (*Myriophyllum aquaticum*) and a geochemical approach (DGT)

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The total contaminant concentration is not a suitable predictor for environmental risk assessment as the response of organisms is typically affected by the fraction which is biologically available over a given time.

The aim of this work is (i) to better understand the bioavailability of copper in sediments as a function of sediment composition and (ii) to assess whether physico-chemical measures of metal bioavailability can predict bioaccumulation in organisms exposed to the sediment.

For this purpose we prepared artificial sediments with different properties (variation of the OECD 207-218 normalised sediment, modified by adding or not organic matter and/or ferric hydroxides - known for the complexation of heavy metals), and spiked them with different levels of copper. The bioavailability of copper was assessed using the passive sampling technique DGT (diffusive gradient in thin film), which provides information on the potential mobility of metals in the sediment and which is supposed to mimics the bioavailable fraction of metals. In parallel, the biological response of the aquatic plant *Myriophyllum aquaticum* (bioaccumulation and growth inhibition) was analysed. Finally the kinetic of copper uptake by the roots was quantified by the use of a numerical mechanistic model based on the Barber-Cushman approach (plant uptake model).

In addition; the processes influencing the lability of metals (sediment composition, physics-chemical parameters) was assessed by using a probabilistic model (DGT-PROF5) to interpret DGT measurements in sediments. Finally the understanding of the processes of absorption of metals by the root was attempted by comparing experimental results and modelling results (plant model uptake).

WE 274

Chronic toxicity and bioaccumulation in the aquatic oligochaete *Tubifex tubifex* exposed to Cu-spiked sediment: assessing critical body residue

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In aquatic ecosystems, organisms are exposed to heavy metals via waterborne and dietary routes, including ingested sediment for sediment dwellers. Metal speciation into different chemical forms (e.g. inorganic salts, ions, methyl-bound metals) and their bioavailability to benthic organisms is difficult to determine. Using metal spiked-toxicity tests, biological adverse effects due to specific sediment metal concentration could be related to metal accumulation in the exposed organisms. Metal concentration in body tissues has been studied through the concept of Critical Body Residue (CBR). In this study, the relationship between copper (Cu²⁺) exposure, bioaccumulation, and chronic toxicity was investigated using the oligochaete worm *Tubifex tubifex*.

Mature worms cultured in the laboratory were exposed to Cu-spiked sediments (both artificial and field collected) in a 28-d chronic toxicity test. Measured chronic endpoints were reproduction, growth and autotomy. Acute 96-h water-only tests were also conducted, for assessment of survival and autotomy. Toxicological effects are expressed by the parameters LC₅₀ and LBR₅₀ (from acute effects), and EC₅₀ and CBR₅₀ (from sublethal chronic effects).

Autotomy showed to be an endpoint of great interest, which can act as a detoxifying mechanism in aquatic oligochaetes. Our results suggest that autotomy is activated at concentrations below the lethality points, at least in an acute exposure. In a chronic exposure, artificial sediments exhibited lower values for reproduction and growth, compared to those measured in worms exposed to natural sediment. Interestingly, *T. tubifex* accumulated Cu in a concentration-dependent manner, both in artificial and natural Cu-spiked sediments. In fact, accumulation patterns were almost similar in both experiments, and reproduction impairment was observed at about the same concentration levels. CBR₅₀ ranged 3.87 to 4.70 µmol Cu g⁻¹ dw in natural sediment, and 4.18 to 4.66 µmol Cu g⁻¹ dw in artificial sediment. Results showed that copper is a highly toxic metal, with a very narrow range of action, that is effective at low levels of metal tissue concentration. Finally, the ecological relevance of copper CBR levels are discussed.

WE 275

Organotropism and toxicokinetics in tilapia *Oreochromis mossambicus* during acute/chronic pulsed waterborne copper exposures

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Laboratory toxicity tests are focused on few organs accumulations and performed with constant exposure scenarios to study vulnerability of fish. In reality, pulsed exposure is ubiquitous in aquatic environments. Moreover, the organ-specific copper (Cu) accumulations in fish are critical indicators of Cu exposure. The purposes of this study were conducting acute and chronic pulsed exposure bioassays to assess toxicokinetics of ten organs and tissues based on Cu burden. The Cu distribution dynamics in tilapia *Oreochromis mossambicus* were also examined. The experimental results found that muscle plays the main distributive dynamics of Cu burden (34.63-50.38%) in the initial exposure time, whereas liver is the most contribution (18.77-64.83%) during the exposure periods. The majority of organs in response to acute pulsed exposure had higher bioconcentration factor (BCF) than those of chronic pulsed exposure, except blood, muscle, and carcass. The gradients of Cu-BCF for ten tissues of tilapia revealed the following pattern: liver > intestine > kidney > stomach > blood > gonad > gills > bone > muscle > carcass for acute pulsed exposure, whereas liver > kidney > stomach > intestine > blood > gonad > gills > muscle > bone > carcass for chronic pulsed exposure. Here we also investigated the correlations between Cu burden of the fish organs in the acute and chronic pulsed exposures, respectively. The results indicated that the positive correlation between Cu burdens of whole body and liver in acute (r=0.748) and chronic exposures (r=0.607). Obviously, liver plays the most important role in storing Cu concentration, leading to trigger protection action in the pulsed Cu exposure. This study provides the organ-specific distribution dynamics of Cu burden and Cu toxicokinetics information to further develop the physiologically based toxicokinetic and toxicodynamic models. Validation with the experimental results to refine the physiological parameters for pulsed Cu exposure in tilapia was also implicated.

WE 276

Uptake, depuration and bioconcentration of arsenic, zinc and copper mixtures in milkfish (*Chanos chanos*)

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The aim of this study is to investigate the uptake and depuration of individual elements by milkfish, *Chanos chanos*, following exposure to a mixture containing arsenic (As), zinc (Zn) and copper (Cu). A 14-day exposure experiment under laboratory conditions was conducted to assess the uptake rate constants (k_u) and depuration rate constants (k_d) as well as the bioconcentration factor (BCF) of milkfish. The values of k_u for As, Zn and Cu in milkfish exposed to single pollutant were 1158.19 ml g⁻¹ d⁻¹, 851.66 ml g⁻¹ d⁻¹ and 13.08 ml g⁻¹ d⁻¹, respectively, while the values of k_d were 6.20 d⁻¹, 2.84 d⁻¹ and 1.24 d⁻¹, respectively. The values of BCF of As, Zn and Cu were 186.71, 299.39 and 10.52, respectively. The interactions among the elements and the subsequent uptake and depuration rates associated with the individual elements were analyzed. The results demonstrate that Zn can reduce the accumulation of As and Cu because the high Zn accumulation ability of milkfish. The competition of Zn toward As and Cu may cause the reduction of accumulation in milkfish of the later toxins. Since Cu will enhance the metabolism of the fish, it might be the reason that milkfish will accumulate more As and Zn, when Cu was added into the stock.

WE 277

Combined effect of uranium exposure and phosphorus deficiency on root exudation, uranium bioaccumulation and toxicity in white lupin (*Lupinus albus* cv. amiga)

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Because of the widespread use that is made of uranium in industry, its concentrations in our environment can locally be higher than background noise. As a heavy metal with radioactive activity, it is characterized by chimio- and radio-toxicity which must be carefully considered to protect human health. Plant species represent a major way of transfer of uranium from soils to food chain, but the element physico-chemical properties and speciation in soils make estimations difficult.

The main goal is the study of rhizospheric processes, in particular those linked with plant nutrition and physiology, and their role in defining uranium phytoavailability in the rhizosphere. We believe that the intense physico-chemical changes occurring there must be of great importance for the future comprehension of U and heavy metals transfer from soils to plant species.

After reviewing roots activities which may influence the physico-chemical properties of soil solid phase and soil solution and equilibria between these two components in the rhizosphere, we decided to focus our experiments on organic acid exudation, specifically citric acid one. Due to its role in phosphate and ferric nutrition and its chelating properties towards metals, we assume that its presence may have back effects on U solid speciation, U desorption from the solid phases, U uptake and U bioaccumulation in plant species. Because of its proteoid roots, the white lupin has the capacity to exude huge amounts of this molecule during phosphorus deficiency periods that makes it one of the best model plant to study these processes.

The work is divided in two parts. In a first one, in hydroponics, citrate exudation by white lupin in different phosphorus and uranium conditions was characterized, as well as toxic effects on lupin development and U localisation in roots. The obtained (and under acquisition) results to be presented will focus in particular on that part, which will then be used to design soil experiments. In this one, phytoavailability of uranium in the rhizosphere of lupin versus simulated rhizospheric effect by adding different amount of citrate (lupin-like amounts) will be studied. For this purpose, we will use RHIZOTest© device and study two contaminated soils where uranium has different origin: a mine soil found in Switzerland and an other one which is regularly sprayed with water containing high concentrations of uranium. The first results of these experiments may be also presented.

WE 278

Speciation, bioavailability and toxicity of uranium in different *Lemna minor* growth media

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Anthropogenic activities have led to a local increase of U concentration to levels that pose potential ecological risks. Uranium toxicity is known to depend on its redox state, speciation and physiological form. The speciation of U strongly varies with parameters such as pH, dissolved organic matter, carbonate and phosphate concentrations and water hardness. The objective of this work was to investigate the bioavailability and toxicity of U to the freshwater macrophyte *Lemna minor* L. using the standard growth inhibition test [1]. However, as the bioavailable fraction of U strongly depends on the medium composition the total U concentration, speciation and growth of *Lemna* in different growth media was evaluated. Three different growth media were selected based on their previous use in *Lemna* growth inhibition tests: (i) the OECD medium [1], (ii) K-medium [2], and (iii) a synthetic freshwater [3]. For each of the media the pH, CO₃ and phosphate concentrations were varied. As expected the OECD medium with normal phosphate concentrations (13.4 mg/L) sustained growth (figure1A). Changing the pH (figure 1A) or omitting CO₃ from the medium did not affect any of the tested growth parameters. On the other hand lowering the phosphate levels in the OECD medium clearly negatively influenced growth rate. In contrast for the K-medium it was shown that lowering the phosphate concentration did not adversely influence the growth rate (figure1B). Only when no phosphate was added the growth rate was below 0.250 average and as such also below OECD guidelines. Finally none of the tested Synthetic Freshwater compositions could sufficiently sustain growth of the *Lemna* plants (average growth rate 0.15/day).

Towards the bioavailability of U only in the K-medium with phosphate concentrations of 0.5mg/L or below U in solution could be retrieved. A dose-response curve for U was

set up (0.05µM up to 500µM). In the K-medium with low phosphate concentrations U concentrations from 50µM or higher induced more than 50% growth inhibition in the plants. In contrast in all other tested media similar concentration did not induce growth inhibiting effects probably due to a changed U speciation. The speciation of U in the different media is currently under investigation.

[1] OECD, 2006 Lemna sp. growth inhibition tests. Guideline 221

[2] Cedergreen, N, et al., 2007 Env. Tox. Chem.. 26(1):149-156.

[3] Charles, AL, SJ Markich, and P Ralph, 2006 Chemosphere. 62(8):1224-1233.

WE 279

Uranium bioavailability in freshwater sediment

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The anticipated increase in demand for nuclear power production will result in the increased mining of uranium (U), which raises the potential for increased U levels in adjacent aquatic ecosystems, especially in sediments. Interactions between dissolved U and solid phases can result in sediment U concentrations that are several orders of magnitude higher than those in the water column. Despite high concentrations of U found downstream from some older Canadian U mining operations, limited data are available regarding the bioavailability of U in sediment and associated effects on freshwater benthic invertebrates. It is well known that physicochemical characteristics of sediment have a profound influence on the bioavailability and toxicity of metals, including U. Here we investigate the influence of key sediment characteristics on the bioavailability and toxicity of sediment-associated U using larvae of the freshwater midge *Chironomus dilutus*. Sediment variables evaluated include sediment particle size, clay content, clay type and organic matter content. We determined, in a series of sediment bioavailability/toxicity tests, that different clay minerals with different surface chemistries differentially influence the bioavailability of sediment-associated U and we have quantified binding coefficients. Particle size and associated differences in surface area also play an important role. Results obtained from experiments with reconstituted sediment were contrasted with observations from a study with U-spiked field sediment. All water (overlying and pore water), whole-sediment and *C. dilutus* tissue samples were analysed for U using ICP-MS and the most probable exposure pathway(s) identified. Knowledge of the different U sorption properties of different sediment phases and components will improve local risk assessment of U contaminated sites, and ideally influence the development of site-specific sediment quality guidelines.

WE 280

Mercury concentrations in nestling eagle owl and their main prey, European rabbit, in Southeastern Spain

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Mercury (Hg) is a toxic and nonessential heavy metal of special concern because it is bioaccumulated and biomagnified through the food chain and it is associated with negative effects. Little is known about the bioaccumulation potential of Hg for terrestrial food chains and it is necessary new information about Hg levels in terrestrial species. The Eurasian Eagle Owl (*Bubo bubo*) could be a sensitive biomonitor of Hg in the terrestrial ecosystem since is a top predator in food chains, has a long life span and is resident and territorial. Hg blood levels detected in eagle owl chicks from 2006 to 2011 in Southeastern Spain (Murcia) can be considered low (18.10-60.45 µg/L), and it is unlikely that Hg pollution can negatively affect the breeding performance. Significant higher mercury concentrations were found in feathers than in blood ($p < 0.001$). Back feathers could be used as biomonitoring tool for mercury contamination in this terrestrial bird species. We have estimated by simple linear regression the equation: $\text{Log Hg in blood (µg/L, ww)} = -0.255 + 0.617 \cdot \text{Log Hg in feathers (µg/Kg)}$. Significant positive correlations ($r = 0.38-0.61$, $p < 0.029$) were found between Hg concentrations in blood of chicks and levels found in muscle of European rabbit (12.47-15.04 µg/Kg ww), their main prey, showing that Hg levels in blood are largely due to current Hg ingested in food collected and fed by the parents. Dietary changes, with a higher proportion of insectivore and omnivore preys, could be associated with higher mercury levels in tissues of eagle owl. Although the studied region is not considered Hg polluted, area under mining influence and the industrial zone seems to contribute in the higher mercury levels in eagle owls and rabbits from the south than levels in owls and rabbits from the north of the study region.

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WE 281

Mercury levels in human scalp and fish consumption near the Mid-Atlantic Ridge (Azores)

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The hydrothermal vents of Mid-Atlantic Ridge (MAR) near the triple junction of Eurasian, African and North American plates are characterized by a highly toxic environment. The hydrothermal fluids are especially rich in sulphides and heavy metals including mercury (Hg). Mercury uptake by marine organisms is a cumulative process resulting of bioaccumulation and biomagnifications through the food webs, predators accumulate higher tissue concentrations than their prey, putting at risk human health. Located near the triple junction in the North Atlantic Ocean and in the path of the Gulf Stream, the Azores islands are distributed in both sites of the MAR where hydrothermal fluids biasedly affect surrounding ocean waters and potentially inducing different contamination levels in local organisms resulting from its location in relation to the MAR.

Fish consumption is usually considered the main route of contamination of mercury in humans, each Azorean consume on average 76.35 kg of fish per year, making the Azores a Portuguese region with the highest rate of per capita consumption of fishery products.

In this study, sediment and human hair were collected from the both sides of the Mid-Atlantic ridge, in the west side, on the Flores island with an annual consumption of 51.2 kg and the east side, on the Faial island with a consumption of 77.5 kg per year and quantified the mercury. The concentrations of total mercury are linked with the weekly consumption of fish.

Biased contamination sources are discussed and the results show a pattern of increased mercury concentration with increasing consumption of fish, after the normalization of other parameters.

WE 282

Uptake, accumulation and toxicity of arsenic in arsenate-tolerant and non-tolerant *Agrostis castellana* L.

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The tailings of Vale das Gatas mine (Northern Portugal) contain high concentrations of arsenic (540.74-5,770 mg/kg) and heavy metals such as lead and zinc, thus colonizing plants, such as *Agrostis castellana* L., may have developed resistance to these elements. The average values of arsenic accumulated in samples of *Agrostis castellana* range from 13.21 mg/kg in the stems to 30.93 mg/kg in leaves.

Due to the great chemical similarity, phosphate and arsenate can compete either for the same adsorption sites on soil particles, either by the same mechanisms of absorption by the roots. Thus, it is anticipated that arsenate uptake by roots occurs by the same mechanism of absorption of phosphate. In this study, plants of *Agrostis castellana*, tolerant and sensitive to arsenate, were tested in relation to As(V) ion as a possible agent of damage to level of root membranes in vivo. Thus, tolerance to As(V) ion was tested by applying a 'root growth test' on specimens from the tailings, with reference to a sensitive population, whose specimens were collected at a location away from the mine. The tolerance index is the ratio of the average 'maximum roots growth' in the presence of As(V) and the average 'maximum roots growth' in the absence of As(V) to several arsenic concentrations (10, 20, 40, 80, 160, 320, 640, 1280 µM). The results allow verifying tolerance indices significantly higher in specimens that grew up in tailings (59.3% maximum), compared with specimens from remote and not contaminated areas (29.5% maximum).

WE 283

Arsenic and heavy metals in soil and vegetation of a contaminated region

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In this work, have been characterized the species present in the old mining area of Cavalo (Oleiros, Castelo Branco, Central Portugal); environment with important W, As and some Cu contamination.

The species were selected envisaging their use as bioindicators, meaning that they are able to detect the pollution present in soils for use in mineral exploration; and seeking their potential use in the restoration with metalotolerant species able to tolerate the geochemical stress imposed by these conditions. The analyzed species included: *Erica australis*, *Erica umbellata*, *Pterispartum tridentatum*, *Halimium ocymoides*, *Arbutus unedo*, *Cistus salvifolius*, *Hypochaeris radicata*, *Pinus pinaster*, *Anarrhinum bellidifolium*, *Conyza canadensis*, *Andryala integrifolia*, *Agrostis delicatula* and *Agrostis curtisii*.

The results of analysis show that the species best suited for biogeochemical indicating are by order of importance: *Erica australis*, *Arbutus unedo* (leaves), *Halimium ocymoides*, *Erica umbellata*, *Cistus salvifolius* and *Pterispartum tridentatum* for As; *Halimium ocymoides* for Cu; *Erica umbellata*, *Arbutus unedo* (leaves and stems), *Erica australis*, *Pterispartum tridentatum*, *Halimium ocymoides* e *Cistus salvifolius* for W.

On the possibility of using these species in revegetation of areas with this type of contamination, it appears that all species accumulate in their tissues the main elements causing contamination (As and W). On the other hand, these species only have medium physiological barriers, not implying a high intake of these elements in the ecosystem.

WE 284

Bioaccumulation of As, Cd, Hg, Pb and Se in the benthic and pelagic food chain of Lake Baikal

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Lake Baikal is located in eastern Siberia, in the centre of a vast mountain region. It is the deepest, the most voluminous and the oldest lake in the world. Arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and selenium (Se) were analyzed in water, plankton, invertebrates and fish samples from Lake Baikal with high resolution inductively

coupled plasma mass spectrometry (HR-ICP-MS). The present study confirms generally low concentrations of potentially toxic chemical elements (As, Cd, Hg, Pb) and Se in Lake Baikal in comparison to other freshwater ecosystems. However, the comparative study of water and biotic components in the ecosystem of Lake Baikal revealed that bioaccumulation potential and distribution of the chemical elements differ between locations with riverine and possible anthropogenic influence (Selenga Shallows) and the reference site (Listvyanka Bay). For example, the concentrations of Pb were significantly higher in water samples from the Selenga Shallows in comparison to Listvyanka Bay, while concentrations of Pb were significantly higher in fish from Listvyanka Bay. This may be related to greater bioavailability of Pb for fish at Listvyanka Bay caused by lower abundance of natural ligands at this location. Similar phenomenon was observed for Cd, Hg and Se were higher concentrations were found in plankton at Listvyanka Bay in spite of lack of differences between concentration in water. In both the pelagic and benthic food chain of Lake Baikal, Hg showed indication of biomagnification while As, Cd and Pb showed indications of biodilution. When comparing fish inhabiting the pelagic and benthic food chain of Lake Baikal, differences in concentrations of As and Hg were found. Arsenic concentrations were twice as high in pelagic fish, while the benthic fish had seven times higher Hg content compared to pelagic fish. The Se:Hg molar ratio revealed that although Baikal is located in a Se deficient region, Se is still in significant excess over Hg. Based on this relationship there is probably no risk of Hg induced toxicity in the endemic fish species of Lake Baikal at the present.

WE 285

The bio-accumulation of selected metals (Cu, Fe, and Mn) in water, sediments and invertebrates (*Physa Acuta*) of Kuilsriver, Western Cape, South Africa

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In this study, metal analysis of water, sediments and freshwater snails from Kuilsriver in Western Cape, South Africa, were carried out with a vision to attain a baseline data of their level and also relating these levels found to possible sources within Kuils River. Samples were collected randomly every two months from selected sites of the Kuilsriver for a period of one year. Samples were digested with 55 % nitric acid. The Inductively Coupled Plasma - Atomic Emission Spectrometer (ICP-AES) were used for metals (Cu, Fe and Mn) analysis. The concentrations of ranges of all the metals measured were found to be below the international guidelines for Sediments. South African guidelines were also used for water comparisons. Then, literature reviews for snails were used. All comparisons done for water and sediment showed kuilsriver to have very low detected metal concentrations; which can be considered as acceptable. However, for Snails these metals are micro nutrients, it was complicated to conclude whether concentrations were toxic or normal to the species.

WE 286

Assessing the combinatorial toxicology of the anthropogenic challenges associated with the marine environment in the proximity of Jeddah city in the Red Sea

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The coastal marine environment of the Red Sea is subject to direct and indirect influences of major populations and industrial facilities found along the coast such as those discovered in proximity to Jeddah City. When evaluating ecological impact of diffuse pollution from urban environments, it is important to consider interactions between contaminate mixtures that individually trigger levels but together may result in significant environmental consequences. To determine whether mixture effects need to be considered, it is essential to define geochemical parameters by performing a survey for major classes of contaminants and to evaluate penetrance into the food chain. Sampling of both sediment and sea water was performed at contrasting sites representative of near-shore (25m) with off-shore (10km) locations. Possible food-chain transference of any contaminant was evaluated by sampling fish (*L. nebulosus*) and plankton at the off-shore sites. All samples were assessed for the presence of heavy metals (ICP-MS) and hydrocarbons (GC-MS).

Our findings revealed that shallow water contained elevated metal concentrations including Pb (100 µg/L), As (47 µg/L) and Ni (32 µg/L). In contrast, metal values in deep water samples showed raised Cu (60 µg/L), As (43 µg/L) and Ni (32 µg/L). Analysis of the sediment pore water found unexpected levels of Al, Pb and As 30-40 µg/kg.

Inorganic profiling of fish tissues (gills and liver) identified Zn and Al to be the most highly represented (~0.04 and ~0.025 µg/g respectively) with Ni at approaching 0.01 µg/g, whilst the balance of metals measured were less than 0.005 µg/g. No detectable metals were identified in plankton and no hydrocarbon residues appeared in any of our analyses.

Results are achieved our objective of defining the contaminant profiles within the water column and transference to the biota. The elevated metals in the off-shore samples were intriguing and may be related to the deep-water brines previously identified in Atlantis II at the axial depression of the Red Sea. Furthermore, uptake of Al from the sediment to the gills and livers of *L. nebulosus* indicated the role of sediment as a source for metal uptake into the food chain. Having identified the major inorganics acting as toxicants within the interface between Jeddah City and the Red Sea and recognising the geogenic input from the deep-water brines, we are in a position to test for consensus among mixture effects using the model organism, *C. elegans*.

WE 287

Dissolved selenium speciation (selenite versus selenate) affects uptake and biotransformation in freshwater periphyton

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WE 288

Heavy metal bioaccumulation trends in the shell of two Bivalve species: a comparative study

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Bivalves are extensively used in biomonitoring programmes because they're known to be efficient accumulators of contaminants and ideal indicators organisms. Various species have been used for assess toxic metals levels in marine environment but the most favourite ones are filter feeding Bivalves such as mussels and oysters. In most cases the laboratory experiments are conducted to evaluate the metals concentration levels in soft tissues: few works concentrate their attention also in shell levels. In the optic of a more exhaustive comprehension of the mechanisms that undergo the transfer of inorganic contaminants to organism's hard parts, it was decided to operate a comparison between two different bivalve species on their ability to accumulate a specific toxic metal. The tests were carried out with standard size organisms in strictly controlled experimental condition in order to assess the extent at which the physiological characteristics of the species affect the metal equilibrium in the organism. The metal concentrations in both shell and soft tissues are analytically determined and subsequently compared to highlight intraspecific correlations and develop a possible explanation of metal partitioning. In addition it was determined the different species responses to bioaccumulation tests to achieve an indication about their ability in accumulate the selected toxic metal in the shell. This skill could be used in future applications concerning the comprehension and the following exploitation of Bivalves role in water basin self-purification mechanisms.

WE 289

Bioaccumulation of heavy metal and trace elements by *Potamogeton pusillus* as bioindicator of aquatic pollution

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Suquia River is the main source of drinking water for the city of Cordoba-Argentina. In recent times, the use of agrochemicals in the basin, rollover of heavy metals and poorly treated domestic waste resulted in increased pollution of its waters.

The aim of this study was to evaluate concentrations of heavy metals and other trace elements in the aquatic macrophyte *Potamogeton pusillus*, matching this information with the corresponding to water and sediment samples from the Suquia River basin. So far, we looked to assess whether this aquatic macrophyte reflects different pollution degrees, generated by anthropogenic pollution.

Potamogeton pusillus is a native species widely distributed throughout the basin, which was selected considering its favorable characteristics for use as a bioindicator (big quantity, widely distribution, easy to harvest and maintain in the laboratory). Plants were collected during the wet season at two stations along the Suquia River basin, having different pollution degrees and anthropogenic impacts. One area is located upstream from the main city (Córdoba). This first area has low population impact, with less pollution in according to previous studies. The second monitoring station is located downstream from Córdoba city. This second area is primarily affected by the input of pollutants from Córdoba sewage, in addition to massive urbanization and intensive agriculture downstream from the main city.

Measured elements include Ag, Al, Ba, Bi, Cd, Co, Cr, Cu, Ga, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sr, Ti, U, V, As, B, Be, Fe, Se, Zn, Ca, Hg, Pd, Pt, Au, Hf, Y, U, Yb, Tm, Th, Tb, Sm, Pr, Nd, Lu, La, Ho, Gd, Eu, Er, Dy and Ce.

Considered studied metals in both water and sediments, it can be seen that most of them have lower concentrations upstream from Córdoba city. Some elements (B, Al, V, Cr, Mn, Fe, Ni, Ga, Rb, Sr, Y, Ba, Ce, Sb, Sn, Pd, Zn, Cu, Hg, Rh and Pb) have significant higher values in water and sediments downstream from Córdoba sewage discharge. Chemometrics demonstrated good matching between heavy metal and trace elements concentrations found in water and sediment with those observed in aquatic plants collected at each monitoring site, indicating the follow up of pollutants from both water and sediment to the plant. So far, we propose the use of *Potamogeton pusillus* as a good bioindicator of aquatic pollution.

WE 291

Investigating the impacts of anthropogenic activities in intertidal mudflats on metal bioavailability to the polychaete *Nereis virens*

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The king ragworm (*Nereis virens*) is an ecologically and commercially important polychaete species of soft sediment inter-tidal communities throughout the northern hemisphere and is known to be impacted by various pollutants. More specifically in many coastal locations, *N. virens* is exposed to a range of heavy metals including copper and zinc which are known to be highly toxic to polychaetes. This species is also collected for bait which affects their abundance and may change the bioavailability of these metals in the sediment, and those available to the worms. To assess the relationship between copper and zinc concentrations in the sediment, pore-water and associated worms, samples will be collected from a number of sites in the UK and France adjoining the English Channel, with different pollution histories and various levels of bait

collection. Bioavailable metal concentrations in the sediment will be assessed using the Community Bureau of Reference (BCR) sequential extraction procedure, whilst the aqua regia extracted fraction will also be assessed followed by Atomic Absorption Spectroscopy (AAS) analysis. The concentrations within the worms and the pore-water will also be measured using standard extraction techniques and analysed by AAS. Initial data from Langstone Harbour in the Solent show relatively low bioavailable concentrations in sediments (10.83 mg kg⁻¹ dry weight for copper and 36.43 mg kg⁻¹ for zinc) and pore-water (0.68 µg l⁻¹ for copper and 3.03 µg l⁻¹ for zinc) despite being a heavily dug site. Concentrations in the tissues would also suggest that *N. virens* accumulates more zinc than copper although inter-individual variability was high. However, no relationship between the tissue concentration and the weight of the worm was observed. These data suggest that, although the site is a dug area, metal bioavailability and accumulation rates are relatively low. It is expected that this work will identify the key drivers of zinc and copper bioavailability under different sediment conditions and, ultimately, will identify chronic exposure routes for this species and how important bait collection is in modifying these processes.

WE 292

Mean concentration of metals level in prawn specimens from Epe Lagoon and Asejire River from Southwest Nigeria

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Whole prawn specimens and water samples from Epe Lagoon, in Lagos State and Asejire River, in Oyo State of Nigeria were analysed quantitatively for the presence of manganese, copper, zinc, lead and cadmium using Techcomp AA 6000 atomic absorption spectrophotometer. The mean concentration of each heavy metal in prawn specimens and water samples from both locations and the bioconcentration factor were determined. The results showed that the mean concentrations of all the metals in prawn specimens were higher (Mn; 94.61 ± 40.45mg/kg, Cu; 72.96 ± 16.26mg/kg, Zn; 97.40 ± 38.10mg/kg, Cd; 0.91 ± 0.26mg/kg) in Asejire River than in Epe Lagoon (Mn; 69.36 ± 78.53mg/kg, Cu; 54.82 ± 13.88mg/kg, Zn; 52.88 ± 4.01mg/kg, Cd; 0.83 ± 0.20mg/kg) except for Lead (5.22±5.08mg/kg in Asejire River and 9.18 ± 7.18mg/kg in Epe Lagoon). Using two sample paired t-test at 95% confidence limit, there were no significant difference (p=0.35, 0.35, 0.20) in the levels of Mn, Pb, and Cd respectively in both locations except for Cu (p=0.01) and Zn (p=0.0004). The mean concentrations of Mn, Cu and Pb in prawn specimens from both locations were above the maximum limits recommended by WHO. While levels of Zn in both locations were lower than the WHO and FAO limits, the levels of Cd in both locations were similar to WHO and FAO limits. The mean concentrations of the metals in water samples from both locations were within the limits recommended by WHO and FEPA. The results showed contamination of the water bodies and bioconcentration of heavy metals in prawns. This portends a serious public health risk. Consequently, continuous environmental pollution monitoring to check heavy metal hazards is hereby recommended.

WE 293

Biomonitoring and assessment of heavy metals in the vicinity of precious metal mining sites in South Africa

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Recent years have been characterised by increasing exploitation of natural resources by anthropogenic activities, especially mining. Not only has it affected the global balance of heavy metals, but a gradual increase in the concentrations in aquatic and soil ecosystems have been observed. There is no doubt that heavy metals are important trace elements in the nutrition of plants, animals and humans, especially zinc (Zn), copper (Cu), chromium (Cr) and nickel (Ni). However, other trace elements such as lead (Pb), cadmium (Cd) and mercury (Hg) are toxic even at low levels, therefore their concentrations should continuously be monitored [1,2]. Of concern in this study is the fact that high concentrations of metals in sediments due to anthropogenic or natural sources can be detrimental to the aquatic ecosystem, since it can be bioaccumulated by benthic organisms and transferred to higher trophic levels. There is thus a great substantial need to understand the bioaccumulation and toxicity of metals in aquatic organisms, since it can provide critical information for the environmental risk assessment of metals in aquatic environments [3,4].

The present study considered the bioaccumulation of heavy metals such as cadmium (Cd), lead (Pb) and zinc (Zn), including precious metals such as platinum (Pt), palladium (Pd) and rhodium (Rh) in sediment and freshwater crabs from sampling sites in close proximity to precious metal mining activities in the North-West Province, South Africa.

WE 294

Does the relationship between trophic level and concentrations of POPs differ between species?

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Seabirds are top predators in arctic food webs, and exposed to persistent organic pollutants (POPs) primarily through diet. POPs undergo trophic transfer and some biomagnify in the food web. The trophic position is therefore used to explain the variability in POPs between species, and stable isotopes (δ 15N, δ 13C) can be used to estimate trophic level and carbon source, respectively. The relationship between δ 15N and POPs is a regression, and from that it is possible to calculate the slope of regression, also known as the trophic magnification factor (TMF).

However, seabirds species differ in physiology and ecology; even though species may have the same trophic position, they may have different concentrations of POPs, or vice versa. Therefore, before overall applying TMF to entire food webs, it is important to investigate the δ 15N-POPs relationship within species.

In present study, muscle samples from three seabird species (*Rissa tridactylis*, *Fulmar glacialis* and *Larus argentatus*) was used to investigate these issues. The birds were sampled by the Norwegian Polar institute at two different sites and two different years (kittiwake and fulmar at Kongsfjorden (Svalbard) in 2006, herring gull at Sommarøya (Tromsø) in 2007).

The POPs investigated in this study were halogenated hydrocarbons: DDT, PCBs, chlordanes (CHL), hexachlorobenzene (HCB), Mirex and the three isomers: α-, β- and γ-hexachlorocyclohexane (HCH). All chemical analyses was done at the Great Lakes Institute of Environmental Research, Canada. The results showed that species had the same trophic level, but different concentrations of POPs, suggesting a different δ 15N-POPs relationship between species. However, the data set was found to be not optimal for further investigations for δ 15N-POPs-regression in either species, possibly due small sample size and lack of variation within the species.

Additionally, the species results for trophic level were in contrast to previous studies of adult birds. The individuals used in present study were chicks, which suggest that chicks may not be an appropriate proxy for adults when using stable isotopes. Also, the POPs did not have a uniform placement in the PCA plot of both species and tissues. Thus, concentrations of specific POPs seem to be influenced by different parameters.

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WE 295

Long-term effects of lead (II) and recovery in synchronized *Chlamydomonas reinhardtii* in the course of the cell division cycle

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It is largely unknown whether the bioaccumulation and effects of toxic substances in freshwater microorganisms varies with their individual physiological state and can lead to a selection towards specific cell states and impact the algal community as well as the dependent biocoenosis. Anthropogenic activities especially the use of leaded gasoline have lead to an increase of bioavailable lead in aquatic ecosystems. Cultures of the model organism *Chlamydomonas reinhardtii* quickly accumulate lead during chronic exposure whereas effects on photosynthetic activity and growth. Toxicity tests are usually performed using non-synchronous cultures and it is not possible to resolve toxic effects regarding the cell division process. In this study *C. reinhardtii* is used to elucidate the influence of the algal cycle on the bioaccumulation and effects of lead. Cultures are synchronized by light/dark adaption (14:10 h) showing a typical growth curve with consecutive divisions of 2, 4 or 8 daughter cells. Synchronisation is verified by flow cytometry of DNA-stained (propidium iodide) algae and the cells are then exposed to free [Pb2+] ion concentrations ranging from 10⁻¹⁰ - 10⁻⁷ M (pPb10-pPb7). Current experiments upon short-term exposition of 7 hours exhibited a growth rate inhibition up to 60% for pPb7 along with chlorophyll fluorescence decrease. pPb 9 and pPb 8 indicated no effects on growth rate and cell cycle states for the short-term exposure. However long-term exposition over 30h shows a limited growth and cell numbers for both. Comparing exposed algae before and during the cell division state exhibits a higher sensitivity for the latter. Hence, the effects of [Pb2+] on different cell cycle states of next generation subcultures are investigated comparing growth rate, photosynthesis and the DNA content along with a chemical analysis. Localisation and distribution of intracellular [Pb2+] by confocal microscopy with previous Pb specific staining is compared to Dynamic Nano SIMS ion imaging along with analysing the total and intracellular Pb content by ICP-MS.

WE 296

Development of an abbreviated in vivo fish bioconcentration test

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Laboratory bioconcentration studies are becoming an integral part of global hazard and risk assessment paradigms. Experimental measurement of the bioconcentration factor (BCF) is often required for any chemical with log KOW greater than 3, if it is produced in quantities greater than 100 t/year. Currently, the expectation is that a BCF will be experimentally determined for any such chemical by performing a fish bioconcentration study according to Organization for Economic Cooperation and Development (OECD) test guideline 305 or U.S. Environmental Protection Agency (U.S. EPA) guideline 850.1730 (OECD 1996, EPA 1996). These standardized guideline studies are demanding of resources and require the use of large numbers of animals. In an attempt to develop an in vivo screening level test, common carp were exposed to chlorpyrifos, methoxychlor, DDT or musk xylene for 7 d, at which point fish were placed in chemical free water for 7d. During this period, liver and muscle tissues were sampled on days 1, 3, 7, 10 and 14. 14 d BCF values for all chemicals were similar to those values obtained using standard protocols and timelines. Statistical and modeling approaches will be applied to these data sets to optimize the experimental design for a robust screening test.

ET05P - Ecotoxicology and ecosystem services: A southern perspective

WE 299

A comparison of the effects of the organophosphate pirimiphos methyl on two freshwater snails *Helisoma duryi* and *Lymnaea natalensis*

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Pirimiphos methyl is a broad spectrum organophosphate used against a wide variety of pests. Like most pesticides it is non-target species specific and it finds its way into aquatic ecosystems via aerial drifts, runoffs and leaching where it affects habitats of aquaeosystems. A field simulated study was carried out to assess the effects pirimiphos methyl on the well being of two different freshwater snail species *Helisoma duryi* and *Lymnaea natalensis*. The snails were exposed to differently polluted dam water, Matopos (relatively pristine) or Umuza (polluted) spiked with 5, 15 or 25 ppb of pirimiphos methyl. for 1, 7 or 14 days. After each exposure period 10 snails were sacrificed and post-mitochondrial fractions prepared. Cholinesterase and carboxylesterase activities were measured. Pirimiphos methyl caused dose-dependent inhibition of esterase activities in both snail species. Inhibition of esterase activity was generally lower in snails exposed to the contaminated dam water when compared to snails exposed to the relatively pristine dam water probably because in the contaminated water there were other chemicals as well as higher microbial load present in the water which enhanced decomposition of the spiked pesticides thereby reducing available pesticides that inhibited esterase activity. The results also showed a time-dependent inhibition of esterase activity with increase in exposure time resulting in increase in inhibition of esterase activity in both snail species. Generally pirimiphos methyl caused higher inhibition of esterase in *H. duryi* when compared to *L. natalensis* suggesting that *H. duryi* was more sensitive to pirimiphos methyl than *L. natalensis*. For snails exposed to 5 ppb pirimiphos methyl in the Matopos dam water, carboxylesterase activity was inhibited in the range 45-72% in *L. natalensis* compared to inhibition in the range 58-88% in *H. duryi* and cholinesterase was inhibited up to 67% in *L. natalensis* and up to 76% in *H. duryi* depending on exposure period. Our results showed that pirimiphos methyl definitely affected the well being of the aquatic snails and this has a negative effect on the entire aquatic ecosystem as snails have a role in maintaining a healthy aquatic ecosystem; they are prey for other organisms such as some fish and frogs while also feeding on a variety of substance thus, contributing to waste removal and they help in maintaining a balanced aquaeosystem, something they can do effectively only if they are healthy.

WE 300

Elemental levels in sediment, fish, and wild bird eggs in the Orange-Senqu catchment, southern Africa

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The Orange-Senqu River basin starts in Lesotho and mouths on the Atlantic coast of southern Africa. Four countries: Botswana, Lesotho, Namibia, and SA, share the basin. Lesotho contributes > 40% of the stream flow, but is a small user. The biggest user, SA, use the water to drive its economy. The Botswana part is covered by desert with little surface runoff, but groundwater contributes to the water demands. The River's estuary is an important wetland, but is on the Montreux Record of threatened wetlands. The Orange-Senqu River Commission (ORASECOM) was established to manage the basin. The results reported here form part of the Transboundary Diagnostic Analysis prepared by an ORASECOM project, funded by the GEF through the UNDP. Sediment, fish, and wild bird eggs were analysed for a variety of elements, including heavy metals. The total concentrations of the elements were determined using ICP-MS. The metal pollution index (MPI) and the geo-accumulation index (Igeo) were calculated for the sediment. Levels were compared to international sediment quality guidelines (SQG). The levels in the fish were compared to international guidelines for human consumption. Levels in sediment, and biota were compared to determine bioaccumulation. Fe and Al had the highest means in the sediment, 23.5 and 20.6 g/kg (d.w.) respectively. The MPI for a site (no. 56) in the North-West Province, SA was the highest and had the most elements with Igeo-values regarded as polluted. The same site was also among the 25% sites with the most elements with the highest concentrations, along with sites in the Riet and Modder Rivers in SA. Shared geology could only partially explain this phenomenon. Two sites in Lesotho with high levels are likely to be due to geology rather than anthropogenic activities. Compared to the SQG only site 56 has a high probability of being toxic to the biota. Only Se had levels in fish higher than international guidelines for human consumption. The elements most likely to bioaccumulate in the fish were Pt>Rb>Au>Sn>Ag>Rh. All the elements had bioaccumulation factors > 1 in eggs, except Cs, but this result was concluded under a number of assumptions. The various bird species bioaccumulated different elements differently but the grey heron bioaccumulated the most. The levels in sediment beg further investigation, considering the Igeo-values for Se, As, and Hg. Further assessment is needed on the bioavailable fractions of the elements at the sites identified.

WE 301

Polybrominated diphenylethers (PBDEs) in biofilms hinder larval settlement of marine intertidal organisms across three phyla

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Polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants over the last three decades, and are now ubiquitous in the marine environment. The South China region is a major global dumping site of electronic wastes and very high levels of PBDEs in seawater, marine sediments, marine mammals, fishes and marine invertebrates have been reported. Studies on mammals and fish have shown that PBDEs can cause abnormal development and reproductive impairment, and this has led to growing environmental concerns. While the harmful effects of PBDEs on mammals and fish are documented, the effects on marine invertebrates remain virtually unknown. Using three model intertidal species from different phyla, including the polychaete *Hydroides elegans* (Phylum: Annelida), the gastropod *Crepidula onyx* (Phylum: Mollusca), and the barnacle *Balanus amphitrite* (Phylum: Arthropoda), this study demonstrated that (a) acute toxicity of PBDEs is low (i.e., 24-h exposure to very high levels of PBDEs up to 10000 µg l⁻¹ did not kill marine larvae; (b) long-term exposure to environmentally realistic concentrations of PBDEs did not affect larval growth, development and settlement, despite PBDEs bioaccumulated in the body; and, (c) PBDEs spiked biofilms reduced the success rate of larval settlement. Since the process of larval settlement governs the population dynamics of benthic invertebrate communities, our results suggested that environmental exposure of PBDEs, which hinder larval settlement of intertidal organisms, may have serious ecological consequences.

WE 302

Effects of hypoxia on the larval growth, settlement and physiologies, and post-settlement performance of the marine invertebrate *Crepidula onyx*

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Increasing anthropogenic activities and the resulting eutrophication in the coastal areas have exacerbated both the occurrence and severity of hypoxia worldwide in the last two decades. Hypoxia occurs when the dissolved oxygen falls below 2.8 mg L⁻¹. The marine gastropod *Crepidula onyx* is found in great abundance in intertidal and subtidal waters over large geographic areas along the Pacific coast of North and South America, China, Japan and Hong Kong. Using *C. onyx* as a model invertebrate species, we tested the hypothesis that while severe hypoxia can result in mortality of marine larvae, mild hypoxia can reduce the growth rate and settlement success and affect the physiologies and post-settlement performance. Our study suggested that the LT₅₀ of 1 mg O₂ L⁻¹ was 2.5 days, and all of the larvae died before they could attain metamorphic competency. When the larvae become metamorphic competent, they settle and metamorphose into juveniles in response to a suitable substratum with biofilms. We found that the ambient DO level did not affect the settlement success of the competent larvae in response to biofilms. Our preliminary data also suggested that long-term exposure to mild hypoxia would reduce the growth rate, lengthen the time required to attain metamorphic competency, reduce the metamorphic success, and affect the lipid content, feeding rate, and post-settlement survivorship.

WE 303

Determination of heavy metals in fish muscles & water of Haraz river

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Objectives: Being the second largest river in the southern Caspian Sea basin, the Haraz River flows northwards through the Alborz mountains in the central region of Mazandaran province. The Haraz basin has specific geological characteristics affecting the river water quality. This area has been a rich source of minerals from times immemorial. About 45 mines (coal, limestone, sand and gravel, etc.) have been operational for the last eight decades. The aim of this study was to determine the concentration of Zn, Cd, Cr, Pb, Cu, Ni in fish muscles & water in Haraz river.

Materials & Methods: In order to determine the heavy metals concentration in all parts of river, 4 station (Haraz, Razan, Chelrood & Amol) were selected. Totally 32 samples were collected from 8 farms (4 sample from each farm and 2 farms from each station). 4 water samples were collected. Biometric were performed, then 10 grams of fish muscle were dissected and samples were prepared according to standard method. Heavy metal concentration were determined by atomic absorption method.

Results & Conclusion: The mean concentration of Zn in fish muscles & water in Haraz, Razan, Chelrood and Amol were 0.72, 0.32, 0.522, 0.5 & 1.72, 1.81, 1.77, 1.7 respectively. Ni didn't detect in fish samples but the mean concentration in water samples in Haraz, Razan, Chelrood and Amol were 1.1, 0.9, 1.1, 1.1 respectively. The mean concentration of Cr in fish muscles & water in Haraz, Razan, Chelrood and Amol were 0.586, 0.492, 0.5, 0.552 & 2.2, 2.2, 2.1, 2.22 respectively. Cd didn't detect in any sample. Pb concentration in fish samples & water in Haraz, Razan, Chelrood & Amol were 0.44, 0.34, 0.37, 0.48 & 0.11, 0.11, 0.11, 0.14 respectively. The mean concentration of Cu in fish muscles & water in Haraz, Razan, Chelrood and Amol were 0.754, 0.372, 0.539, 2.3 & 0.11, 0.21, 0.17, 0.37 respectively. Cu concentration in the fish muscles and water was increased significantly in Amol station. The results of this study showed that heavy metal concentration in fish muscles and water are lower than standards.

WE 304

Mycotoxin Zearalenone induced apoptosis in BEAS-2B cells through generation of ROS and activation of JNK and p38 MAPKs signaling pathways

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Human exposure to Zearalenone (ZEA, a non-steroidal estrogenic mycotoxin) through inhalation has raised considerable concern. However, the potential health risk and the mechanism of actions of ZEA are not well understood. In the present study, we used BEAS-2B, cultured human bronchial epithelial cells, as well as Cygb stably transfected BEAS-2B cells to study the cytotoxic effects and the toxic mechanisms of ZEA. Our results indicated that ZEA decreased cell viability, induced apoptosis and promoted ROS level in BEAS-2B cells. Oxidative stress was clearly evident, as shown by an elevated mRNA expression levels of oxidative stress markers (Hsp70 and Hsp27) and endogenous antioxidants (SOD2 and Gpx). Stable transfection of Cygb significantly increased the level of Cygb but reduced level of ROS and the percentage of apoptotic cells induced by ZEA. Cells pretreated with either p38 or JNK inhibitors showed no attenuation in ROS level, but the percentage of apoptotic cells was lower than cells treated with ZEA.

alone. Overall, our results indicated that ZEA induces apoptosis, possibly through generation of ROS and activation of JNK and/or p38 MAPK signaling pathways.

WE 305

Bacteria associated with skeletal tissue growth anomalies in the coral *Platygyra carnosus*

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Scleractinian corals with growth anomalies, often referred to as 'tumors', have been reported globally. A recent survey of Hong Kong waters showed that more than 60% of *Platygyra carnosus* colonies developed tumors. Here we report for the first time, the bacterial community associated with tumors in *P. carnosus* over different seasons and locations in Hoi Ha Wan Marine Park and Port Shelter. Culture-based methods for strain isolation and molecular techniques of 16S rRNA analysis for strain identification were used, as well as the culture independent technique terminal-restriction fragment length polymorphism. We tested the hypothesis that the community composition would be considerably different between healthy and tumor corals and aimed to investigate whether potential differences because of tumors would override the seasonal and spatial influences. Our analysis detected only minor differences between the communities associated with the healthy and tumor corals, indicating that tumors are not associated with major changes in the bacterial community structure. In contrast, community structure was strongly influenced by the location and season, with greater Alphaproteobacteria diversity in the winter than in the summer. This study demonstrated that the coral-associated bacterial community composition was more related to environmental variables (i.e. season and location) than to disease (i.e. tumor).

WE 306

Identification of alternate solutions to mitigate river health degradation and their effect on ecosystem services in low flow river watershed

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Global change impacts on water availability, water quality and ecosystem services in Mediterranean river basins of the Iberian Peninsula, as well as their impacts on the human society and economy makes it a key issue on the EU agenda. It is likely that the first impacts of climate change will be felt in the Mediterranean water resource system through increased frequency of water shortages and decline in water quality. Excessive nutrient loading from agriculture, industrial and domestic sectors is expected to become a growing threat to low flow river Francolí, located in Catalonia (Northeastern Spain). Water quality degradation is getting severe in areas where water is scarce due to reduced capacity of contaminants' dilution. Provision of additional water resources in the low flow river watershed can mitigate the severe effects of river health degradation. Some of the alternate solutions to meet the increasing demand may include desalination, inter basin transfers, using ground waters and ground water aquifer recharge. In parallel to technical solutions, an attractive aspect for river basin managers is the promotion of responsible water use in the basin. To evaluate and improve water management in the basin, two steps are followed. First, different hydrological scenarios are simulated using MIKE BASIN software. Then, spatially explicit modelling tool, called Integrated Valuation of Ecosystem Services and Tradeoffs (InVEST) is used to assess the ecosystem services of different modelled hydrological scenarios. The evaluated services are water yield and supply, nutrient and sediment retention. Ecosystem based approach to river basin management, that consider the trade-offs between different inland water ecosystem services are more likely to ensure sustainable solutions. The results of this work are expected to improve the understanding of the management problem, and help to identify the best management solutions for the studied basin.

WE 307

Tailoring of InVEST model to assess climate change impacts on ecosystem services at a Mediterranean river basin

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The Mediterranean basin is considered one of the most vulnerable regions of the world to climate change and with a high potential to present important problems in water availability in the next few years. The predicted future scenarios for this region present an increased frequency of floods and extended droughts, especially at the Iberian Peninsula. These events are likely to produce important effects in the ecosystem services provided by Mediterranean river basins.

To evaluate the impacts of climate change on ecosystems services of Mediterranean river basins, we apply InVEST, a spatially explicit tool consisting of a suite of models that use land use and land cover patterns to estimate ecosystem services from a biophysical and economical point of view. This tool was applied to evaluate and quantify water provisioning, water purification and erosion protection services. For the application of this tool for the Mediterranean areas, some improvement of the model and the inclusion of some peculiarities of the studied region were made. These changes regard the incorporation of water extraction and return points, and the possibility of valuing water for uses other than the hydropower production. These improvements are key for the model application to the studied areas, since they strongly influence the dynamics of Mediterranean basins.

To evaluate the appropriateness those improvements, the modified tool was applied to the Llobregat river basin, located at Catalonia (NE of Spain). The Llobregat River is the second longest river in Catalonia, and one of Barcelona's major drinking water supplier. This catchment receives extensive urban and industrial wastewater discharges as well as surface runoff from agricultural areas. The results of this study will be validated with real data from the studied basin.

WE 308

Testing strategies in Ecotoxicology: two examples for non target arthropod testing from China

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Registration of plant protection products may vary by region and national legislation requirements. Independent from the regional aspects, use patterns may vary from South East Asia to northern Europe requiring significantly different approaches.

As an example of this aspect, two terrestrial ecotoxicology test systems are presented, both standardized for Chinese registration processes and approved by the national Institute for the Control of Agrochemicals, Ministry of Agriculture (ICAMA). Setup, handling and results of tests with the parasitoid wasp genus *Trichogramma* (most frequently used species: *T. cacoeciae*, *T. evanescens*) and the silkworm *Bombyx mori* are presented, both species required by ICAMA and described in Chinese guidelines for ecotoxicological testing:

- *Trichogramma* has a worldwide geographical distribution and plays an important role as natural enemy against lepidopterous pests on a wide range of agricultural crops. It is regarded as a sensitive indicator species, suitable for identifying plant protection products that are unlikely to be harmful to non-target arthropods in the field. The testing guidelines of ICAMA (2004) and IOBC (2000) differ strongly considering the duration of the exposure phase and the type of test cages.

- *Bombyx* was originally found in China and adjacent countries. Today, the core countries for culturing of silkworms and silk production are China, Cambodia, Vietnam, Japan, India, Southern Europe and especially Brazil. Due to their economic importance and sensitivity to xenobiotics, larvae of *B. mori* are regarded as suitable for hazard assessment for plant protection products and biocides.

Both examples will demonstrate that the registration of plant protection may depend on regional requirements either by using region-specific testing procedures (see *Trichogramma*) or even region-specific test species (see *Bombyx*). The differences in handling, required test species, regional emphases as well as cultural aspects show the difficulties that can appear within ecotoxicology testing and global registration of plant protection products.

WE 309

Selenium cycling in an estuarine ecosystem receiving inputs from coal-fired power station

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Release of trace elements, especially selenium, from coal power plant fly ash is of concern due to the potential for environmental contamination and accumulation in food webs. Lake Macquarie has two power stations located on its shore and has been contaminated with selenium (Se), cadmium (Cd), lead (Pb) and zinc (Zn). The presence of these elements are a direct result of runoff from nearby coal power plant fly ash. This project investigated the history of trace metal contamination in sediments in Lake Macquarie using Pb210 analysis to evaluate whether selenium concentrations have decreased after improved fly-ash handling procedures have been implemented at the power stations. Selenium accumulation and biomagnification in the seagrass food webs were examined by using C and N isotopes to establish food chains. As well the volatilization of selenium from sediments was measured. This work clarifies the current and historical inputs of selenium and characterizes how selenium may be lost from sediments by volatilization or remobilization into marine food webs.

WE 310

Amazon deforestation, ecotoxicology and ecosystem services: valuing mercury contamination

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The Amazon rainforest provides many ecosystem services that are important locally, regionally and internationally. These ecosystem services represent the good and processes that are important for human well-being, obtained directly or indirectly from ecosystems. A service that is little known or considered is the protection that the forest provides against contamination of aquatic ecosystems by mercury. Much of the mercury in the Amazon is stored naturally in the soil. With the removal of vegetation mercury is transported to aquatic ecosystems enabling its transformation into methylmercury. This substance has, among other mercury species, the greatest potential for

bioaccumulation and biomagnification in different trophic levels, starting by aquatic organisms. This study was conducted using the Purus River Basin as a model to simulate the damage promoted by the input of mercury in aquatic systems produced by deforestation, and the valuation of those damages. Data of soil types, concentration of mercury in soils, methylation rate and concentration of MeHg in fish were collected from literature. Hence, the model estimated the potential costs of mercury poisoning treatment, as a consequence of forest clearing, by using a function with the following variables: the deforestation, the concentration of mercury in the soil, the rate amount of soil eroded, the level of mercury methylation, MeHg concentrations in fish, fish consumption by humans, damage to health and costs expended in diagnosis and treatment of poisoning. Assuming a deforestation rate of 8% of the basin per year, which is the current rate of deforestation, the potential damage costs would be approximately \$ 1 million/year. This estimated cost represents the value of ecosystem service of entrapment of mercury in the soil due to protection provided by the forest against erosion.

WE 311

Hypoxia effects on intertidal community: from microbes to invertebrates

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Eutrophication due to ever-increasing anthropogenic activities (especially sewage discharge and the wide use of agricultural fertilizers) has exacerbated the duration, intensity and extent of hypoxia in coastal areas worldwide. Hypoxia is generally defined as the dissolved oxygen falls below 2.8 mg L⁻¹. Substratum preference for larval settlement may govern the population dynamics of benthic invertebrate communities. Larvae of barnacles, polychaetes, bryozoans, oysters and gastropods have been reported to settle and then metamorphose in response to biofilms, which vary in the microbial community composition with the prevailing environmental factors. In this study, we tested the hypothesis that hypoxia can alter the microbial community composition of biofilms and subsequently, the settlement response of larvae, thereby shifting the benthic invertebrate communities. Biofilms were first allowed to develop in Clear Water Bay at the lower intertidal level for one week, and then exposed to hypoxia (i.e. 1.5 mg O₂ L⁻¹) or normoxia (i.e. 6 mg O₂ L⁻¹) in the laboratory. After one week exposure to hypoxic or normoxic condition, the biofilms were tested for their effects on larval settlement of the polychaete *Hydroides elegans* and the slipper limpet *Crepidula onyx*. Our results showed that the hypoxic biofilms significantly reduced the larval settlement rate of *H. elegans*, but not of *C. onyx*. These biofilms were also tested for their effects on recruitment of new settlers in the field. We found that the hypoxic biofilms attracted much less *H. elegans* and another polychaete, the Christmas tree worm *Spirobranchus giganteus* than the normoxic biofilms, therefore shifting the benthic invertebrate community structure. At the same time, some hypoxic biofilms were allowed to recover in normoxia in the laboratory for one week and subsequently, tested for their effects on larval settlement. Our results showed that the biofilms once exposed to hypoxia, but returned to normoxia no longer reduced the larval settlement rate of *H. elegans*. Furthermore, our preliminary data suggested that hypoxia altered the microbial community composition of biofilms, explaining the observed difference in larval settlement response.

WE 312

The use of an ecosystem services performance index to describe the status of water quality impacted by small scale farming communities in Limpopo, South Africa

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A vast percentage of South African water resources are degraded but people in rural areas, especially small scale farmers, often rely directly on good water quality. Thus to improve the implementation of resource protection into decision making processes the focal point need to be shifted away from biodiversity and ecosystem health towards ecosystem services and associated benefits for society.

Water quality in natural aquatic ecosystems provides different services including provisioning, recreational (aesthetic), cultural and supporting services. The different services related to water quality in rural small scale farming areas in Limpopo, South Africa are discussed in this paper. The well function of the river systems are utilized by various beneficiaries (local communities, agriculture, industry etc). The South African Water Quality Guidelines recognize these different groups plus the aquatic ecosystem itself as beneficiaries and allocated specific guideline values to each group of beneficiaries.

A further concept in the assessment of rivers statuses is the comparison to reference sites. According to the level of different variables, river sections are divided into natural, good, fair or poor quality. This can be aligned with the concept of Ecosystem services performance index which calculates the distance value between an observed state and a reference state for selected parameters and which has been used for the assessment of soil integrity. In terms of agriculture e.g. it is the desire to achieve sustainable land and natural resource management thus we compare the index between different agricultural management systems.

The entire project will look at the impact of different small scale farming management practices (change to agro ecological principles) on the status of ecosystems This will be measured by the development of an ecosystem service index adapted to rural areas, since they are more reliant on the services than other communities.

However this paper will only discuss the ecosystem services of water quality being important for small scale farmers, the input parameters for the index (including social and economic parameters if available) as well as the status of the ecosystem services as a baseline assessment. The input variables for the index are obtained through Participatory Action Learning workshops with the small scale farmers communities.

WE 313

Hypoxia is an endocrine disruptor and threatens the reproduction and sustainability of fish populations

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Hypoxia caused by eutrophication affects thousands of km² of water worldwide, and has caused declines in fish populations and major changes in aquatic communities worldwide.

For the first time, we demonstrated that hypoxia is an endocrine disruptor, and can affect specific hormones, neurotransmitters and receptors along the Hypothalamus-Pituitary-Gonad (HPG) axis. In particular, down-regulations of 3 β -HSD, CYP11A, CYP19A and CYP19B associated with an increase in the testosterone/estradiol ratio were observed. The hormonal disruption subsequently leads to a male-biased population in the F1 generation. A male-dominated populations caused by hypoxia, taken together with our earlier findings that hypoxia can cause significant reproductive impairments, will reduce reproductive success and pose a significant threat to the sustainability of natural fish populations.

Since the genes and hormones regulating the HPG axis is highly conserved across all vertebrates, hypoxia may also cause endocrine disruption and reproductive impairment in higher vertebrates, including humans. Recent epidemiological and medical research offer peripheral evidence to support this postulation.

WE 314

The application of a wetland biotic index to assess the pollution control functionality of the Nylsley wetland, South Africa

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Wetlands form a vitally important part of the river systems around the world. They perform vital functions that help maintain the quality and quantity of fresh water in many regions. One of the essential services that wetlands provide in developing countries is related to pollution control. This provides a natural cleansing function for safe water by downstream users. Bio-assessments are based on the premise that the biotic communities will reflect the health or integrity of the wetland system. The aim of this study was to determine whether the Wetland Biotic Index (WBI), which was developed specifically for riverine floodplain wetlands, was able to demonstrate the pollution control function of the Nylsley wetland in the Limpopo province of South Africa. The Nyl River and floodplain are subjected to various impacts such as mining and farming as well as runoff from formal and informal settlements. Sampling sites were situated along a pollution/impact gradient to determine the change in WBI scores in relation to changes in water quality as the Nyl River moves through the wetland. The WBI index consists of three metrics, i.e. a macroinvertebrate index based on sensitivity scores, a habitat quality and land-use rating. The WBI scores were determined on a seasonal basis from six sites in the Nylsley system. Concurrent water and sediment quality analyses were undertaken at the selected sites. Water quality parameters measured included nutrients, microbial content, suspended matter and dissolved metal concentrations. Sediment samples were analysed for metals and pesticides. Low WBI scores were obtained in the upper catchment that is subjected to runoff from urban settlements, agriculture and mining activities. As the water flows into the wetland the level of pollutants decrease and this is reflected in the increased WBI scores. During the rainy season the sites upstream of the wetland have much lower WBI scores with concomitant higher microbial, nutrient and metal loads. However the water flow is attenuated by the wetland system resulting in higher WBI scores and lower pollutant concentrations. The highest index scores and best water quality is found at the most downstream site, which is situated in an impoundment that is used to supply potable water to a nearby town. We demonstrated that the WBI is a useful monitoring tool to determine the effectiveness of the pollution control functioning of ephemeral floodplain wetlands.

WE 315

An innovative approach for the biological component of an ecosystem services analysis: a case study for fruit production in Southern Europe

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Practical application of an ecosystem services analysis requires a scientifically sound biological component. Critical components for using ecosystem services in developing best management practices (BMPs) and conservation/mitigation planning are: 1) the estimate of what environmental effects (management actions) are limiting sustainable populations and where (geographically) those limitations are more substantial, and 2) the estimate of the resultant benefits of implementing BMPs or conservation/mitigation measures. This information forms the basis for determining net ecosystem services (benefits and costs) associated with different management scenarios. As ecosystem services analysis becomes a common tool to manage ecosystems, the biological component must have comparable scientific rigor to other components such as biophysical, economic and social. Some of the more common criticisms related to the biological component center around the facts that commonly used methods: 1) do not have a quantitative basis for the estimates, 2) are not transparent to allow thorough review and stakeholder input, and 3) are not reproducible to allow use for future improvements, estimates, and

subsequent adjustment.

In this case study, a net ecosystem services analysis (NESA) was applied in support of the re-registration of a well-known insecticide used in Southern European countries. The European Food Safety Authority (EFSA) recently published a framework for developing specific protection goals for the environmental risk assessment of pesticides that includes an ecosystem services approach. Our approach builds on an analytical framework developed over several years and used on other ecosystem service analysis and conservation planning projects. The NESA framework allows for the formal quantification (semi-quantification) of biological ecosystem service values associated with different land cover types (e.g. orchards or scrub vegetation) and the management actions associated with each cover type. The results produce a composite measurement of baseline conditions expressed in a standard metric (currency) allowing ecosystem service levels to be compared. Results are then estimated for conditions after a change in a management action (such as the application of a pesticide and incorporation of conservation measures) and the net ecosystem service gains and losses between scenarios or condition levels are compared, including future values.

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WE 317

Proteom analysis of blue mussel tissue by two dimensional gel electrophoresis and MALDI - MS

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Contaminants are continually entering the aquatic environment and hence the tissue of resident biota such as mussels, which are used worldwide as sentinel bioindicator for chemical stress in the environment. In order to detect early changes on the molecular level and to identify prognostic protein biomarker related to the impact of hazardous substances the methodologies and tools of proteome investigation provide interesting possibilities for environmental research. In particular variations in protein expression could indicate the exposure to and effect of contaminants. In contrast to frequently used assay systems, which allow the detection of selected markers at the protein level, proteomic techniques enable the detection of changes of protein expression profiles, which allow a much better insight into contaminant related effects, simultaneously. In consequence a set of proteins can be identified as indicators for environmental stress. To reduce the number of proteins to be analyzed, tissue specific responses can be detected by dissecting organs involved in different metabolic pathways or different exposure routes. In this study especially gills of the Blue Mussel *Mytilus edulis* (L.) have been analysed by two dimensional gel electrophoresis (2D GE). The process of protein extraction and purification was especially designed for this tissue type. The gill proteome of mussels from differently exposed sites have been evaluated by gel imaging and 2D GE software (Delta 2D). Differentially expressed spots have been selected and analyzed by matrix assisted laser desorption/ionisation - time of flight - mass spectrometry (MALDI-ToF-MS). Hints for proteins involved in the metabolism of the amino acid cysteine, especially enriched in Metallotheionins as indicators for metal exposure, have been found. Furthermore an altered expression of cytoskeletal proteins could be detected. Currently the identification of further potential biomarker as well as the analysis of other tissue types such as the digestive gland of physiologically well defined mussel groups is in progress.

WE 318

Can environmental realistic concentration of ibuprofen alter the protein profile in *Mytilus galloprovincialis*?

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In an ecotoxicological point of view, the proteomic approach has been successfully applied on the early detection of molecular events implicated in potential adverse biological effects. Being a good bioindicator to assess the impact of aquatic contaminants, mussels *Mytilus galloprovincialis* have also been used in a variety of proteomic studies.

Some emergent contaminants, such as pharmaceutical compounds (namely ibuprofen -IBU) have been an object of enormous attention by the scientific community since their presence in the aquatic systems gradually increases and diverse ecotoxicological effects were observed. Although have been detected adverse responses in mussels exposed to IBU, molecular biological aspects related to the expression of protein profile changes are lacking.

IBU is a nonsteroidal anti-inflammatory drug (NSAID) included in the therapeutic class of analgesic/antipyretic medicines. It is highly consumed by the general population and their polarity and stability make it bioavailable for the aquatic organisms.

Protein expression profiles were studied in digestive gland fractions from unexposed and IBU (250 ng/L) exposed mussels using two-dimension gel electrophoresis after a short-term exposure (7 days). Proteomes obtained were analysed using the PDQuest[TRADEMARK] software.

IBU induced significant alterations in the digestive gland protein profile of exposed mussels. Hence, IBU condition induced the expression of 143 new proteins and suppressed 48 (unique in unexposed digestive gland). 45 proteins were two-fold over expressed after IBU exposure, whereas 27 proteins were down-regulated (2-fold). The development of this research will allow the identification of novel protein biomarkers related to NSAIDs contamination (particularly, IBU) in *M. galloprovincialis* which may be more sensitive or precise than the presently available molecular biomarkers array.

WE 319

A first ecotoxicological study on the oxidative post-translational modifications in *D. polymorpha* proteome after exposure to Benzoylcegonine

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Drugs of abuse and their human metabolites have been recently recognized as emerging environmental contaminants since these compounds have become pseudo-persistence in aquatic environment due to their huge production and widespread use. Notwithstanding these kinds of contaminants have some features in common with pharmaceuticals, and these characteristics make them potentially dangerous not only for humans (through a direct usage) but also for aquatic ecosystems, their ecotoxicology has not been investigated yet. One of the most abundant drugs founded in rivers and wastewaters is Benzoylcegonine (BE), the main metabolite of Cocaine (CO).

In the present study, we applied a Redox proteomics approach to evaluate the changes in the proteome of *Dreissena polymorpha* gills as effect of oxidative stress on protein thiols and aldehyde/ketone groups after an *in vivo* exposure (14 days) to BE. Moreover, we choose to test two different concentrations of BE (0.5 and 1 µg L⁻¹) in order to evaluate possible dose-dependent changes in the proteome. One dimensional electrophoresis didn't reveal a reduction in protein thiols content, while underlined a significant increase of protein carbonylation at both doses tested. We then performed protein profiling using two-dimensional gel electrophoresis with subsequent Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) and TOF/TOF with LIFT technique and LTQ-Orbitrap (Linear ion trap combined with Orbitrap mass spectrometer) yielding *De novo* protein sequences suitable for database searching. These preliminary results and the identification of affected proteins suggest the presence of oxidative stress caused by BE, whose mechanism of action is discussed.

WE 320

Long term exposure of Atlantic halibut to an acid environment at different temperatures

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The oceans are becoming acid as a result of a rapid increase in atmospheric carbon dioxide (CO₂) concentrations and its subsequent dissolution into the seas. Increasing CO₂ concentrations in the atmosphere are also leading to a significant increase in surface temperatures. Consequently, marine organisms will be exposed to these two stressors simultaneously. Such anthropogenic effects have been considerably studied in calcifying organisms but effects on marine fish are almost entirely unknown, especially long term exposures. The present study has used a proteomic approach to analyze samples of gills and blood plasma of Atlantic halibut (*Hippoglossus hippoglossus*) exposed to pH of 8.1 (present situation) and 7.7 (predicted by 2100) at temperatures of 12°C and 18°C. This study focus on the effects of low pH combined or not with higher temperature, and the fish exposure lasted for three months. The proteome was separated by two-dimensional (2D) gel electrophoresis, and as the DNA of Atlantic halibut is not fully sequenced the peptides identities were obtained via electrospray ionization-tandem mass spectrometry (ESI-MS/MS). Preliminary results show several proteins differentially expressed when compared to controls. Results on gill samples from both temperature exposures indicate upregulation of peptides involved in metabolic pathways, changes in calcium related peptides and changes on the expression of proteins related to immunological responses. Blood plasma samples from both temperature exposures presented high expression of a peptide linked to innate immune response, among other changes. Our results indicate that an acidic environment combined or not with high temperature, induced significant biological changes in halibut proteome, especially regarding immunological response and metabolic status. This is possibly the first study showing effects of acidification in marine fish.

WE 321

Searching for biomarkers: a literature review about proteins found in fish proteome experiments- The Top 20 uncovered

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The increase in ecotoxicological publications in the area of proteomics generates huge amount of informations. These include the many different proteins found in miscellaneous approaches and proteomics experiments. As the notion increased that very often similar proteins are identified (Petrak et al. 2008) a literature review was done to find the most often identified proteins in fish experiments. The two internet databases PubMed® and Web of Knowledge® were searched with identical keywords. While both databases found about the same number of publications (140) the overlap was only 2/3 e.g. 80 publications were duplicates, while the rest was only found in either one or the other database. From these publications only the ones were selected for further analysis which had a certain test design and experimental approach and used adult fish or other life stages of fish. About 20 publications were identified. From these publications only the proteins were selected for further analysis which were found in at least 10 % of all 20 identified publications and which were shown to be of significantly different abundance than the respective controls. From more than 400 differentially expressed proteins about 17 protein types or groups could be identified. These were for example Heat Shock Proteins, muscular proteins, Enolase, Keratin, Glutathione S-transferase, NKEF, Transferrin and Cytochrome P450. The list of proteins could be of help to differentiate between the usual suspected high abundant proteins and proteins pointing to more specific pathways.

WE 322

Hepatic transcriptomic profiling identifies concentration-dependent mechanisms of depleted uranium toxicity in Atlantic salmon (*Salmo salar*)

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Uranium (U) is a naturally occurring heavy metal widely used in many military and civil applications. Uranium contamination and the associated potential adverse effects of U on the aquatic environment have been debated during recent years. Depleted uranium (DU), a by-product of U enrichment with reduced specific activity and identical chemical properties, has become a good model to study the chemical toxicity of natural U. In order to understand the effect and toxicological mechanisms of DU in vivo, juvenile Atlantic salmon (*Salmo salar*) were exposed to 0.25 mg/L, 0.5 mg/L and 1.0 mg/L waterborne DU in a static system for 48 hours. The U concentrations in the gill and liver were analyzed and the resulting biological effects were determined by a combination of analysis of gene expression and micronuclei formation. Twelve biomarker genes representing oxidative stress (GCS, GR and GPx), DNA damage and repair (P53, P21, Gadd45, PCNA and Rad51), apoptosis (BAX, Bcl-x, Caspase 6) and protein degradation (ubiquitin) were first evaluated by quantitative real-time polymerase chain reaction (q-rtPCR). Then a hepatic transcriptomic profiling was performed using a custom 60k salmonid oligonucleotide microarray. The results clearly showed accumulation of DU in the gill and liver with increasing concentrations of U in the exposure water. The effects of DU on hepatic biomarker gene expression occurred in a concentration-dependent manner, although deviations from ideal concentration-response relationships were observed at the highest U concentration (1.0 mg/L). All biomarker genes tested were found to be up-regulated by DU. Microarray analysis clearly showed that the number of differentially expressed genes as well as the biological processes significantly regulated by U also appeared to be concentration-dependent. Micronuclei assay on blood cells showed an apparent, albeit non-significant increase of micronuclei formation at 0.25 mg/L U. The results suggest that DU may have concentration-dependent mechanisms of toxicity in Atlantic salmon, giving rise to the regulation of different biological processes as well as the potential induction of several toxicological pathways, which commonly can be established within the framework of an early stress-response regulatory network. However, the early responses at the transcription level were not found to lead to immediate detrimental effects (e.g. micronuclei formation), potentially due to the short exposure duration in this study.

WE 323

Toxicogenomics - a tool for the assessment of sediment quality?

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The analysis of gene expression in the context of ecotoxicology is of growing interest, because in principle it allows the simultaneous analysis of exposure and effect and can thus help to answer the question if a contamination might be a pollution or not. This question has high relevance for the risk assessment of contaminated river sediments. The sediment is an ecosystem (benthos) with organisms reacting to particle bound pollution. Sediments can act as both, potential sinks but under other conditions as potential sources for many hazardous substances. Therefore, several methods for sediment contact tests with various species were developed in the recent years. In the present study sediment contact tests with rice (*Oryza sativa*) were performed. *Oryza sativa* can be grown under emerse conditions and has - as a prerequisite for a comprehensive expression analysis - a sequenced genome. In order to initially characterize macroscopic effects of Ni on rice development (*Oryza sativa*), sprouted rice grains were exposed on OECD sediment 218 spiked with NiCl₂ at various concentrations. The most sensitive end point for exposure to Ni was the root length; in addition to growth inhibition a different morphology of the root was observable. Roots of exposed plants were less branched and thicker compared to the unexposed control. For the analysis of gene expression the NOEC (50 mg Ni²⁺/kg, n=8) and LOEC (150 mg Ni²⁺/kg, n=7) levels for nickel were chosen for exposure in addition to the unspiked control (n=11). After isolation of the total RNA from the roots the Array hybridization was done after single colour labelling using 4x44K DNA Arrays for *Oryza sativa*. For the identification of potential biomarkers of exposure the control samples were compared with the Ni-exposed samples. For the identification of biomarkers of effect the control- and NOEC-samples were compared with the expression at LOEC-levels. With this approach genes were identified which a) respond to the presence of Ni in the sediment, irrespective of an observable ecotoxicological effect and b) can be correlated to an adverse effect. In fact, several genes were identified the function of which is linked to the growth of the root and morphology. The presented work will be continued in a research project in order to challenge this approach with further model compounds, mixtures and natural sediments.

WE 324

Natural toxins and their molecular and life-history effects on non-target soil invertebrates

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Soil invertebrates that belong to the detrital food web are essential for proper soil ecosystem functioning, as they control carbon and nutrient flows and stimulate plant nutrient uptake. Soil ecosystem functioning is challenged by many anthropogenic toxins which can disrupt the soil ecosystem through mortality or reduced reproduction of soil organisms. Natural toxins, on the other hand, are rarely considered a threat to the environment yet can be lethal at low dosages. Natural toxins are organic compounds that are produced as secondary metabolites in fungi, bacteria, algae, plants or animals.

Glucosinolates (GSL) are natural toxins produced as secondary metabolites by many commercial crops (e.g. Broccoli and Cabbage). Tissue damage hydrolyses GSL into several toxic compounds such as isothiocyanate (ITC) and nitriles, which is mediated by the enzyme myrosinase. Current interests in ITCs focus on the toxic characteristics that have, for instance, been exploited for alternative pest management methods, so-called biofumigation, but also the possible chemopreventive nature of ITCs. Due to these social-economic benefits, novel crop varieties with enhanced levels are likely to be introduced in the future. This may introduce a potential risk for soil ecosystem processes by exerting detrimental effects on beneficial non-target soil organisms.

This study focused on the effects of ITC on the detritus food web, using *Folsomia candida* and *Eisenia andrei* as model species for beneficial soil invertebrates that maintain essential soil functions. Data from standardized ecotoxicological tests was combined with gene expression profiles obtained with microarrays to have more insight into the molecular mechanism of the toxic responses.

Results will focus on:

- 1) Toxic effects on survival and reproduction of allyl ITC *Folsomia candida* and *Eisenia andrei* using ecotoxicological experiments.
- 2) Gene expression analysis that reflect negative effects of allyl ITC on soil invertebrates, using microarrays.
- 3) Comparative genomic analysis to identify commonalities in stress response pathways between *Eisenia* and *Folsomia* when exposed to the same stress factor.

WE 325

Establishment and application of Chinese Rare Minnow (*Gobiocypris Rarus*) endocrine system functional genome cDNA microarrays

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Functional Genomics will be a powerful tool to exhibit the function of endocrine system in fish. Unfortunately, there are relatively few functional genes in GenBank, which limits the use of molecular tools to study disruption of endocrine responses in fish. In this research, we will select Chinese rare minnow as a model fish and develop functional genes database of hypothalamic-pituitary-gonad (HPG), hypothalamic-pituitary-thyroid (HPT), and hypothalamic-pituitary-adrenal (HPA) axis in fish using gene clone and RT-PCR methods. Molecular targets of three endocrine axis will be confirmed after exposure to a certain endocrine disruptor, and molecular toxicological mechanism of endocrine disruptor will be elucidated. Endocrine system functional gene cDNA microarrays of Chinese rare minnow were established. The feasibility of cDNA microarray was proved by real-time PCR methods after short-term exposure to 100 µg/L E2. The changes of gene mRNA expressions in cDNA microarrays were basically in accordance with real-time PCR methods. Normal development of larvae may be affected by butachlor. The results of lesion livers and gonads indicated that butachlor may pose threat to normal growth and reproduction in fish. In addition, the alterations of mRNA levels displayed the estrogenic and antithyroid activities. These results could be useful as a basis for toxicogenomics of endocrine disruption in rare minnow.

WE 326

Toxicogenomic analysis of benzene, toluene and formaldehyde on *Drosophila melanogaster* and *Caenorhabditis elegans*

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Indoor air quality has been recognized as a significant health and environment issue. Some air pollutants are reported to occur more frequently and at a higher concentration in indoor air than in outdoor air, including volatile organic compounds (VOCs). In this study, toxicogenomic analysis was conducted on the most representative indoor air pollutants, such as, benzene, toluene and formaldehyde, on non-mammalian model species, *Drosophila melanogaster* and *Caenorhabditis elegans*. Microarray data indicated the numbers of genes differentially expressed by benzene, toluene and formaldehyde were 53, 37 and 130, respectively in *D.melanogaster*, whereas, 228, 50 and 1100 in *C.elegans*. Pathway analyses conducted on the differentially expressed genes revealed inflammation related pathways are involved in VOC toxicity. Functional genomic tools (gene knock out and RNAi) are used to assess toxicological meaning of up- or down-regulated gene expression due to VOC exposure. This can provide indicators of the toxic mode of action at the single gene level up to that of an entire organism at relatively low cost. Moreover, the responses of *D. melanogaster* and *C. elegans* to VOCs exposure were screened across various mutant strains and sensitive mutant strains were selected to be developed as biomonitoring tools for indoor air pollutants.

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WE 327

Effects of nickel and dimethoate on genetic markers of the terrestrial isopod: porcellionides pruinosus

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Molecular genetics provides an opportunity to evaluate the mechanistic basis underlying modes of action of specific compounds. Integrating these data with life history impacts and functional parameters, such as energy metabolism, permits a more holistic systems approach to predictive ecotoxicology.

In order to study these detoxification processes, terrestrial isopods (*Porcellionides pruinosus*) where exposed to nickel at 50 mg/kg soil (maximum limit concentration for

agriculture soils, by the Canadian Environmental Quality Guidelines) and 250 mg/kg soil (5x the previous concentration) and to the pesticide dimethoate at 0.4 mg/kg soil (dose advised for field application) and 10 mg/kg soil (concentration below the EC50 value for isopod species). Organisms were exposed for a 28 day period, followed by a 14 day post exposure period upon transference to clean soil. Samples were taken at the start of the exposure (T0), after 96 hrs and 14, 28, 14 days post-exposure. To evaluate toxicity and detoxification processes, changes on gene expression (e.g. HSP70, metallothioneins, lipid peroxidase, glutathione peroxidase and DNA damage) were studied using QPCR.

The results obtained in this work sheds light on the detoxification process in this ecologically relevant detritivore and toxicity induced by the two classes of chemicals (a metal and a organic pesticide). These data are related to previous works on biomarkers including evaluation of energy reserves.

WE 328

Implementing a functional genomic approach to decipher the mechanisms behind glyphosate hormesis in *Zea mays*

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The toxicological phenomenon termed hormesis is described as a biphasic dose-response, characterized by low-dose stimulation & high-dose inhibition. Although hormesis has often been observed for the effect of the herbicide glyphosate sprayed on plants at doses of 10-40 g a.i. ha⁻¹, the physiological mechanism(s) behind this phenomenon are currently poorly understood. Functional genomic studies and microarrays in particular are very helpful in identifying and characterizing groups or families of genes that are transcriptionally regulated, in response to a physiological stimulus. The availability of genomic information for *Z. mays*, as well as the existence of a genetically modified glyphosate target-site resistant mutant (GMO), makes this species ideal for such a study. In this study we present microarray analysis of mRNA isolated from GMO and wild type (WT) *Z. mays* treated with 27 g a.i. glyphosate ha⁻¹ compared to plants sprayed with surfactants only. Plant material for mRNA analysis was harvested 24 h after spraying. Plant dry weight 10 days after spraying was ($\pm$ standard deviation) 0.17 g $\pm$ 0.02, 0.19 g $\pm$ 0.02, 0.17 g $\pm$ 0.02 and 0.24 $\pm$ 0.02 for glyphosate treated and un-treated GMO and WT plants. The fact that the glyphosate resistant *Z. mays* did not respond to the glyphosate treatment as the WT plants did, shows that the hormetic response is directly related to glyphosate binding to its target enzyme. Microarray analysis of both surfactant (untreated) and glyphosate treated plants showed qualitative differences in gene regulation between target site resistant plants (GMO) and susceptible (WT) plants. Annotation of these genes is currently underway using publicly available *Z. mays* genomic information, and will provide clues to the cellular mechanisms involved in the observed increase of dry weight in WT plants following treatment with 27 g a.i. ha⁻¹ glyphosate.

WE 329

Reference gene selection for Real-Time qPCR in *Bidens laevis* L. during pesticide and cold stress

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Environmental monitoring demands the usage of new species and sensitive methods in order to get accurate regional biomonitoring. The aquatic macrophyte *Bidens laevis* L. (fam.: Asteraceae) has desirable growing characteristics for use in laboratory assays, has proven to respond positively to genotoxic compounds and showed enzymatic response when exposed to the pesticide endosulfan. Nowadays, gene expression offers crucial information when analysing responses to xenobiotic compounds. It is well known that Real Time PCR (RT-qPCR) is the preferred method for studying gene expression because of its sensitivity, precision and robustness but it requires the use of reference genes with stable expression among different treatments, as well as different tissues and growth stages.

The aim of this study was to analyze the expression of two candidate genes, Elongation Factor 1 alfa (EF1a) and Actin (ACT) in *B. laevis* in three tissues (root, stem and leaves) under four conditions: 1-control: Large plants (>1500mg), temperature 22°C, media: Hoagland solution; 2 -short-: Short plants (<250mg), temperature 22°C, media: Hoagland solution; 3 -xenobiotic-: Large plants, temperature 22°C, media: Hoagland solution + endosulfan 10ug/L; 4 -cold- Large plants, temperature 5°C, media: Hoagland solution. All plants remained 24h under each condition with 3 replicates tested for each one. Firstly, the partial sequences for both genes were obtained from degenerate PCR products. The two sequences showed an identity of 82% (EF1a) and 81% (ACT) compared to other members of Asteraceae family. From these sequences a set of primers for each gene was designed and the RT-qPCR was optimized.

The results showed that EF1a had no significant variation in expression among the three different tissues. This expression remained stable between control and short plants. However, a significant expression increase was observed in plants exposed to endosulfan or cold. In contrast, ACT expression did not show significant differences among tissues or under any condition tested in this study.

From these results we can conclude that Actin expression did not seem to be influenced in short plants or during endosulfan or cold exposure and can thus be used as a reference gene for RT-qPCR in any tissue, while Elongation Factor-1 alfa should not be used when analysing stress conditions such as cold and xenobiotic exposure.

WE 330

Defining the transcriptome of the fathead minnow liver using high-throughput sequencing: effects of chemical and environmental stressors

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The fathead minnow (*Pimphales promelas*) is a popular small fish model routinely used in regulatory testing and basic research in environmental toxicology. More information about chemical toxicity has been derived from studies with the fathead minnow than any other species of fish. In the past decade, following the advent of high-throughput 'omics' technologies, the fathead minnow has been used as a model species for proteomics, metabolomics and transcriptomics. The vast majority of the transcriptome profiling studies have utilized one of several available cDNA microarray platforms. However, to date, no studies had been performed by use of recently developed open-format high-throughput transcriptome sequencing technologies such as Illumina RNAseq. As a consequence, large-scale characterization of the transcriptome of the fathead minnow liver had not been performed. We are actively characterizing the transcriptome of the liver of the fathead minnow in order to quantify transcriptional responses to various natural and anthropogenic environmental stressors. In one series of studies we have been exploring the transcriptional responses on fathead minnows acclimated to low temperatures. In another series of studies we have been characterizing transcriptional responses in fathead minnows exposed to oil sands process affected water (OSPW) generated during the extraction of bitumen from the oil sands deposits of Alberta, Canada. We have collected approximately 575 million 100bp paired-end reads from livers of fathead minnows exposed to these environmental stressors. These reads have been assembled into greater than 50,000 contigs greater than 100bp in length that have been annotated using Blast2GO software. The transcriptome was further annotated using Kyoto Encyclopedia of Genes and Genomes (KEGG) mapping. Analysis of transcript abundance has identified a suite of genes that are differentially expressed in response to acclimation to low temperature and exposure to OSPW.

WE 331

Transcriptomic responses in Japanese medaka, *Oryzias latipes*, exposed to mixtures of sex steroids

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The advent of OMICS technologies allows us to dramatically expand the scope and depth of perspective on toxicity. The OMICS studies may also help toxicologists to understand the mechanism of physiological impairments caused by long-term exposures to various chemicals. In this study, we investigated long-term effects of sex steroids using transcriptomic analyses. Larval Japanese medaka, *Oryzias latipes*, within 12h post-hatch, were exposed to sex steroids, 17 β -estradiol (E2) and 17 β -trenbolone (TB), and mixtures of E2 and TB. Chronic effects were evaluated on medaka exposed to E2, TB and mixtures for 60 days posthatch. Sex reversal occurred by the single exposures of E2 or TB at all concentrations used in this study. In contrast, at low concentration of mixture, estrogenic and androgenic effects appeared to cancel each other, resulting in no apparent reproductive impairment. At high concentration of mixture, reproductive activities were severely damaged for both sexes, although the sex reversal rate was much lower than that of single exposure. Gene profiling of medaka was performed using oligonucleotide-based DNA microarray containing 36,398 probes (Agilent Technologies). The gene expression profiles obtained were in line with biological experiments and histological analyses. The gene expression profile of the mixture at low concentration was similar to that of control compared with other exposure groups, in agreement with the chronic effects. Various genes induced by either E2 or TB were neither up- nor down-regulated by the mixture exposure, implying that these genes may be candidates for the genes involved in sex reversal. Interestingly, the expression levels of vitellogenins (VTGs) and choriogenins (CHGs) in the mixture-exposed samples were similar to those in the E2-exposed group, suggesting that the expressions of VTGs and CHGs are controlled simply by E2. We also tried to estimate degrees of endocrine disrupting effects the gene expression data. Results suggest that physiological outcomes caused by sex steroids, even mixtures, could be estimated using the transcriptomic analyses.

WE 332

Use of Javanese Medaka (*Oryzias javanicus*) cDNA microarrays in endocrine disrupting chemical risk assessment

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Endocrine disrupting activity of environmental chemicals has been one of the major public concerns since it could cause reduction in reproductive success and affect human and wildlife populations. Up to this date, only biomarkers used to detect chemicals' potential endocrine disruption (estrogenicity) in experimental fish species are vitellogenin (a female-specific glucolipoprotein yolk precursor) and choriogenin (a precursor of egg envelope protein), and this fact motivated us to look for other useful biomarkers to assess endocrine disrupting effects of the environmental chemicals. Furthermore, several reports have shown that fish exposed to environmentally relevant concentrations (up to 100 ng/L) of 17 β -estradiol (E2) for the period prior to sex differentiation and the time of sex differentiation caused disruptions in the process of sexual differentiation (oocyte development in genotypic male, significant change of the sex ratio towards the female sex, etc.), and alteration in the egg production pattern in the subsequent adults. This clearly indicated that early life stages of fish development were sensitive to low concentrations of EDC, and such exposure is now known to lead to distinctive pathological endpoints. Therefore, we were interested in the endocrine disruption studies using both adult and embryo of medaka that were exposed by EDCs. Naturally, development of a bioassay tool was important in order to reduce time, cost, and labor of single biomarker- and pathological observation-dependent test methods, thus, we developed adult Javanese medaka cDNA microarrays which contain 2,500 cDNA probes. In this presentation, experiments using these microarrays and EDC-exposed medaka will be introduced in details, and their analyzed results will be discussed as well.

WE 333

Molecular characterization and expression profile of hemoglobin cDNA from *Chironomus riparius* (Diptera: Chironomidae)

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The hemoglobin (Hb) of invertebrates is responsible for essentially the same function as those of vertebrates: carrying oxygen from the environment to the respiring tissues. One of the particularity of aquatic midge, *Chironomus* is its possessing Hb during its larval stage. *Chironomus* Hbs exist throughout the four larval stage with stage-specific and tissue-specific single-chain globin syntheses, which play important role in survival in extreme environment, such as hypoxia response or pollution tolerance. In this study, Hb cDNA of *C. riparius* (CrHb) were identified by screening through an expressed sequence tags data base developed from the fourth instar larvae of *Chironomus riparius* using 454 pyrosequencing. Here we report identification and characterization of hemoglobin genes. CrHb1 (tentative gene name) has the full-length cDNA of 777 bp long and contains an open reading frame (ORF) of 504 bp. The deduced CrHb protein has 169 amino acids. Expression pattern of CrHbs were studied using real-time polymerase chain reaction after short term exposure to environmental pollutants and the results suggest that the expression profile of CrHb may potentially contribute to the development of a biomarker for ecotoxicity monitoring in *C. riparius*.

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WE 334

Stress response gene expression in *Chironomus riparius* exposed to various environmental pollutants

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Aquatic organisms are exposed to various kinds of environmental pollutants having different modes of action released from various anthropogenic sources which can disrupt many biological functions. Recently next generation sequencing (NGS) technology has been increasingly used in wildlife species gene characterization in an attempt to identify them as stress response genes, which has high potentials of being used as biomarkers in ecotoxicological studies. SOD is the prime antioxidant enzyme and has been widely used as oxidative stress biomarker in ecotoxicology, whereas, p38MAPK has rarely been approached in an ecotoxicological context, although, the roles of p38MAPK in stress responses have frequently been reported in various biological systems. In this study, we identified and characterized SOD and p38 MAPK in *C. riparius*, by screening through an expressed sequence tags (EST) database developed from the fourth instar larvae of *Chironomus riparius* using 454 pyrosequencing. We also investigated the response of previously characterized stress response genes toward various environmental pollutants and their responses will be compared with higher level effect such as development and reproduction. This comprehensive approach will provides an insight into the stress response mechanism of important sediment toxicity model species, *C. riparius* and also offers information on the relationship between stress response gene expression and higher level effect.

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WE 335

Risk assessment of wood smoke particles using a test battery

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Biomass burning is a major source of air pollution and is a reason for enhanced hospital admission and mortality. Data on the MOA exhibited by wood smoke particles are insufficient. At present we cannot provide an adequate risk assessment for wood smoke. For this we intend to implement a method. *C. elegans* appears as an optimal surrogate to achieve a profound risk assessment for humans and the environment. It is a popular model organism because it is well established in ecotoxicology and molecular studies.

Gene expression analysis of selected biomarkers which are involved in supposed mechanisms are used to give insight into the mode of action exhibited by wood smoke particles. Results won by assays with *C. elegans* are complemented by microbial biotests that are conducted by a colleague.

Wood smoke fine dust won by an electrostatic precipitator from different furnaces was used to establish a nematode test adapted to the use with wood smoke particles in suspension. Gene expression analysis was conducted via realtime PCR of 4 previously selected genes that are involved in xenobiotic detoxification, inflammation and oxidative stress.

Contrary to expectations sample B from complete combustion i. e. with low PAH content exhibited higher toxicity than sample A from incomplete combustion and high PAH content. We suspect low bioavailability of PAH from sample A and high availability of zinc from sample B to cause the toxicity levels. The results from gene expression analysis shown in 2 support the argumentation drawn from the nematode test. Cyp35C1 reflecting the bioavailability of PAH is strongly expressed in sample A. Expression differences of Cyp35C1 in sample A and B do not reflect PAH contents. We can assume low bioavailability of PAH from sample A. Enhanced expression of superoxide dismutase (Sod3) in sample B supposes oxidative stress probably caused by zinc, which is supported by the standard nematode test and a battery of microbial biotests.

The adaption of the nematode test to wood smoke particles was successfully implemented. The results suppose that chemical analysis and common predictions do not adequately project fine dust toxicity. Further studies on the molecular mechanisms of fine dust toxicity are going on.

WE 336

Effects of munitions compounds and breakdown products on Peroxisomal Proliferator activated receptors Alpha, Beta/Delta, and Gamma

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Exposure to munitions (e.g., nitroaromatics) compounds has been shown to alter energy stores and biomolecules in wildlife, some of which are due to chemical-mediated alteration of PPAR-regulated genes. The objective of this project was to determine the role of munitions compounds on gene activation via PPAR and its heterodimer RXRC. PPAR activation was measured according to the cell-based nuclear receptor activation kits (Indigo Biosciences, State College, PA). Briefly, 0.01-10 mg/l munitions compounds (2,4,6-trinitrotoluene [TNT], 2,4-dinitrotoluene [2,4-DNT], 2,6-dinitrotoluene [2,6-DNT], hexahydro-1,3,5-trinitro-1,3,5-triazine [RDX], 2,4,6,8,10,12-hexanitro-2,4,6,8,10,12-hexazaisowurtzitane [CL-20], 1,3,5-trinitrobenzene [1,3,5-TNB], 2,4-dinitroanisole [DNAN], and 5-nitro-1,2,4-triazol-3-one [NTO]) and TNT breakdown products (2,4-amino-6-nitrotoluene [2,4-DANT], 2-amino-4,6-dinitrotoluene [2-A-4,6-DNT], 4-amino-2,6-dinitrotoluene [4-A-2,6-DNT]) were added to wells containing transfected cells containing high constitutive levels of PPAR isoforms (α , γ , δ) or RXR α . Cells were also exposed to positive controls for the respective nuclear receptors (GW590735 for PPAR α , rosiglitazone for PPAR γ , GW0742 for PPAR δ , and 9-cis retinoic acid for RXR α). After 24 h exposure, levels of nuclear receptor activation were measured by luciferase assays. Cytotoxicity was measured by the Live Cell Multiplex assay (Calcein-AM). PPAR α activation was suppressed by TNT and other nitroaromatic compounds (0.33-0.41-fold of control at 10 mg/l) and DNAN (0.73). PPAR γ activation was increased by TNT (6.7-fold of control at 10 mg/l), but suppressed by 4-A-2,6-DNT (0.41). PPAR δ activation was suppressed by 12 of the 17 energetics studied (0.25-0.7). RXR α activation was increased by NTO (1.45), but suppressed by 4-A-2,6-DNT (0.54). Cell survival was only markedly decreased by 1,3,5-TNB. These data demonstrate the ability of primarily legacy munitions to moderate nuclear receptors that regulate numerous genes involved in lipid and glucose homeostasis. This in vitro systems toxicology approach may provide insight into molecular initiation events that can be linked to adverse outcomes at increasing levels of biological organization.

WE 337

Molecular and behavioral endpoints in zebrafish early life stage tests: increased sensitivity and link to delayed effects

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Fish early life stage tests are based mainly on morphological changes and mortality, endpoints that might lack specificity and sensitivity to evaluate modes of action and environmentally relevant concentrations. In this work we investigate if additional parameters improve the sensitivity and prediction potential of tests. Gene expression and behavior were combined to morphological endpoints to characterize toxic effects of PCB126, model dioxin-like compound. Endpoints were evaluated in two tests: (a) ELS, early life stage test until 5 days post fertilization (dpf); and (b) pELS, prolonged ELS test. In both, fish were exposed from 3h until 5dpf to 3, 30, 100, 300 and 3000ng/L PCB126 plus controls. In the ELS, by end of exposure swimming behavior was recorded on video, then fish were euthanized and preserved in liquid nitrogen for gene expression. In the pELS, after exposure fish were transferred to clean water, raised according to standard procedures, and observed until 14dpf, when behavior record, euthanasia and preservation were done as in the ELS. Morphological endpoints were based on previous publications. Behavioral endpoints consisted of behavior observation and video tracking analysis (EthoVision®). Gene expression was performed on whole fish RNA with RT-PCR using Sybr Green. Target genes were selected based on mode of action (cyp1a) and observed toxic effects (ptgs2a: edema; shha: swim-bladder; sox9b and col11a2: cartilaginous tissue; hspa8: heat shock protein; plus genes for cardiotoxicity and pigmentation), with bcl2l1 as reference. Until 3dpf no significant difference between exposed and control could be detected morphologically. At 5dpf, dose-dependent decrease in swim-bladder pigmentation and inflation occurred in concentrations above 30ng/L. Behavior at 5dpf reveal that reduced swimming and equilibrium loss occurred above 300ng/L. Gene expression of cyp1a was higher than controls in concentrations above 100ng/L, while expression of shha and sox9b was reduced above 100ng/L compared to controls. In the pELS test, delayed mortality was observed in concentrations as low as 30 ng/L. Observed delayed mortality agrees with pELS results in other species. The behavioral and molecular endpoints contributed to increase the sensitivity of the ELS and to predict delayed effects in the pELS. Further research is needed for the interpretation of multiple endpoints. Expression of additional genes and the video analysis are currently under evaluation.

WE 338

Mechanistic profiling of flame retardants in general systemic stress and endocrine disruption within the INFLAME project

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Indoor Contamination with Flame Retardant Chemicals: Causes and Impacts (INFLAME), is a Marie Curie Initial Training Network research project funded by the EU. Its goal is to study the leaching of flame retardants (FRs) from everyday consumer goods and construction materials and their ability to enter and further influence the human body. The research of INFLAME is not solely limited to a chemically related group of FRs but targets a broad range of compounds, such as brominated, chlorinated and organophosphate FRs, including both widely used chemicals as well as newly emerging compounds.

In this presentation, we outline the concept of an INFLAME subproject dedicated to the assessment of the FRs' potential to cause general systemic stress and to act as endocrine disruptors. Mechanistic toxicity profiles of pure compounds, as well as indoor dust samples, are evaluated with multiple *in vitro* assays. A dedicated set of bacterial and human reporter gene assays are used to examine the general stress potential. These assays indicate a variety of different stresses such as specific DNA-, protein- and membrane damage, as well as osmotic and oxidative stress. In order to assess the endocrine disrupting potential of FRs, we selected the MCF-7 E-screen and the H295R

steroidogenesis assay. To further understand the molecular mode of action leading to the observed effects, orbitrap-based proteomics data is gathered and combined with transcriptomics and metabolomics data acquired by other INFLAME partners. In this way, a systems biology approach is achieved. The selection of the pure compounds and sample extracts to be studied is based on chemical analysis of indoor dust samples and consumer goods. Possible mixture effects can be determined and samples, where biological effects can not be explained by the amount of bioactive FRs present, can be subjected to further analysis. We will attempt to isolate and identify any unknown bioactive substance from these samples by combining LC fractionation and mass spectrometry with the bioassays. With this approach, more information on the specific mode of action of FRs can be obtained, that could serve as the base of future risk assessments.

ET15P - Pesticide fate and ecotoxicology

WE 341

Effects of the organophosphates, azinphos and chlorpyrifos, on juvenile fish (*Oreochromis mossambicus*)

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Pesticides sprayed in orchards and vineyards often cause pollution of surrounding areas due to run off and spray drift and can thus reach freshwater bodies in the vicinity. The concentrations of organophosphates in the water vary with the intensity and frequency of the spraying procedures being followed. The aim of the present study was to determine whether juvenile fish occurring in such water bodies were negatively affected. Juveniles were used as little is known of their susceptibility to organophosphate pesticides and it was presumed that juveniles would be more susceptible to damaging effects of toxicants than adults. Specimens of the fish species, *Oreochromis mossambicus*, endemic to the waters of the study area in the Western Cape, South Africa were exposed in a laboratory experiment to field relevant concentrations of Azinphos-methyl and Chlorpyrifos. Acute tests were done and specimens were also subjected to an intermittent exposure regime, corresponding with the programs followed by deciduous fruit farmers in the area. These programs consist of bi-weekly periods between consecutive spraying events, which are repeated up to four times during the season. LC_{50} values were determined and compared to values from the literature for adult fish. Exposure concentrations of 5 and 25% of the LC_{50} were calculated and used as exposure concentrations. The endpoints used were the whole body characteristics, mortality and growth (length and weight gain), and the biomarker, acetylcholinesterase activity. In addition, feeding response time and food consumption were measured. The results showed that Azinphos-methyl was more hazardous to the fish than Chlorpyrifos if applied on a bi-weekly basis. For the majority of endpoints investigated exposure interval played a more important role in inducing effects than exposure concentration. With shorter exposure intervals the majority of endpoints showed no difference between a higher and lower exposure concentration, while with longer exposure intervals the effects of exposure concentration became more prominent. The results of the LC_{50} tests indicated that juvenile *O. mossambicus* are more sensitive to the pesticides than adults and that different pesticide application regimes have different effects. This information could be used in the compilation of spraying programs that are used in the agricultural industry to sustain fish populations in surface waters.

WE 342

Carbaryl and dimethoate induced alterations in the antioxidant defense system of the aquatic snail *Helisoma duryi*.

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We studied the effects of carbaryl and dimethoate, individually or as a mixture, on the antioxidant defense system of *H. duryi*. Adult snails were exposed to 25 ppb of carbaryl or/and dimethoate for 72 hours. Post mitochondrial supernatants were used to determine the activity of antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), DT-Diaphorase (DTD), glutathione perox- idase (GPX) and glutathione S-transferase (GST). Thiobarbituric acid reactive substance (TBARS) levels were measured in tissue samples. The TBARS and SOD, CAT, GPX and GST activity were elevated (up to 100%) after exposure to individual pesticides as well as the mixture. The effects were observed after exposure to individual pesticides but were more pronounced after exposure to the mixture. Thus, carbaryl and dimethoate exposure induces an 'oxidative stress' in *H. duryi*. Altered antioxidant enzyme activity supports the use of *H. duryi* for the development of a potential biomarker for detecting the presence of carbaryl and dimethoate in contaminated water samples. The observed 'oxidative stress' suggest a potentially harmful environmental impact of these pesticides on aquatic macroinvertebrates.

WE 343

Does measuring cell number inhibition improve an existing high throughput algal bioassay?

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Herbicides applied in agriculture or weed control are regularly detected in samples of surface waters. Bioassays with algae can be used to assess the effects of these herbicides to non-target aquatic organisms. For routine monitoring, such bioassays should be able to screen large amounts of low volume samples and give reproducible and accurate results within a short time period.

For this purpose Escher and Rutishauser (2008) developed a 24h-biotest using 96-well-plates for simultaneously assessing the photosynthesis inhibition and effects on growth in green algae measured as optical density (OD). For herbicides inhibiting the cell division of algae, such as chloroacetanilide herbicides, this test might have a blind spot since OD mainly detects effects on cell volume growth and is less sensitive for effects on cell division. To analyze the quantitative importance of this blind spot the above mentioned biotest was expanded to the additional endpoint cell number after 48h. Two substances were selected as model compounds for this study: the phenylurea herbicide diuron, which is a known photosynthesis inhibitor and is already used as a model substance in the 24-h biotest, and the chloroacetanilide herbicide metazachlor.

Comparing the EC50 values of the different endpoints photosynthesis-, growth- and cell number inhibition identifies cell number to be the most sensitive endpoint for metazachlor, followed by growth and photosynthesis. What does that mean concerning the sensitivity of the 24h-biotest for herbicidal effects of realistic water samples where photosynthesis inhibiting substances as well as substances acting on cell division are likely to occur together? To clarify this question in a second step mixtures of diuron and metazachlor in different mixture ratios were analyzed. In a final cost-benefit analysis the increase of sensitivity due to the extension of the 24-h test to a 48h test including the endpoint cell division is compared to the increased costs with respect to longer test duration and higher amount of working hours needed.

WE 344

Oviposition preference of *Chironomus riparius* females - potential sublethal behavioral endpoint for in-vivo water toxicity testing

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This study examines the potential use of insect behavior for detection of contaminants in water samples. Females of non-biting midge *Chironomus riparius* make choice of habitat for their offspring when laying eggs after fertilization in a swarm. The ability of fertilized females to detect samples contaminated with sublethal levels of chromium and chlorpyrifos in water samples was assessed in two experiments. In the first experiment, females were given a choice between control and samples contaminated with hexavalent chromium (0.1-100 mg/L) or organophosphate insecticide chlorpyrifos (1-100 ng/L). In the second experiment (no choice experiments), fertilized females were provided with either clear control water or contaminated water sample only. In the second experiment, we investigated differences in the reproduction output of females, i.e. no. of eggrope laid into the control vs. contaminated sample. The first experimental data indicate potential preference of chironomids towards clean water but further investigation is in progress, and the full outcomes will be discussed in detail. The study contributes to understanding of insect habitat selection, an important ecological feature in fresh-water ecosystems, with respect to contamination.

WE 345

Use of *Chlorella vulgaris* (Chlorophyceae) to test wood preservatives at a laboratory scale

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Most of the surfactants and antimicrobials are discarded after use in industrial effluents or sewage and can reach rivers, lakes and oceans, driving the development of new surfactants that are less harmful to the environment. We present the results obtained after testing a novel wood preservative compound through acute and chronic toxicity tests using *Chlorella vulgaris* (Chlorophyceae). Two compounds were studied: a gemini synthesized and isolated at the laboratory (A5D, 80-85% purity), and a TCMTB based commercial (solution 30%). TCMTB is an oil soluble active agent used to preserve wood, very efficient due to its high penetrating power. We calculated the growth rate (μ) (PROBALG2 program), the percentage of growth inhibition (%I) and EC50 96h for each of the compounds, all essays with three replicates per concentration. With TCMTB the following concentrations were tested: 1.562×10^{-4} mg/L, 3.125×10^{-4} mg/L, 6.250×10^{-4} mg/L, 1.250×10^{-3} mg/L, 2.5×10^{-3} mg/L, 5×10^{-3} mg/L, 0.01 mg/L, 0.02 mg/L and a control. A5D gemini concentrations were: 0.32 mg/L, 0.64 mg/L, 1.68 mg/L, 2.56 mg/L, 5.12 mg/L, 10.24 mg/L, 20 mg/L, 48 mg/L and a control. The culture conditions were constant, continuous illumination with white light 3000 lux, $23 \pm 1^\circ\text{C}$.

With TCMTB we obtained: $EC_{50} = 2.172 \times 10^{-4}$ mg/L. Between the lowest and the highest concentration we obtained 50.300 and 4000 cells/ml; μ between 0.0096 and -0.016 and (%I) between 36 and 206.

With the gemini compound we obtained: $EC_{50} = 0.6568$ mg/L (three orders of magnitude smaller than the commercial product). Between the lowest and the highest concentrations we obtained between 4800 - 54400 cells/ml; μ between 0.010 and -0.014 and (%I) between 33.0 and 193.3. These results highlight the importance of promoting the synthesis of compounds less harmful to the environment.

WE 346

DNA-based toxicity assay for pesticides in the environment

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Environmental pollution and poisoning owing to the widespread use of pesticides in agricultural and domestic pest control may be detrimental to the health of handlers, non target organisms and consumers. For example, some farmers preserve harvested grains by treating them with some chemicals. Pesticides or their residues are ubiquitous contaminants in the environmental media (air, soil, water), and in humans, plants and animal tissue samples. Pesticides uptake occurs through the skin, eyes, by inhalation, or by ingestion directly or through the food chain. Here we assess the genotoxic effect of dichlorvos, a widely used pesticide in Nigeria, on the DNA of poultry birds (*Gallus domesticus*). This study explored different special representation of treated groups using a three-patch matrix model incorporating dichlorvos contamination. Thermal denaturation of the DNA from the exposed birds resulted in a significant reduction ($p < 0.01$) in the DNA melting temperature from 87.2°C to 81.7°C while the GC/AT ratio was also significantly reduced ($p < 0.01$) from 0.77 in the control to 0.42 in exposed birds respectively. Electrophoresis of isolated DNA in 0.8% agarose gels gave variations in band intensity between the control DNA sample and DNA from exposed birds. These variations in band intensity were more pronounced in the RAPD-PCR products amplified with OPE-01 primer, where there is complete disappearance of DNA bands in the birds exposed to 0.04% pesticide. Thus deletion of DNA segments of birds exposed to dichlorvos can be modelled as a molecular biomarker of genotoxicity. This may also suggest that insecticides are capable of impacting genotoxic effects on non-target populations with consistent, long-term use.

WE 347

Use of passive samplers extracts for toxicity assessment of environmental realistic mixture of pesticides on natural biofilms communities

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The objective of this study was to evaluate the potential use of passive samplers extracts in order to highlight effects of representative pesticide mixtures on natural biofilm communities.

Chronic and acute impacts of pesticides in mixture were evaluated using POCIS (Polar Organic Chemical Integrative Sampler) extracts on natural biofilm communities. Biofilms were exposed to POCIS extracts (PE+) or placed in clean water (PE-) in order to maintain or to model a removal of a toxic pressure in relation with a realistic pesticide mixture directly isolated from the field.

After 13 days of exposure, a significant decrease of DW, AFDM and diatoms density on downstream biofilms exposed to PE compared to non exposed biofilms was observed; moreover mean chl a amount was lower for biofilms exposed to PE compared to non exposed biofilms. Acute toxicity tests at day 13 revealed a significant higher tolerance for PE+ compared to PE- biofilms.

Moreover a decrease of tolerance of downstream biofilms from day 0 to day 13 was observed for both treatments; with 51±13 and 43±2% of inhibition at day 13 compared to 25±3% of inhibition at day 0 in the highest concentration (d0). This global decrease of tolerance to PE with time could be explained by differences between channel and field conditions (light intensity, PE composition different from real river exposure due to POCIS selectivity, pesticides concentrations lower in channels than in river, [3DOTS]) Nevertheless, tolerance of downstream biofilms at day 13 still be much more higher than tolerance of biofilms originated from upstream site (significant inhibition of Fv/Fm for d0, d1, d2 and d3 for upstream biofilms).

Our results underline impacts of pesticides in mixture on both growth (DW, AFDM and diatoms density) and community tolerance of a natural biofilm. In the one hand, removing the toxic pressure stimulates global biofilm growth but in the other hand it leads to a decrease of biofilms tolerance. A global decrease of tolerance over time for both treatments was observed; nevertheless without reaching the tolerance levels of upstream biofilms.

This study highlights the potential use of passive sampler extracts combined with acute toxicity tests in order to evaluate effects of more realistic pesticide mixtures on natural biofilms communities, and then the future and likely applications of such approaches for ecological risk assessment.

WE 348

A chronic exposure test method for two aquatic insects, the mayfly *Cloeon dipterum* and the caddis fly *Anabolia nervosa*

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Species sensitivity distribution (SSD) models are an important tool in protecting aquatic ecosystems. Most SSD models rely on acute toxicity endpoints. Some of the reasons for not utilizing chronic endpoints are cost and the limited number of species available having reliable chronic endpoint study designs. We have evaluated a 28-day study design with two common European aquatic insects, the mayfly *Cloeon dipterum* and the caddis fly *Anabolia nervosa*. May fly nymphs and larval caddis flies were field collected and brought back to the laboratory where they were acclimated to natural filtered water at 17 ± 1 °C. Feeding experiments were initially conducted using three diets: flaked fish food suspension (10 mg/L), periphyton grown on microscope slide and a green algae (*Ankistrodesmus* sp.). Following a 10-day feeding trial that looked at survival and weight, the flaked fish food suspension along with the green algae was chosen as an appropriate diet for both species. The final study design was 28 days in duration, 5 replicates/treatment and 8 individual/replicate, under static renewal conditions (renewals on Monday, Wednesday and Friday) in laboratory dilution water and laboratory dilution water with acetonitrile as a co-solvent at approximately 50 µL/L. May fly survival/emergence was 95% in the dilution water and the dilution water with co-solvent. Emergence began on day 6 and continued through day 28. The caddis fly experiment was terminated early on day 23. Dilution water survival was 100% however, survival in dilution water with co-solvent had dropped to 50%. Considerable fungus growth was observed on the caddis fly cases in the treatment with co-solvent, which was absent in the dilution water only treatment. The fungus accumulation was assumed to be a significant factor in the poor survival. Recommendations for refining the caddis fly test method include testing alternative co-solvents like acetone or dimethylformamide (DMF). The mayfly *Cloeon dipterum* is a very promising aquatic insect for use in chronic exposures.

WE 349

Behavioural toxicity of endosulfan and a novel approach to the study of its ecotoxicity in aquatic microcrustaceans

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Despite the organochlorine insecticide endosulfan is currently banned in over 30 countries, it is still widely used in most Latin American countries. Recently, values from 0.2 to 13.5 µg L⁻¹ have been found in neotropical wetlands, exceeding the maximum permitted concentration. Its continued use might respond to the hegemonic agro-economic model, despite several works have demonstrated its negative consequences to humans and environment. In addition to its direct effects on non-target organisms it has also been reported indirect effects, such as alterations in predator-prey interactions, in aquatic and terrestrial organisms. The importance of these last studies lies in providing relevant information on the actual ecological processes that take place after anthropogenic interventions. The purpose of this work was to demonstrate an indirect effect of the early impact of an endosulfan based-insecticide on the escape behaviour of four subtropical microcrustaceans: two copepods (*Notodiaptomus conifer*, *Argyrodiaptomus falcifer*) and two cladocerans (*Pseudosida variabilis* and *Ceriodaphnia dubia*). The experimental assays were carried out using a novel hydraulic device designed to mimic three-speed predator capture behaviour. This allows to quantify the organisms' ability to perceive and react to the approach of possible predators. Two environmental relevant concentrations of endosulfan were used. They were prepared from a commercial formulation containing 35% of the active ingredient (Zebra Ciagro®). Endosulfan concentrations in water was quantified by GC-ECD showing recoveries >95% of the nominal value. The exposure time was 15 (±5) minutes. Analyses of data were carried out using the Deviance as variability means (ANODEV). The magnitude and the kind of responses were different depending on species, capture speed and toxic concentration, however the following pattern was recognized: endosulfan made cladocerans more capturable ($p < 0.05$) but copepods enhanced their escape ability at lower concentrations ($p < 0.05$), which might be the result of an early toxicant stimulation. The immediate apparent advantage of this last effect will be discussed in the context of ecophysiological theory, since an increase in one physiological or behavioural process may be at the expense of other biological attributes. Considering the comparatively high sensitivity of the escape, the proposed method is suggested as an effective complement (and ecologically relevant) in ecotoxicology.

WE 350

Characterization of a controlled release formulation of Novaluron

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Mosquitoes pose a serious risk to public health around the globe as vectors of human diseases such as the West Nile fever, malaria and dengue fever. In 2009 there were 225 million cases of malaria worldwide; these cases resulted in the death of 781 000 people. Consequently, it is extremely important to control mosquito populations especially at their breeding sites, i.e., standing water. A number of pesticides including novaluron, a member of benzoylphenyl urea chemical family, are effective at controlling mosquitoes; however, in many cases reapplication is required to provide effective season-long control. A new slow-release, wax-based formulation of novaluron has been developed by Pestaloto Inc. to regulate the release of the active ingredient in standing water allowing for a prolonged mosquito control period. This novaluron formulation was added to a set of 12000-L mesocosms to measure its efficacy against target (mosquito larvae) and non-target (zooplankton) species. Mesocosms were divided into 5 groups: novaluron (0.12%;300g/mesocosm) with added sediment, novaluron (0.12%;300g/mesocosm) without added sediment, novaluron (0.48%;300g/mesocosm) with added sediment, novaluron (0.48%;300g/mesocosm) without added sediment and no novaluron with added sediment. The concentrations of released novaluron measured in each mesocosm by HPLC over a 120 day period were compared using an *Aedes aegypti* bioassay. Regardless whether sediment was or was not present, the 0.48% treatment provided 100% control of *Aedes aegypti* throughout the 120 day study while the 0.12% treatment provided 100% control for only 28 days after application. Other monitored parameters were affected by the addition to novaluron to the mesocosms; dissolved oxygen and chlorophyll concentrations were higher in sampled water containing novaluron, while zooplankton abundance was lower.

WE 351

Effects of dicamba on mRNA transcription of thyroid hormone regulation in Chinese rare minnow (*Gobiocypris rarus*)

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Dicamba (3,6-dichloro-2-methoxybenzoic acid) is a benzoic acid herbicide and widely used in agriculture, which has been detected in surface-water and groundwater. In this study, to determine the endocrine disruption effects of dicamba, adult fish were separately exposed to concentrations 0, 0.05, 0.5, 5, and 50 µg/L for 40 days. The results showed that no effects on length in adult males, whereas the length of adult females were significantly decrease in 50 µg/L dicamba treatment ($p < 0.5$). Moreover, GSI and

HSI of adult females were significantly decreased in high-dose treatment. However, GSI and HSI of adult males were increased except 5 µg/L dicamba treatment, and HSI of adult males were significantly increased in low-dose treatment ($p < 0.5$). In addition, the transcription of thyroid hormones (THs) related genes including thyroid hormone receptor C (trC), transthyretin (trr), thyroid peroxidase (tpo), deiodinase (d1, d2, d3) from adult livers were evaluated. After 40 d exposure, the mRNA expressions of d3 were significantly down-regulated in the livers of male and female ($p < 0.01$), whereas trr and d1 were significantly up-regulated ($p < 0.01$). Meanwhile, significantly up-regulation of trC, tpo and d2 were observed in the livers of females ($p < 0.01$) but significantly down-regulated in males ($p < 0.05$). These results indicate that dicamba had negative effects on thyroid system in males and females. Therefore, dicamba could be a potential endocrine disruptor.

WE 352

Evaluation of endocrine disruption and molecular level toxicity to pesticides in *Misgurnus anguillicaudatus*

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Pesticides have contributed significantly to improving quality of life and safeguarding the environment. However, potential toxicities of pesticides like persistent organic pollutant (POPs) to non-target species have been reported, when they were inadvertently discharged into ecosystem. In this study, 21day exposure tests to alachlor, benomyl, carbaryl, endosulfan, methomyl, metribuzin, 2,4-D were performed using *Misgurnus anguillicaudatus*, which inhabit widely rice paddy in Korea, under continuous flow-through system. Following range-finding test, test concentrations for 21d exposure test were determined below the lethal level as much as possible. We also observed various indicators and biomarkers such as condition factor (CF), gonado-somatic index (GSI), liver-somatic index (LSI), vitellogenin (VTG), DNA damage using comet assay, acetylcholinesterase (AChE), and ethoxresorufin-O-deethylase (EROD) at middle and end of the test for identifying the effect of endocrine disruption and sub-lethal toxicity. VTG in blood plasma was significantly greater in male fish exposed to 0.002 mg/L endosulfan, in female and male fish exposed to 0.4 and 2 mg/L methomyl, in male fish exposed to 5 mg/L carbaryl, and in female fish exposed to 10 mg/L metribuzin for 21d than in control ($p < 0.05$). While CF, GSI, and LSI weren't affected by exposure to test chemicals, DNA were damaged in fish exposed all test pesticides ($p < 0.05$). AChE and EROD activity in *M. anguillicaudatus* were also influenced by exposure of carbaryl, endosulfan, methomyl and alachlor, endosulfan, 2,4-D, respectively ($p < 0.05$). In conclusion, biomarkers used in this 21day exposure test are helpful to evaluate influence on endocrine disruption as well as molecular level toxicity, and this results warrant further studies on pesticides supposed potential endocrine disruptor, for example methomyl and endosulfan.

WE 353

Acute exposure of a glyphosate-based herbicide affects the gills and liver of the Neotropical fish, *Piaractus mesopotamicus*

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The glyphosate (N-phosphonomethyl glycine) herbicides, post-emergent herbicides with a broad-spectrum and low selectivity, have been intensively applied on numerous field cultures, urban and industrial areas to control unwanted plants, including the macrophytes in aquatic systems. Recently, a new glyphosate formulation Roundup® Ready (RR), specifically developed for genetically modified glyphosate-tolerant soybean crops (RR crops), was commercially available in Brazil. This study determined the acute toxicity of such herbicide in *Piaractus mesopotamicus*, and evaluated the effects on the morphology of the gills and liver of exposed surviving fish. Fish were exposed to 3.0, 3.5, 4.0 and 4.5 mg L⁻¹ of glyphosate, and the LC50/48h was estimated at 3.74 ± 0.2 mg L⁻¹. Gill histopathology was rare, and the activity of Na⁺/K⁺-ATPase enzyme did not change, suggesting normal function. However, the damage to the liver was classified as moderate to severe. Cytoplasmic vacuolization, lipid accumulation, nuclear and cellular membrane alterations and glycogen depletion were found in the liver, suggesting a reduction in the liver function. The glyphosate in the RR formulation was classified as moderately toxic for *P. mesopotamicus*, and the severe damage in the liver may affect the detoxification and/or tissue repair process and contribute to fish death.

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WE 354

Effects of the fungicide tebuconazole on fungal and bacterial communities in the sediment of outdoor freshwater microcosms

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Only a few studies have been published on the non-target effects of fungicides on aquatic microbial communities. One reason for the lack of information regarding effects of fungicides on non-target micro-organisms may be the basic dossier requirements for EU registration. So far, only information from ecotoxicity studies performed on vertebrates, invertebrates and primary producers are asked for the aquatic effect assessment. Aquatic fungi and bacteria, however, play an important role in freshwater ecosystems, contributing significantly to decomposition processes. Hence, aims of the present work were (1) to evaluate the impact of tebuconazole on sediment microbial community structure of an experimental freshwater ecosystem, and (2) to evaluate whether the current effect assessment procedure (based on standard tests with fish, aquatic invertebrates and algae) is protective for aquatic fungi and bacteria. To this end, outdoor microcosms were set up in an experimental ditch and four test systems served as controls while tebuconazole was applied to four other microcosms at an intended concentration of 238 µg a.i./L. This treatment level is in line with the HC5 (based on acute toxicity tests with aquatic organisms) for tebuconazole, as described by Maltby et al. (2009). Throughout the experiment sediment samples were taken from each microcosm and used for total DNA isolation. The isolated DNA was used to access bacterial and fungal community structure by Denaturing Gradient Gel Electrophoresis (DGGE) and bacterial community structure and composition was also accessed by 454-pyrosequencing of PCR-amplified ribosomal RNA gene fragments. Results showed that fungal community structure was not affected by the treatment, where controls and treated systems presented a similar DGGE profiles over time. DGGE profiles of the bacterial community showed similar community structures between controls and treated systems. Nevertheless, DGGE profiles of controls and treated systems showed differences on band intensities, indicating possible effects of the fungicide exposure on the bacterial community.

WE 355

Chronic toxicity of acetone, DMF and DMSO used in sediment-water test systems on *Lumbriculus variegatus*

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Organic solvents are frequently used for the application of test items with low or no water solubility. According to the OECD Guideline 225 (Sediment-Water *Lumbriculus* Toxicity Test Using Spiked Sediment) a suitable organic solvent for the application of test items should be used. However, until now no data on the toxicity of solvents listed in OECD series No 23 on *Lumbriculus variegatus* are available. Toxicity data for the following organic solvents will be presented: Dimethylformamide (DMF), Dimethylsulfoxide (DMSO), Acetone.

WE 356

Oxidative stress, acetylcholinesterase and physiological status as responses in clams transplanted into the Mar Menor lagoon (Spain) to assess the biological impact of anthropogenic activities

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The Mar Menor Lagoon (SE Spain) is subjected to several forms of disturbance, amongst which chemical pollution associated with anthropogenic activities (industrial production, high levels of urbanization, tourism, ports etc.). In fact, previous works showed the input of high levels of triazine and organophosphate pesticides to this lagoon through the El Albuñón watercourse from different sources (groundwaters, wastewater effluent, etc.). Therefore an environmental monitoring is necessary to protect biological/ecological systems, and study the overall biological effects of exposure to potentially harmful substances.

In this context, an active biomonitoring experiment (ABM) was performed using clams *Ruditapes decussatus* collected at a pristine site, and transplanted to four locations inside the lagoon, two of them located near to the Albuñón wadi mouth, for 7 and 30 days in autumn 2009. The primary aim of this study was to select a set of relevant biomarkers in transplanted clams for the biological assessment of water pollution in the Mar Menor. Oxidative stress, neurotoxicity and physiological responses were studied in these bioindicator organisms; levels of catalase (CAT), glutathione reductase (GR), glutathione S-transferase (GST) and lipid peroxidation (LPO) were measured in digestive gland tissues, acetylcholinesterase (AChE) in gills of clams before and after the transplant experiment. The physiological status of the animals was assessed by the scope for growth (SFG).

Clams transplanted near the Albuñón appeared to be the most impacted by the pollutant discharges. Biochemical responses displayed a high oxidative stress, with significantly higher levels of LPO, GST and GR than those found at time 0 and other sampling sites, whereas AChE activity was significantly inhibited by the input of organophosphate pesticides. Physiological rates were also affected at the sites near the pollutant discharges where a significant decrease of 30% on the SFG were recorded. Thus these results suggested that ABM approach have a potential for application in the Mar Menor pollution monitoring.

WE 357

The effects on non-target species of novel products applied together with herbicides and insecticides in relation to transgenic soybean crops in Argentina

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Aquatic environments of the pampasic region of Argentina receive chemicals from agricultural activity due to an increase in a glyphosate tolerant transgenic variety of soybean crops. In the last year the extension of the soybean crops in the region has reach to about fourteen million hectares. Non target organisms, as aquatic primary producers are severely affected by this agricultural contamination. The cultivation of transgenic soybean is an activity of real importance to the national economy, thus the use of chemicals in order to reduce losses that occur as plant and animal pests has increased. The research for new products for this purpose is under constant development.

The present study is aimed to compare the toxicity of herbicide Glyphosate and insecticide Endosulfan and Chlorpyrifos prior and after the addition of three novel products which formulations is based on nonylphenol ethoxylates as adjuvant. These products are mixed with pesticides according to rates indicated in the product containers, whose function is to enhance penetration, avoid drift and evaporation improving the biological activity of pesticides. The not target organisms selected were the aquatics primary producers green algae and the floating macrophyte *Lemna minor*, as they are essential to ecosystem providing the basic energy for food webs. The comparisons were made taken in account different endpoint as biomass, growth, anatomic parameters, oxidative stress and lipoperoxidation biomarkers. Algal growth and growth rates of *Lemna minor* were severely affected. The colony architecture of *Lemna minor* was modified as the stipe was observed more often and permanent in the case of insecticide Chlorpyrifos. Glyphosate, Endosulfan and Chlorpyrifos increased and altered the activity of oxidative stress enzyme and lipoperoxidation of *Lemna minor*. The addition of novel products to pesticides increased the adverse effects already observed. The major concern of using these products is the increase of the fate of this persistent compound as it acts as endocrine disruptor with consequences on health effects, reproductive abnormalities and impaired development in wildlife species. While the addition of these products allows lower doses of pesticide application, the action of nonylphenol results in more severe and serious effect of major significance at the population and ecosystem level.

WE 358

Evaluation of toxicities of herbicides using short-term chronic tests of alga, daphnid and fish

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To calculate the possible adverse effect of pesticides on our ecosystem, the concentrations and application window of herbicides in the environment were need to be monitored. We investigated the effect of herbicides on aquatic organisms, and the herbicides released in environment were characterized by chemical analysis. The agricultural drainage water collected from 14 different spots of rice field in Japan. Water samples were collected directly after 7 days of herbicide application. The short term chronic toxicity tests were carried out using alga (*P. subcapitata*) growth inhibition test, daphnid (*C. dubia*) reproduction test, and fish (*D. rerio*) short-term toxicity test on embryo and sac-fry stages. In this study, the effects on alga and daphnid were observed in 9 spots and 2 spots, respectively. The effect on hatching, survival of fish was not found in any spots examined. We also performed simultaneous analysis of the GC-MS for the water samples, and 92 different herbicides were detected in this study. Bromobutide was detected at high concentration in almost all fields. Dimethametryn and pretilachlor were also detected in the same spots. One month later from herbicides application, the concentrations of herbicides detected in this study decreased significantly. Our results suggested that the herbicides disappear immediately by disintegration or proliferation in the environment. Moreover, we examined the short-term chronic toxicity on 3 species for ten candidate herbicides, which were detected in the water samples. In this study, the strongest effect was obtained in the alga. Toxicity on the alga was found in pretilachlor, cyanazine and simetryn at 1-10 mg/L. The effects of herbicides on daphnid were weaker than on alga, with the only exception of bendiocarb, which showed adverse effect on daphnid at 10 times lower concentrations compared to an alga. The effects of herbicides were found in order of alga > daphnid > fish. In the present study, it suggested that the short-term chronic toxicity tests using the aquatic organisms is effective in predicting the effect of herbicides released in environment. For the chemical safety and control of environmental chemicals including herbicides, further study is needed to detect synergistic effects with herbicides and other substances.

WE 359

Mutagenic and genotoxic effect of Roundup Transorb β herbicide in gill cells and erythrocytes from *Poecilia reticulata*

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The effects of mutagenic and genotoxic substances in the fish genome have been the subject of many studies, especially those that seek to establish the response of genes to environmental stimuli. This study aimed to conduct a study on mutagenicity and genotoxicity in fish *Poecilia reticulata*, by exposure to Roundup Transorb β using the micronucleus test (MN) and Comet assay. Erythrocytes were obtained by centrifugation of the gills being added fetal calf serum to precipitate, and then dripped onto wafers to make the smear and electrophoresis. Fifteen fish were used for each treatment at the following concentrations: 0 μ l / L, 1.41 μ l/L, 2.83 μ l/L, 4.24 μ l/L and 5.65 μ l/L at 24 hours of exposure. For the analysis of slides, 75.000 cells were counted and agreed to the frequency of occurrence of Nuclear Morphological Changes (NMAs). In the MN test and Comet assay could detect significant genotoxic and mutagenic effects in erythrocytes and gill cells, indicating a gradual increase in the number of cells with NMAs and DNA damage under higher concentrations, indicating a concentration-dependent effect. These results suggest that the formulation of the herbicide tested showed genotoxicity and mutagenicity tests for the MN and Comet assay, and they are very efficient in detecting low levels of contamination.

WE 360

Acute toxicity of the herbicides ametryn, Glyphosate and it is association (Glyphosate+Ametryn) to *Daphnia magna*

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In Brazil it is very common the application of herbicides in association (tank mix) to improve the range of action and to reduce the costs. This practice is not previewed in law, and the environmental behavior of associations is not very known in literature. The glyphosate is non-residual effective herbicide, and has been hardly studied, being known as “environmental friendly” with quick mineralization and low toxicity. Ametryn is an herbicide of residual effect, with moderated toxicity and environmental persistence. The association between this both herbicides could be synergic or antagonistic about its environmental mineralization, degradation, persistence and toxicity. The aim of this study was to evaluate the acute toxicity of the herbicides Ametryn, glyphosate and ametryn + glyphosate to the microcrustacean *D. magna*. Firstly, was realized a preliminary test for each product in order to establish the concentrations to be used in the definitive acute test, which were: 10, 20, 40, 60, 80 e 100 mg/L for each product and to the association (glyphosate+ametryn). All concentrations were conducted in two replicates with five organisms each; plus control (reconstituted water). The test duration was 48 hours, in a dark incubation chamber without food. After this period the Median Effective Concentration (EC50) (48h) for each product was calculated using the statistic program Trimmed Spearman Karber (Hamilton et al, 1977). The EC50 (48h) for ametryn, glyphosate and ametryn + glyphosate were respectively (mg.L⁻¹): 41.55; 28.8 and 28.28. We conclude that glyphosate and the mixture ametryn + glyphosate were more toxic than ametryn. The mix of ametryn and glyphosate did not increase or reduce the toxicity of the test, being the toxicity of glyphosate predominant. A chronic test is being conducted and the results should be presented at the poster in the congress.

WE 361

Inhibition of oxidative stress enzyme activities and genotoxicity of the insecticide imidacloprid in the freshwater fish *Australoheros facetus*

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Imidacloprid (IMI) is a neonicotinoid insecticide with neurotoxic action that is at the moment one of the insecticide with the world's fastest growing sales. In this study, biochemical and genetic parameters of the freshwater fish *Australoheros facetus* after acute exposure to IMI are presented. Glutathione-S-transferases (GST) and catalase (CAT) activities, malondialdehyde content (MDA) in liver and gills and micronucleus frequency (MN) in blood were analyzed. Fish were exposed to: 0 (negative control), 1, 10, 100, 1000 y 10000 μ g/L during 24 h. Biochemical parameters were measured espectrophotometrically. Peripheral blood smears, two per fish, were made by applying a drop of blood on clean slides, fixed in absolute methanol; air dried and stained with 15% Giemsa solution. Two thousand erythrocytes, 1000 per slide, were analyzed from each animal under 1000 X magnification. In gills GST and CAT activity was inhibited at 10, 100, 1000 μ g/L and at 1, 10, 100, 1000 μ g/L, respectively (p<0.05). In liver, no effects on biochemical parameters were detected. The MN was increased at 100 and 1000 μ g/L with respect to the control (p<0.05). Although detected environmental concentrations have been reported in the range from 1 to 14 μ g/L and the CL50 for fishes are in the order of 200 mg/L; our results show a negative effect on the oxidative stress defense enzymes at mostly all the concentrations tested as well as mortality of fishes at 10 mg/L after 3 hours of exposure. The responses observed in our work when fishes were exposed to environmentally relevant concentrations, alert about the possible impact of these levels in aquatic ecosystems and highlight the different sensitivity of the fish species.

WE 362

DNA damage induced in fish (*Anguilla anguilla*) by aminomethylphosphonic acid (AMPA) - the major breakdown product of glyphosate

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Most of studies on the effects of pesticides in aquatic organisms are focused on the active ingredients of the comercial formulations. However, the assessment of the direct impact of chemicals that may occur in the environment as breakdown products of the parental compound could be considered even more ecotoxicologically relevant.

Glyphosate is the active ingredient of various well-known herbicide preparations applied worldwide, that easily reaches the aquatic compartment where it can be naturally degraded into aminomethylphosphonic acid (AMPA). This major breakdown product has been shown to have greater environmental mobility and persistence than glyphosate.

Hence, the main goal of the present research was to assess the DNA damaging potential of AMPA in fish and compare it with that of glyphosate. Therefore, the comet assay was used as genotoxicity test, being applied to blood cells of *Anguilla anguilla*, following short-term exposures (1 and 3 days) to AMPA (11.8 and 23.6 μ g.L⁻¹) and glyphosate (17.9 and 35.7 μ g.L⁻¹), adopting environmentally realistic and equivalent molar concentrations.

Analyzing the results after 1 day exposure, it was observed that both concentrations of AMPA and glyphosate displayed significantly higher DNA damage, when compared with control. The 3 days exposure revealed that only the lower concentration of glyphosate showed to induce DNA damage. Thus, the present findings confirmed the genotoxicity of AMPA, for the first time in fish. However, this metabolite appears to be more dangerous after 1 day, since it was possible to observe a temporal recovery along the experiment. Moreover, there are no evidences that AMPA is more genotoxic than its precursor glyphosate.

WE 363

Genotoxic evaluation of Roundup ® formulation and its constituents in fish (*Anguilla anguilla*)

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Herbicides represent a diffused and hazardous group of environmental contaminants, affecting non-target organisms. Though it is recognized that these agrochemicals

represent a potential threat to fish, their genotoxic potential remains largely unexplored in this animal group. Moreover, genotoxic studies have been almost exclusively focused on the active ingredients, whereas the effects of adjuvants are frequently disregarded. Therefore, the present work addressed the broad-spectrum herbicide - Roundup® - as one of the most popular formulations worldwide, aiming the evaluation of the relative contribution of the active ingredient (glyphosate) and the surfactant (polyethoxylated amine; POEA) to the genotoxicity of the commercial mixture on *Anguilla anguilla*. Fish were exposed to equivalent concentrations of Roundup® (58 and 116 µg.L⁻¹), glyphosate (17.9 and 35.7 µg.L⁻¹) and POEA (9.3 and 18.6 µg.L⁻¹), during 1 and 3 days. The comet assay was applied to blood cells. After 1 day exposure, it was observed that all treatments, with the exception of the lower concentration of Roundup®, displayed significantly higher DNA damage in comparison with the control. In relation to 3 days exposure, significant increases were observed for the higher concentration of Roundup® and the lower concentration of glyphosate, as well as for both POEA exposed groups. Overall, the results confirmed the genotoxicity of Roundup®, also demonstrating the DNA damaging potential of glyphosate and POEA individually. Though both components contributed to the overall genotoxicity of the pesticide formulation, the sum of their individual effects was never observed, suggesting an antagonistic interaction. Though POEA is far to be considered biologically inert, did not increase the risk associated to glyphosate. The present findings emphasize the need to define regulatory thresholds for all the formulation components and recommend, in particular, the revision of the hazard classification of POEA.

WE 364

Bioaccumulation and toxicity of the herbicide Prowl® in *Daphnia magna* exposed to contaminated food

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The intensive use of pesticides leads to the contamination of soils and nearby water systems due to runoff, leaching or spraydrift from agricultural areas. Pendimethalin - the active ingredient of Prowl® - is an herbicide used before crop emergence or planting, since it inhibits the development of weeds in seedlings. Therefore, besides attaining freshwater reservoirs, pendimethalin may also affect green algae in particular, what in turn may influence the quality of the food for the majority of herbivorous zooplankton, affecting their ecology and community structure.

The aims of this work were: i) to evaluate the nutritive quality of phytoplankton previously exposed to sub-lethal concentrations of Prowl®; ii) to assess changes on the reproductive output of *D. magna* cultured in non-contaminated ASTM medium and fed with *Pseudokirchneriella subcapitata* previously exposed to sub-lethal concentrations of Prowl® and iii) to evaluate the bioaccumulation of pendimethalin by *D. magna*.

First an algae growth inhibition test was performed to evaluate the ecotoxicity of Prowl® for *P. subcapitata*. EC50 and EC20 values obtained for growth inhibition were 18.3 µg L⁻¹ and 10.7 µg L⁻¹, respectively. Thereafter, *P. subcapitata* was cultured in MBL medium containing a concentration of pendimethalin equivalent to the EC20 value, to obtain contaminated algae. These were used to feed *D. magna* during the chronic assay. After 26 days, the results expressed a significant decrease in the fecundity of daphnids that were fed with contaminated algae. However, no significant differences in the growth rate of *D. magna* were recorded, when compared to the control (with no contaminated food). In what concerns the bioaccumulation of pendimethalin, it was observed that this herbicide had a significant bioaccumulation potential (> 4.20 mg/g) in daphnids. The current results highlighted that low concentrations of Prowl®, that could be found under real-scenarios of agricultural areas, can affect the reproductive performance of *D. magna*, due to the change of their food quality.

WE 365

Differential gene transcription in *Poecilia vivipara* exposed to the herbicide Atrazine

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Atrazine is the most common herbicide found in freshwater ecosystems. *Poecilia vivipara* is a euryhaline fish found in fresh and brackish water with different degrees of anthropogenic impact. The aim of this study was to identify genes that are up and down-regulated in the liver of *Poecilia vivipara* after exposure (24 h) to atrazine (10 µg/L), employing the suppressive subtractive hybridization method (SSH). Results showed that 147 genes were differentially transcribed. Among them, 67 up-regulated genes and 48 down-regulated genes were identified by homology using when compared to sequences (blastx) available at the genbank database sequences. The metabolic functions of up- and down-regulated genes are associated with immune system, structure, energy production, general metabolism, signaling and transport. Therefore, this study provides a basis for studying the response of fish to atrazine exposure and allows the characterization of new potential molecular markers for atrazine contamination in natural ecosystems.

WE 366

Effects of Plant Protection Products on *Lemna minor* growth - active ingredient vs. formulated compounds

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The increased industrialization and agricultural practices is becoming a threat to ecosystems. Although soil is the primary recipient of pesticides, water bodies adjacent to agricultural areas can be contaminated due to spray drift, surface runoff and/or leaching and accidental spills of pesticide residues. This can be a problem for the integrity of non-target wildlife, as far as pesticides are biologically active compounds. In particular, aquatic duckweeds are sensitive to toxic compounds and are currently required for regulatory toxicity testing of pesticides and other chemicals.

As such, this work aims to assess and compare the effects of the active ingredient and respective formulated product of an herbicide (pendimethaline vs. Prowl®) and a fungicide (copper sulphate vs. Calda Bordalesa®) on the growth of *Lemna minor*. The ecotoxicological evaluation of formulated products gives a hint of potential risks under real agricultural scenarios. The biomass production of *L. minor* used for the calculation of growth rate was based on dry weight measures.

Overall, dose-response relationships were determined for all the compounds tested. In general, the formulated products were more toxic to *L. minor* growth than the respective active ingredients. In particular, Prowl® induced quite significant growth rate impairments on *L. minor*, since it inhibits microtubule formation during cell division. In spite of this, the active ingredient of the fungicide was more toxic than that of the herbicide, evidencing a lower EC₅₀ value. Anyway, the generally reduced effect concentrations determined (at the µg L⁻¹ level) for the formulated products strengthen their potential risks for aquatic duckweeds during their agricultural application. Future studies should extend the testing for other sub-lethal responses and macrophyte species, which usually have important role-plays in ecosystem balance namely related with the natural mitigation of agrochemicals entering the aquatic systems.

WE 367

Assessment of the effects of the 2010-2011 Victorian locust control program on aquatic ecosystems

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In spring 2010, Victoria, Australia was threatened with a potentially serious locust plague. In response, the Victorian Government launched the Australian Plague Locust Response (APLR) to mitigate locust impacts by biological and chemical control. The aim of this study was to monitor aquatic ecosystems for impacts of APLR-approved pesticides. 81 sites that were likely to be subject to contamination in the event of pesticide applications were monitored. Surface waters and fine (<64µm) sediments were collected and chemically analysed for APLR-approved pesticides (fenitrothion, chlorpyrifos, diazinon, malathion, carbaryl, fipronil and its breakdown products, cyfluthrin and cypermethrin). A suite of non APLR-approved biocides, heavy metals, hydrocarbons and nutrients were also determined. Ecotoxicological assays were conducted on sediments and surface waters. Endpoints were one or several of: toxicity (mortality, reduced size, reduced emergence) of sediment to the midge *Chironomus tepperi*, inducement of morphological deformities in midge larvae by sediment and surface waters, and impacts to populations of the fish *Craterocephalus fluvialis*. Macroinvertebrate communities were monitored, but this component of the study was compromised by flooding. Novel in situ and laboratory bioassays were also trialled for three sites, assessing the acute toxicity of sediments and surface waters to the freshwater snail *Potamopyrgus antipodum* and the freshwater amphipod *Austrochiltonia subtenuis*. Contamination yielding a toxic result was investigated to determine whether or not the contamination-causing pesticide application of the APLR. To determine the likelihood of a site being impacted by the APLR, chemical, ecotoxicological and investigation results were incorporated into a decision tree. This determined sites as being "unlikely", "possibly", "likely" or "very likely" impacted by the APLR. Chemical analysis found 11 incidences of APLR-approved pesticide contamination: four in sediments (chlorpyrifos and cyhalothrin) and seven in surface waters (carbaryl, fipronil and its breakdown products and diazinon). Three chlorpyrifos contaminated sediment samples yielded toxic results to test midges. Investigations found APLR was not the cause of these contamination events. No other contamination event yielded toxic results. The APLR was therefore deemed successful in avoiding aquatic ecosystem impacts, but this result may also have been influenced by atypical climatic events.

WE 368

Ecotoxicity of triclosan, triclocarban and other antimicrobial agents to aquatic organisms and benthic invertebrate

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Antimicrobial agents triclosan and triclocarban are found to be strongly toxic for aquatic organisms and their ecological risk is a growing concern. There are several other antimicrobials commercially available and they may enhance the ecological risk of this class of micropollutants. Not much has been known about the ecological risk of the other antimicrobial agents such as 2-phenoxyethanol and p-thymol. Additionally, triclosan and triclocarban was relatively hydrophobic and accumulative in sediment, and several researchers have detected them from sediment.

In this study, we investigated acute and chronic or sub-chronic toxicity of six compounds (Triclosan, triclocarban, p-Chloro-m-xylenol, 4-isopropyl-3-methylphenol, 2-

phenoxyethanol and resorsinol) using conventional three aquatic organisms, fish, daphnid, algae and a benthic invertebrate chironomid in addition to the measurements of these compounds in urban streams in Tokushima, Kyoto, and Saitama, Japan. Combining these ecotoxicological and monitoring data, we conducted screening level ecological risk assessment of these compounds.

Triclosan was found to be very strongly toxic for algae with 96 h-EC50 of 2.8 µg/L but moderately toxic for daphnid and fish. Triclocarban showed the similar trend but was more strongly toxic to daphnid and fish than triclosan. Other antimicrobial agents were less toxic than these two with a few exception such as resorsinol, which was relatively toxic to daphnia (8 d-NOEC of 310 µg/L). As presented above, the toxicity of triclosan and triclocarban was found to be two to three orders of magnitude stronger than the other antimicrobial agents. Maximum MEC/PNEC values were over 1 for triclosan (algae) and triclocarban (daphnia) while that for p-thymol and the other antifungal agents were under 0.1 based on acute toxicity tests (data not shown). Based on chronic/sub-chronic toxicity tests, MEC/PNEC value of TCS was 0.01-6.3 and there is a threat for aquatic organisms while that of 2-phenoxyethanol was 0.00017-0.013 and the emergent risk for the aquatic organisms might be limited. However, further investigations on these antimicrobial agents are necessary because the information on the fate and ecological risk in the urban area is still limited. In addition, these compounds may have additive effects as we observed for parabens, another class of preservatives.

WE 369

Acute ecotoxicity evaluation of plant extracts such as pepper extract, zanthoxylum extract, clove bud oil, rosemary oil, and origanum oil

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Environment-friendly agro-materials tend to be preferred to chemical insecticides recently. For this reason, many studies are conducted to develop environment-friendly insecticides containing natural materials. The purpose of this study was to assess ecotoxicity for 5 plant extract and essential oils (pepper, Zanthoxylum, clove bud oil, rosemary oil, and origanum oil) expected to prevent from pests and be used for agro-materials. Target species used to assess acute toxicity were aquatic invertebrate (Daphnia magna), fish (Oryzias latipes), honeybee (Apis mellifera L.) and earthworm (Eisenia fetida). The EC50 value of pepper, zanthoxylum, clove bud oil, and rosemary oil to Daphnia magna were 0.1, 0.13, 1.61 and 8.72 mg L⁻¹ respectively and these values were moderately toxic according to standard of USEPA. The EC50 of origanum oil was more than 10 mg L⁻¹ then they were considered as slightly toxicity. In case of acute toxicity test to fish, LC50 of pepper extract, clove bud oil, and rosemary oil were more than 10 mg L⁻¹ respectively and LC50 of origanum oil 7.5 mg L⁻¹, then their toxicity of fish was category in Korea. The LC50 of zanthoxylum was less than 1 mg L⁻¹ so it was more than category. Acute contact and oral toxicity test to Honeybee were conducted. As a result, LD50 of pepper, clove bud, and origanum were >100 a.i. bee-1 in both of tests. LD50 of zanthoxylum and rosemary were 9.3 and 99.1 a.i. bee-1 in contact toxicity, 15.2, 69.2 a.i. bee-1. In case of acute toxicity test to earthworm, LC50 of all the plant extracts were more than 1,000 mg kg⁻¹. In conclusion, 4 plant extracts except zanthoxylum extract are expected to be used for environment-friendly insecticide materials with low risk against ecosystem and contribute to developing environment-friendly agro-materials.

WE 370

Effects of the sulfonylurea herbicide amidosulfuron on target and non-target terrestrial and aquatic organisms

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The usage of herbicides is increasing in agriculture and herbicides have significant environmental and human health impact. Risk assessment of chemicals is often based on single species tests under optimal laboratory conditions for tested organisms. One of the major drawbacks of these tests is that they do not account for species biological interaction in community at natural conditions which are often non-optimal. The effects of the sulfonylurea herbicide amidosulfuron on target and non-target organisms: plants (Chenopodium album, Hordeum vulgare; Lemna minor), algae Pseudokirchneriella subcapitata and aquatic microinvertebrates Daphnia magna were tested using single-species and multi-species systems. The terrestrial target Ch. album and non-target H. vulgare plants, growing separately and together in the microcosms at the combination 1:1, were sprayed with herbicide sprays solutions equivalent to 0.001-0.1 of field application rate. The aquatic organisms were affected by the same concentrations of herbicide in their growth medium separately and in the microcosms (L. minor + P. subcapitata; L. minor + P. subcapitata + D. magna). Target species Ch. album had a strong phytotoxic response to herbicide application (inhibition of growth, biomass; reduced content of photosynthetic pigments and increased content of malondialdehyde). Less pronounced but significant effects were also shown by non-target H. vulgare: herbicide treatment has provoked oxidative stress and adversely affected the photosystem.

Some differences between the response of individually grown terrestrial plant species and the same species grown in the microcosms were observed. In two-species tests the impact of herbicide to the growth and photosystems of Ch. album was less pronounced than in single-species tests, as in the microcosms the herbicide at the same time was taken up by Ch. album and H. vulgare. The impact on the growth of non-target H. vulgare was in the same order in both single- and two-species tests. The aquatic organisms were extremely susceptible to herbicide. L. minor was more sensitive than P. subcapitata and the EC50 value for growth inhibition of L. minor was one order of magnitude lower than for algae. The microinvertebrates were acutely affected by herbicide directly in single-species test (mortality was > 80%) and in the microcosms the indirect impact was also observed due to reduced food availability.

WE 371

New born pesticides based in natural products derivatives to ants control with no action to non-target species

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The application of pesticides can control the incidence of insects and diseases in a particular crop, but has a number of undesirable side effects. Residues of chemicals employed can adhere to the plant and contaminate foods, as well as rivers and other water font, when lixiviated to them.

The concept of biorrational pest control, a way to control insects and diseases with the use of natural products and their derivatives, is proposed in order to minimize the environmental impacts. The symbiosis between leaf-cutting ants and the fungus Leucoagaricus gongylophorus can be used to control the ants, since if the fungus die no plants material will transformed in food to the nest and it will be disappear.

Based on this concern, this communication proposed the use of chemical transformation of flavonoid isolated from citrus pulp to control ants without to affect others insect. The flavonoid undergone a conversion of its carbonyl function to an imine. The obtained compound presented high inhibition of L. gongylophorus growth. This compound was complexed to metal and the final compound has better inhibition of L. gongylophorus.

FAPESP, CNPq, CAPES, INCT-CBIP

WE 372

Effect of pesticides used in apple orchards on biochemical properties and predation behavior of the natural enemy Forficula auricularia L.

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In the European context aiming at reducing the use of pesticides, biological control against pests has to be encouraged. Thus studying the effects of agricultural practices on current natural enemies is necessary. Forficula auricularia, a generalist predator, is distributed worldwide, shows high abundance and is active over a long period of the year. In order to assess pesticide impacts, earwig populations were sampled in apple orchards in Provence conducted under different pesticide management strategies. Two different types of biomarkers were used: the activity of B-esterases, acetylcholinesterase (AChE), and carboxylesterase (CaE) as biochemical biomarkers, and predatory activity as new behavioural biomarker. Location and characterization of B-esterase and sensitivity of both AChE and CaE activities to organophosphorus (OP) and carbamate (CA) insecticides were investigated. AChE was preferentially located in the head. CaE activities measured with three different substrates to evaluate multiple isoenzymes, were present in the different body parts (head, thorax, abdomen) but were preferentially concentrated in the abdomen. Both AChE and CaE were concentration-dependent inhibited by OP and CA. In female, CaE activities exhibited a lower sensitivity towards both pesticides compare to AChE. In male, CaE activities are more sensitive to OP than AChE. Predatory activity of earwig at three different stages of development (third and fourth instar larvae and adults) on rosy apple aphid, was investigated after 12h-exposure to pesticides. Four insecticides were evaluated at the normal application rate: Pyrinex (OP), Decis (deltamethrine), Suprem (acetamiprid) and Spinosad (organic insecticide). No difference on the predatory activity of control earwigs was observed depending on the stage of development. Pyrinex and Spinosad affect significantly the predatory activity, while no effect was observed after exposure to Decis and Supreme. All these results suggest a potential role of male CaE in AChE protection toward OP but point out variability in sensitivity depending on sex that may disturb population dynamics of the earwig. Moreover, pesticides modify the predatory activity of earwig, which play an important role in pest control, especially in organic farming. This study emphasizes the need for complementary biomarkers able to assess modifications of behavior that influence natural regulation of pest control.

WE 373

Long-time effect of abamectin on the horse chestnut leafminer (Cameraria ohridella) in leaves of Aesculus hippocastanum following trunk injection

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The trunk application of pesticides is an alternative method for the control of pests and diseases in the canopy of trees at urban sites. But little is known about transport of pesticides into leaves after trunk application. Recently the control of horse chestnut leaf miner was tested with the application of systemic insecticides via trunk spraying and tree tape with different success. Another method is the trunk injection at the beginning of flowering. 120 years old horse chestnut trees (*Aesculus hippocastanum*) were treated once with abamectin in 2004 - 2006. The active substance was injected at one position in a 2 cm deep drilled hole. The efficiency of abamectin against *Cameraria ohridella* was studied several times the year up to now. Residue analyses of the two abamectin homologs, avermectin B1a and avermectin B1b, and also the major photodegradation product 8,9-Z-avermectin B1a were conducted in leaves with LC/MS from 2006 to 2010. Pesticide concentration in leaves is also important from the ecotoxicological point of view to assess the effects on beneficial organisms and on the decomposition of fallen leaf litter. Sufficient control of leafminers was achieved only at one part of the canopy. The leaves at the other part were damaged and fall from trees earlier. The residue analysis confirmed this result caused by the insecticide. Mainly avermectin B1a (B1b and 8,9-Z-B1a only traces) were measured only in leaf samples without damages by leafminers. It seems that leaves were only protected when they provided with xylem sap of the drilled vessels. A horizontal distribution was obviously not occurred in the horse chestnut trunk. But it is remarkable that the control of the leaf miner *Cameraria ohridella* could be observed after only one injection over seven years up to now. Avermectins could be found in leaves over the whole period with maximal concentrations of

approximately 10 mg/kg seven days after application and were reduced on the limit of determination after six years.

WE 374

The effect of insecticide resistance to environment in grape production

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European Grapevine Moth (EGM) is the most common pest in the vineyards of all over the European and Mediterranean countries. The moth reduces yields and quality of vineyards. Insecticides are used season to control the moth in vineyards up to 5 times from mid-season to end of the and effect nontarget organism and pose risk in the environment. The most important danger is insecticide resistance of EGM because of higher application rates and use frequency of insecticides. The aim of this study was to expect the risk factors of the pesticides in the environment, beneficials, bees etc. by using Environmental Impact Quotient (EIQ). The larvae were collected from 3 different places; Alaçehir, Salihli, Sarıgöl, where the intensive viticulture farming of the Aegean region. All population were maintained in a climate chamber. EC50 values of insecticides were measured by using enzymatic and bioassay methods. Due to the occurrence of resistance, how often pesticide applications are required and the effect of this situation on non target organisms will be estimated by using EIQ.

WE 375

Tributyltin (TBT) bioremediation evaluation in soil after *Aeromonas molluscorum* Av27 inoculation

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Due to its wide application on antifouling paints and industrial agriculture processes Tributyltin (TBT) has become the most significant pesticide in marine ecosystems. Despite the global ban of its use on paints in 2008 by the International Maritime Organization, TBT remains a worldwide pollution problem due to its high persistence, toxicity and bioaccumulation. Owing to its high affinity to suspended particles, harbour sediments and sewage sludge can show high TBT contamination and their disposal constitutes an important pathway for this compound to reach soils and affect soil fauna and flora. Considering this, biological degradation seems to be one major tool to remove TBT from the environment. *Aeromonas molluscorum* Av27 is a bacterium that is able to degrade TBT in aqueous solutions into its less toxic byproducts, dibutyltin (MBT) and monobutyltin (MBT). Therefore, it can be used as a powerful natural tool to diminish contamination of TBT in the environment. In this study we aimed to evaluate the ability of *A. molluscorum* Av27 to bioremediate TBT contaminated soil. With that purpose, after remediation of soil by Av27, two bioassays were performed: i) the seed germination and growth test with the rapid cycle turnip *Brassica rapa*, ii) the feeding inhibition test with the terrestrial isopod *Porcellionides pruinosus*. Decrease in soil toxicity was also evaluated using a simple method based on the inhibition of growth of an indicator *Micrococcus luteus* strain. Several trials were carried out using different approaches for *A. molluscorum* Av27 inoculation.

The following results were obtained: in the plant bioassays, in the presence of *A. molluscorum* Av27 it was observed a decrease in the number of germinated seeds however, it stimulated plant growth within the range of TBT concentrations; the feeding bioassay revealed higher consumption ratios on isopods exposed to soil inoculated with Av27, when compared to animals exposed to soil without bacteria, within the TBT concentration range. These experiments can bring new insight for bioremediation methodologies for TBT contaminated soils.

WE 376

Details on occurrence and frequency of guttation in different crops in Germany

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In crop species of economic relevance in Germany, several glasshouse and field trials were conducted from 2009 to 2011 by the Julius Kühn-Institut in Germany and by cooperating research partners (DWD, IfZ, BDP and UFOP) to determine the frequency and occurrence of guttation. Several crop species such as sugar beet, winter oilseed rape, maize, barley, potatoes, oat, onions, carrots, peas and cucumber were investigated and an assessment of the occurrence, frequency and intensity of guttation (size/number of guttation drops, number of guttating plants) in the tested crop species was conducted. Also the formation of dew was assessed. Observations were conducted from the beginning of early plant emergence until at least the flowering stage. During this period, the climatic conditions (relative air humidity, air and soil temperature) were recorded. In the glasshouse trials, the size of guttation drops was determined by weighing of the guttation drops. In the field trials, sky cover and soil humidity were estimated. In the field, the guttation frequency of adjacent vegetation was also assessed in order to facilitate a comparison to the investigated crop species. Daily assessments were conducted in the glasshouse while assessments in the field trials were only conducted under climatic conditions suitable for guttation.

Guttation occurred mainly at young growth stages in the tested crop species. Only maize, cucumber and potatoes produced guttation droplets up to emergence of inflorescence.

Monocotyledonous crop species such as maize and cereals showed a higher guttation frequency than dicotyledonous crops such as sugar beets. Other dicotyledonous crops such cruciferous species (oilseed rape) and potatoes showed a much higher guttation frequency than sugar beets. Even under climatic conditions suitable for guttation, guttation could only be observed on 50 % of the preselected observation days for crop species that showed frequent guttation events in the glasshouse and for the adjacent vegetation.

WE 377

Assessment of exposure to polycyclic aromatic compounds (PACs) in avian embryo hepatic β -oxidation system

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Polycyclic aromatic compounds (PACs) are considered to be ubiquitous environmental pollutants. For instance, many polycyclic aromatic hydrocarbons (PAHs) are well-known to be potentially carcinogenic to humans and ecotoxicological effects have been shown in many studies. However, PAHs can also be oxidized into more soluble oxygenated derivatives (Oxy-PAHs) and these derivatives have been reported to have hormone disturbing potency, showing mutagenicity in bacterial and human cell and also being potentially carcinogenic. Oxy-PAHs form, not only primarily during incomplete combustion, but also following secondary oxidation of PAHs in the atmosphere and during chemical and biological degrading processes of PAH-contaminated soil. Although some studies have addressed questions concerning the health impact of PAHs in avian over the years, the number of studies investigating avian toxicity of oxy-PAHs is practically non-existent. We have designed a method to investigate the effects of organic pollutants such as PACs on the C₁₆ oxidation of fatty acids in hepatic embryonic tissue from chicken (*Gallus domesticus*). Our previous studies provide direct evidence that the metabolic effect on the hepatic C₁₆ oxidation system is clearly reduced after exposure in ovo to a 16 PAHs mix, showing decreased oxidation values compared to control. The focus for this present study is (a) exposure to a 13 oxy-PAHs mix, (b) single exposure to some selected PAHs and (c) single exposure to their oxygenated derivatives, injected separately on day 4 of incubation into the air cell. This is then followed by in vitro incubation of the embryonic livers on day 10 with a tritiated fatty acid mixture (palmitate 16:0). The fatty acids undergo oxidation and repetitive cleavage of carbons before entering the tricarboxylic pathway and cycles of C₁₆ oxidation. As a result of this lipid oxidation tritiated water is produced. The radioactive water (3H₂O) is collected and measured in a scintillator counter. Studies presenting effects on the fatty acid metabolism in avian embryos due to exposure of PACs are absent. Using our assay the effects of PACs and their derivatives on the enzyme function regulating the hepatic fatty acid metabolism can be studied in vitro. This approach could be a very useful trying to find out the mechanisms behind avian toxicity including disturbance of fatty acid oxidation and lipid homeostasis. To the best of our knowledge, this is the first time such a study has been performed.

WE 378

Butachlor DNA binding and genotoxicity in human peripheral blood mononuclear cells

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N-(butoxymethyl)-2-chloro-2',6'-diethyl acetanilide, commonly known as butachlor, is a widely used herbicide in Asia, South America and Africa, for control of a range of annual grasses and some broad-leaf weeds. Extensive use of this herbicide over the years has led to the deleterious effects on non-target organisms. In this study, we have assessed the genotoxic potential of butachlor in human peripheral blood mononuclear cells (PBMCs), using sensitive techniques like single cell gel electrophoresis (comet) assay, cytokinesis blocked micronucleus (CBMN) assay, and flow cytometry. Fluorescence data demonstrated significant quenching at the highest butachlor-DNA molar ratio of 1:2. The fluorescence-quenching constant (K_{SV}), affinity constant (K_a) and number of butachlor binding sites on DNA (n) were determined to be 1.19 × 10⁴ (r² = 0.99), 1.2 × 10⁴ M⁻¹ and 1.02 (r² = 0.99), respectively. The data revealed the binding of butachlor at minor groove of DNA. The results of comet assay showed a dose dependent increase in the size of comet tail with concomitant reduction in head size and exhibited 26-fold enhanced DNA migration. A concentration dependent increase in the total number of binuclear-micronucleated (BNMN) human PBMCs was observed upon 24 h treatment with butachlor. The data of CBMN assay indicated significantly higher (3.77 fold, p<0.05) BNMN formation. The nuclear division index (NDI) of 1.80 was estimated at 100 μM butachlor vis-a-vis 1.92 in untreated control cells. Flow cytometric analysis of butachlor treated PBMCs showed enhancement in fluorescence of both the DCF and Rh123 probes at 25 to 100 μM butachlor due to higher ROS production. Furthermore, at higher concentrations of 250 and 500 μM butachlor, ROS production decreased due to substantial mitochondrial membrane damage and cell necrosis. Also, the cell cycle analysis of PI-stained butachlor treated cells exhibited an increased sub-G1 peak with concomitant reduction in G1 phase, and significant G2/M arrest, as compared with solvent control. Thus, it is concluded that butachlor treatment to PBMCs may induces oxidative stress due to intracellular ROS production, which triggers mitochondrial dysfunction and DNA damage.

WE 379

National requirements in fate and behavior of plant protection products in zonal authorization in Poland

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The new - zonal authorization of plant protection product in Member States considers the submission of the dossier in accordance with proper zone: South, Central or North (Regulation 1107/2009).

The requirements of fate and behavior are defined in the Regulation and in proper guidelines. For assessment of predicted environmental concentration (PEC) in particular

environmental compartment the FOCUS working group has established the tools. For ground water - the PECGW calculations should be done by using of FOCUS PEARL and FOCUS PELMO models. There are more than 10 scenarios, but for Poland only three are obligatory. For surface water the assessment of PECGW and PECSW also should be done by tier approach using the FOCUS tools. The scenarios cover the drift, drainage and run off (D1 - D6 and R1 - R4). For Poland, Central Zone, the D3, D4 and R1 are required.

WE 380

Fate of pesticides and metabolites in the Yangtze River

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The aim of this project is to determine the fate and behavior of a model pollutant in Yangtze water sediment systems.

We investigate the fate of pesticides in Yangtze water and sediment in agriculturally influenced areas of the Three Gorges Reservoir while non-agriculturally influenced areas are used for obtaining control samples. We select propanil as model substance, which is known to release the priority pollutant 3, 4-dichloroaniline (DCA). DCA is also formed by many other pesticides and therefore an important model pollutant for our studies. DCA has been reported to form a toxic and bioaccumulative metabolite in soil and sediment, i.e., 3, 3', 4, 4'-tetrachloroazobenzene (TCAB; H. Chisaka & P.C. Kearney 1970). We will synthesize this azo-compound by chemical means in order to study its fate. We will apply flooding conditions with sediment, with sediment collected in the dry season of the reservoir as well as in the temporarily flooded season, and soil at the river bank as well. We will incubate propanil, DCA and TCAB in water sediment collected from the Yangtze River, applying radioactively labeled compounds in order to establish mass balances.

As preliminary studies we investigated the herbicide clodinafop-propargyl and propanil in water sediment from the river Rhine, two compounds with different physico-chemical properties. Clodinafop-propargyl is moderately adsorbed to the sediment, mineralization was low, and formation of non-extractable residues was also moderate. Besides, first results of the propanil study in water sediment systems have shown that several metabolites are formed that will be identified.

By investigating the fate of a model substance known to form toxic metabolites we will address one important part of the environmental risk assessment which will be complemented by two further projects of our group on the ecotoxicity and the bioaccumulation of the pollutants.

WE 381

Comparison of the dissipation rates of an insecticide in laboratory and field studies

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Historically, laboratory-based water/sediment systems (e.g. OECD 308) have been used to estimate the dissipation rate of an agrochemical in the freshwater aquatic environment. Although these studies provide important information for the regulatory assessment of a substance, for some the dissipation rates generated may be over estimated and unrepresentative of those that are likely to be found in the real world. In the more complex laboratory and field microcosm systems, many factors can influence the rate of dissipation from the water column and the extent of its adsorption onto sediment. We have compared the published dissipation rates of an insecticide determined in OECD Method 308 and those found in a sediment:water laboratory microcosm study, with the dissipation rates and distribution of the substance that we determined in field microcosms.

The field microcosm study was conducted in four outdoor freshwater microcosms (water depth: 30 cm) which were designed to represent the worst case of a shallow static or slow-flowing ditch. Each microcosm was established in early Spring by the addition of a clay base sediment, a layer of lake sediment (approximately 5 cm depth, 4% organic carbon) and a diverse community of aquatic macrophytes planted along one axis. Continuous aeration allowed the establishment of similar and stable invertebrate communities and physico-chemical conditions. Approximately 14 days before the substance was applied, a number of aluminium trays containing further sediment were inserted into the base layer of each microcosm to aid sediment removal during the course of the study. Following the application of the insecticide, samples of water, sediment and aquatic macrophytes (Elodea) were taken for analysis by liquid chromatography with tandem mass spectrometry (LCMS-MS) and the results compared to those obtained in OECD 308 and the laboratory microcosm study.

The DT50 from the water column in the field microcosm study (ca. 21 days) was approximately half of that found in either the OECD 308 study or the laboratory sediment:water microcosm (48 - 49 days). Adsorption onto plant material was found to be a major factor responsible for the initial rapid dissipation of the substance from the water column followed by adsorption and degradation in the upper layers of sediment. Our poster compares the physico-chemical properties of each test system and examines the implications of the results to the design and analysis of field microcosm studies.

WE 382

Determination of environmental levels of glyphosate and AMPA in water, sediment and soil samples of the Pampas Region, Argentina

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In the last 30 years, agriculture in Argentina and other developing countries has markedly expanded. In particular, the surface of soybean crop has increased 10-fold since the 80's. The Pampas Region is the main agriculture district of Argentina. According with current agriculture practices, Glyphosate is the major soybean herbicide. Due to the molecular multifunctional structure, this compound presents a complex environmental behavior. Its speciation and complex coordination depends on the pH, MO and free ions (i.e. metals). Regional variations in the nature of the chemical matrices demands adjustments of the analytical methods for glyphosate determination depending of the soils and water chemistry. The aim of this presentation is to report environmental levels of glyphosate, and its major metabolite the aminomethylphosphonic acid (AMPA), obtained in the last five years using methods optimized in our laboratory for the analysis of water, sediment and soil samples of the Pampas Region, Argentina. Detection method was based on HPLC-MS after alkaline derivatization using FMOC-CL. Isotopic tracers were used for quality assurance. A total number of 250 water samples (surface and groundwater) and 70 sediments and soil samples were analyzed. Chromatographic conditions, particularly MeOH/H₂O gradient, were critical for eliminating matrix interferences. The highest instrumental sensitivity was obtained using ESI interface in negative mode, monitoring the M-H⁺ product of the derivatization (glyphosate-FMOC: m/z 390, AMPA: m/z 332). Detection limits for both analytes were 0.5 µg/l for water samples and 5 µg/Kg for soils and sediments. Recoveries were higher than 95% for water samples and between 85-95% for the solid matrices. In surface waters, 25% of the samples were positive with concentrations between 1-400 µg/l. None of the groundwater samples yielded positive results. Levels in soils and sediments were between 2-300 µg/Kg, with 40 and 10% of the samples presenting levels above the detection limit, respectively. Optimized methods were suitable for detecting glyphosate and AMPA in different environmental matrices, demonstrating the occurrence of these compounds in surface waters, sediments and soils of the Pampas Region. On the other hand, glyphosate and AMPA, seems not to readily leach and reach groundwater.

WE 383

Distribution and degradation of triclosan in a pond mesocosm system

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The antibacterial and antifungal agent triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is a registered pesticide. But it is more frequently used in consumer products such as detergents, toothpaste, cosmetics, and antibacterial textile coating, even though it does not provide any extra benefit to health with the exception of toothpaste (US Food and Drug Administration (FDA), 2010, [1]) and concentrations above effect levels have been measured in surface waters [2].

Triclosan is highly toxic to algae and various microbial species. Both triclosan and the metabolite methyl triclosan have a high potential to bioaccumulate in aquatic organisms [2]. The effects of triclosan on single species are well studied. However, knowledge on fate and distribution in water, sediment, and biota is scarce.

In 2011, a combined fate and effect pond mesocosm study was carried out by the German Federal Environment Agency. After single dosing of the free water, concentrations of triclosan and the transformation products methyl-triclosan and 2,4-dichlorophenol were measured for 120 days in the compartments free water, sediment, aufwuchs, macrophytes, and snails (*Lymnea stagnalis*).

Besides dissipation curves of triclosan and the metabolites in water and sediment, first results of selected measurements in biota will be presented.

WE 384

Current-use pesticides (CUPs) in surface sediments from the Bohai and Yellow Seas, North China

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With the goal of reducing human and ecosystem exposure, nine organochlorine pesticides (OCPs) included in the UNEP Stockholm Convention POPs list are eliminated or undergoing substantial reduction of consumption. The levels of these OCPs are declining gradually in Europe, North America and the Arctic. However, some current-use pesticides (CUPs) have been observed not only in or close to agricultural areas where they are applied but also in remote areas (high mountains and the Arctic) where no pesticides have been used. Environmental fate and impact of CUPs deserve more concerns. Until now, data of CUPs in the marine environment are relatively sparse.

To improve our knowledge of CUPs' fate in the marine environment, surface sediment samples from the Bohai and Yellow Seas were analyzed for 7 CUPs (trifluralin, endosulfan, chlorpyrifos, chlorothalonil, dicofol, dacthal, quinozole) and 1 metabolite (pentachloroanisole). Samples were collected during 2009 to 2010 using a grab sampler or a box corer, and then freeze-dried, homogenized and Soxhlet extracted for 16 h using dichloromethane. The extracts were reduced to 2 mL using a rotary evaporator and further purified on a silica column. Elution started with 20 mL hexane (fraction 1), followed by 30 mL dichloromethane/acetone (1:1) (fraction 2), the extracts were evaporated both of fraction 1 and 2 to a final volume of 30 µL. Then all samples were measured with a GC/MS-system in electron capture negative chemical ionization mode (ECNCI).

Levels, spatial distributions and possible sources of these pesticides will be discussed in this study. To better understand the environmental behavior of CUPs, some legacy organochlorines, such as Hexachlorobenzene (HCB) and hexachlorocyclohexanes (HCHs) were also analyzed for comparison with CUPs.

WE 385**Fate and behaviour of the strobilurin fungicide Azoxystrobin in four UK soils under laboratory conditions**K.A. Kostas¹, K.A. Fenlon², K.T. Semple², K.C. Kevin³¹CYPRUS UNIVERSITY OF TECHNOLOGY, Lemesos, Cyprus²Lancaster University, Lancaster, United Kingdom³LANCASTER UNIVERSITY, Lancaster, United Kingdom

Studies investigating the fate and behaviour of azoxystrobin in natural soils are limited. This study aimed to investigate the fate and behaviour of azoxystrobin in four natural soils under laboratory aerobic conditions and for a period of 100 days. Fate and behaviour in this study were described in terms of losses due to biodegradation and extractability into three extractions solutions and the respective formation of non extractable residues. Losses of the azoxystrobin were observed in all four soils and were ranged from zero to approximately 23% of the azoxystrobin associated 14C-activity. Extractability of the azoxystrobin showed to diminish with incubation time. Non extractable 14C-residues increased with the incubation time and at the end of the incubation period more than 75% of the azoxystrobin associated 14C-activity was not extractable with CaCl₂ or HP-GCD. The mineralisation extent in all soils was very limited. This study illustrates the variability of the azoxystrobin fate and behaviour in four natural soils. Biodegradation, sorption/desorption and experimental set up affect the fate profile in lab exercised experiments and thus special consideration is required when interpreting such data for risk assessment based studies.

WE 386**Plot-scale runoff study using portable rainfall simulator**

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Short-term, edge-of-plot runoff collection studies provide many challenges due to design constraints, and also environmental factors. While developing a proper study design is crucial, designing and creating equipment and instrumentation to achieve the necessary objectives is of great importance. This plot-scale runoff study employed a low maintenance, portable rainfall simulator along with custom runoff collection systems for each plot. In addition, a quick-install wind shelter was utilized when necessary. The study design used 3 plots per replicate, with each plot measuring approximately 1.5 m (5 ft) wide by approximately 6 m (20 ft) long. Replicate blocks were as similar as possible with respect to soil and topographic parameters. Each test plot was bordered by an untreated soil area, and isolated by metal flashing. The overall slope of the sites tested ranged from 2 to 5%, with the length of the plots parallel to the slope of the field. The plots were subjected to a simulated rainfall event to generate runoff (i.e., approximately 2.5 cm (1 in) of rainfall per hour). This study was conducted at multiple sites, with the treated plots at each site receiving simulated rainfall using the same simulated rainfall parameters, to provide storms of similar intensity and duration. Runoff generated during the simulated rainfall events was manually collected on timed intervals and as a complete volume of runoff.

WE 387**Distribution of aged 14C-atrazine residues in soil following 22 years of environmental exposure**K.A. Kostas¹, N.D. Jablonowski², K.C. Kevin³, K.T. Semple⁴¹CYPRUS UNIVERSITY OF TECHNOLOGY, Lemesos, Cyprus²Agrosphere Institute, ICG IV, Forschungszentrum, Jülich, Germany³LANCASTER UNIVERSITY, Lancaster, United Kingdom⁴Lancaster University, Lancaster, United Kingdom

Distribution and localisation of pesticide residues into the different physical fractions of soil may reveal information on processes taking place in soil. Soils amended with 14C-atrazine, were aged for 22 years under environmental conditions in a lysimeter in Germany. The soil was sampled and subjected to physical and chemical fractionation before and after incubation with the earthworm *Apporectodea caliginosa* for 7 days. No significant change in the soil physical and chemical fractionation of the 14C-atrazine residues and organic carbon was observed in this study as a consequence of the activity of the *A. caliginosa*. The low organic carbon of the soil, the absence of relatively fresh organic matter and the long ageing time might explain the limited bioavailability of the 14C-atrazine to the earthworm. Approximately 9% of the applied 14C-atrazine associated activity was found to be present in the top soil layer 0-10 cm of the lysimeter. This amount represented approximately 36% of the total amount of 14C-atrazine associated activity presented in the soil after 22 years of environmental ageing. Soil half lives calculated in this study taking into account the non-extractable 14C-atrazine residues were much greater than those reported from previous studies. This finding is of particular importance given that the soil used here was aged under natural environmental conditions compared to laboratory studies.

WE 388**Earthworm tolerance to residual pesticide contamination in agricultural landscapes : from field assessment to physiological responses**N. Givaudan¹, C. Wiegand², B. Lebort³, F. Binet¹¹Université de Rennes 1, Rennes, France²University of Southern Denmark, Institute of Biology, Odense, Denmark³Ecole des Hautes Etudes en Santé Publique, LERES, Rennes, France

Worldwide use of pesticides in modern conventional agriculture has led to chronic contaminations of cropped soils and thereby of their biota such as earthworms (Lumbricidae). In particular, the fate and environmental relevance (in terms of biological activity) of pesticide residues for soil organisms has been largely debated due to the formation of bound residues. The ability of earthworms to cope with such xenobiotics, e.g tolerance, is reflected by their ubiquitous presence in agricultural fields, albeit in reduced numbers and lower species diversity. Tolerance to contaminants can induce compensatory mechanisms in the energy metabolism of organisms due to detoxification and excretion, as well as damage mitigation due to oxidative stress. Such physiological costs for individuals have been shown in laboratory studies but scarcely in field-based assessments. In a field study, we propose to analyse residual pesticide levels in 5 agricultural soils along a gradient of pesticide input and link them to detoxifying and antioxidant enzyme activities, along with energy reserves, in three earthworm species (two endogeic and one anecic species). On each field plot (three conventional fields, an organic cultivated field and an organic permanent pasture), soil cores were sampled along with earthworms at five 5x5 m sampling points. On the 57 different active molecules applied on the fields during the last 10 years, up to 14 molecules and three metabolites were selected for analysis in the five soils. Then physiological parameters such as enzymatic activities related to detoxification and oxidative stress mitigation (glutathione-S-transferase and catalase), along with energy resources (glycogen and lipids) were measured in individual animals. With regard to the beneficial effects of earthworms for the soil, these results will help to understand the impacts of multiple residual contaminants on living lumbricid populations.

ET18P - Tropical ecotoxicology**WE 392****Threats posed by chemical use in tilapia cage aquaculture in Southeast Asia: the case of the Moon River (Ubon Ratchathani, Thailand)**R. Oliveira¹, S. McDonough², A. Rico³, A.M.V.M. Soares⁴, A.J.A. Nogueira⁵, I. Domingues¹¹Universidade de Aveiro, Aveiro, Portugal²School of Environment, Resources and Development, Asian Institute of Technology, Pathumthani, Thailand³Aquatic Ecology and Water Quality Management Group, Wageningen University, Wageningen, Nederland⁴University of Aveiro & CESAM, Aveiro, Portugal⁵CESAM & Department of Biology, University of Aveiro, Aveiro, Portugal

Aquaculture is one of the most effective alternatives to meet the growing demand of food by the world population. However, modern aquaculture faces new challenges such as maintaining both profitability and environmental sustainability. In this study, we performed a survey to obtain baseline information on the current status of chemical use and aquaculture practices on 15 tilapia cage farms located in the Moon River (Maenam Mun) located in the Ubon Ratchathani Province in north-eastern Thailand. The data collected in this survey included information about routinely applied chemical products and dosages, socio-economic data and infrastructure of the studied farms. A total of 37 chemical products divided into three main groups were identified: i) antibiotics (used by the 56.8% of the interviewed farmers); ii) parasiticide compounds such as disinfectants (5.4%), fungicides (2.4%) and anthelmintic compounds (2.4%); and iii) food supplements, including probiotics (18.9%) and vitamins (13.5%). Antibiotics applied mixed with feed or in bath treatments were the most commonly used group of compounds, with 16 different products. Oxytetracycline (OTC), one of the most commonly used veterinary antibiotic worldwide, was the most commonly used by the interviewed farmers. In addition, it was noticed that OTC was often applied simultaneously with enrofloxacin and sulphonamides. Several imprudent use practices were identified such as the non-use of masks or gloves while handling or applying the drugs, inadequate storage, and disposal of empty packages on the river banks and reuse of empty packages for food storage. Considering the extensive use of chemicals at the studied area, a more restrictive use of antibiotics (e.g. avoiding prophylactic use) and a more intensive use of probiotics, vitamins and natural products are strongly recommended for preventing negative impacts on human health. In addition, antibiotics can persist in the sediment of aquatic environments for months, can induce the development of bacterial resistance and pose toxic effects for non-target organisms inhabiting surrounding freshwater ecosystems. Further research should focus on assessing the effects of single-compound and mixture exposures of antibiotics on tropical freshwater ecosystems. In this presentation, the results of the chemical use survey will be presented together with the results of an antibiotic residue monitoring campaign carried out during the same time the surveys were conducted.

WE 393**Comparative biomarkers responses of molluscs exposed in situ to different effluents in a tropical zone**J.C.L. Ladewig¹, F. Morgado¹, J. Rendón-von Osten², A.M.V.M. Soares³¹Aveiro University, Aveiro, Portugal²Epomex (Centro de Ecología, Pesquerías y Oceanografía del Golfo de México), Campeche, México³University of Aveiro & CESAM, Aveiro, Portugal

Bivalve molluscs are organisms widely used to assess stress and chemical contamination in the aquatic environment. In order to assess the health of aquatic organisms, biomarkers that measure changes at the biochemical, cellular and physiological levels have been used as effective early warning tools in ecological risk assessment and marine

environment monitoring. Some of the most successful types of biomarkers are those linked to the physiology responses which will offer a very high level of sensitivity, making it possible to detect a low-level cellular response to organic or inorganic pollutants. The potential toxic effects of some effluents were investigated using transplantation of bivalves (*Crassostrea virginica* and *Isognomon alatus*) in two scenarios of the Gulf of Mexico (lagoon and marine) during an experiment of three months, this effluents have a historical pollution through pesticides. To achieve these objectives a battery of biomarkers were analysed in the digestive tract of this bivalves and representative biomarkers from different metabolic pathways were chosen (acetylcholinesterase (Ache), catalase (CAT), lactate dehydrogenase (LDH), glutathione-S-transferase (GST), lipid peroxidation (LPO), superoxide dismutase (SOD) and determination of ethoxy-resorufine-O-de-ethylase (EROD)). The molluscs showed inhibition of the oxidative stress enzyme CAT and for the GST. No changes in LDH activity were noticed in the results. No significant differences were noticed to the values of Ache and LPO. The response of EROD and SOD showed a diversity of responses during the time of the experiment in situ. EROD is a biomarker more sensitive due to its linkage to the genetic response of an organism to chemical pollutants, due to that it is a better tool to assess the effect of the effluents. Linkages between the enzymatic effects found and parameters at higher levels of organization are essential for better understanding the mechanisms of adaptation and detoxification of molluscs in the environment.

WE 394

Changes in Glutathione-S-transferase levels and Lipid Peroxidation in Amphibians exposed to sub lethal concentrations of lead

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The study evaluates changes in glutathione-S-transferase (GST) and lipid peroxidation levels in the liver of *Hoplobatrachus occipitalis* and *B. maculatus* exposed to lead. The amphibians were exposed to sub lethal concentrations (0.25, 0.50, 1.00 and 2.00 mg/l) of the heavy metal in the laboratory. There were three replicate tanks per treatment and three individuals per tank including control groups. Hepatic GST levels and lipid peroxidation were determined after 28 days of exposure. There was a dose-dependent increase ($p < 0.05$) in GST relative to the control groups in both amphibians studied. This could be due to increase in production of the enzyme since it is used as a catalyst in the degradation of leucotrienes. Lead-induced tissue inflammation is primary to tissue death via apoptosis. Thiobarbituric acid reactive substances (TBARS) which is an index of lipid peroxidation increased as concentration of lead increased. The increased level of TBARS in the liver of frogs exposed to lead was an indication of increased membrane lipid peroxidation which could lead to cell damage. The release of lead into the environment could possibly affect the levels of GST and the health of amphibians where they live. It is therefore imperative to protect amphibians from lead pollution.

WE 395

Using whole-sediment mesocosm respiration rates and nutrient fluxes as a measure of community functional response to environmental stress in urban streams in Bogota, Colombia

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Urban streams in Colombia, such as the Rio Bogota and its tributaries, are severely contaminated by coarse garbage, oxygen-consuming organic material, sediments, metals and hydrocarbons, due to inadequate wastewater treatment and a predominance of combined sewage and stormwater lines. As a consequence, biochemical oxygen demand tends to be high and macrobenthic species diversity tends to be low and restricted to hardy and/or opportunistic species, such as oligochaetes, diptera larvae, amphipods and leeches. While individual species of these high-stress-adapted communities generally show low sensitivity in acute whole effluent toxicity tests (in terms of survival), their metabolic performance tends to be more sensitive to environmental stress and other ambient conditions. This study evaluated how whole-sediment respiration and remineralization rates (i.e. nutrient fluxes) of urban macrobenthic communities respond to added environmental stress, such as elevated temperature, wastewater discharge and hydrocarbon pollution. For this purpose, sediment cores, collected from a variety of urban streams from Bogota, Colombia, were incubated in benthic mesocosms under varying environmental conditions, to determine oxygen consumption and nitrate fluxes as an integrated measure of micro- and macrobenthic community function. If whole sediment respiration and remineralization rates respond in a dose-dependent way to environmental stress, this integrated functional response can be used as a gauge to benchmark the viability and vigor of the corresponding benthic community.

WE 396

Use of bioindicators for assessing the health status of fish

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The reservoir of the hydroelectric plant Corumbá IV is located in Corumbá river Brazil (16 °20'47"S 48°10'44' W) with a surface area of 173km² at a maximum normal operating and total volume of just under 3,7billion cubic meters. The reservoir has notorious environmental services, social and economic by providing tourism, fisheries production, real estate valuation and leisure and contributing to regional development and constituting a refuge for biodiversity. Upon the occurrence of episodes of fish mortality a study was carried out on the health status of different species. Fish were collected from the place of occurrence of mortality in the reservoir toward the river Descoberto one of the main tributaries of the reservoir. Samples were collected with net and periodic inspections and fixed for taxonomic identification and light microscopy analysis. The characterization of physical and chemical (N, pH, COD, BOD e P) and microbiological (cyanobacteria) quality of water was performed to identify possible causes of histopathological lesions. The species collected were: *Pimelodus maculatus*, *Pimelodus blochii*, *Leporinus friderici*, *Geophagus brasiliensis*, *Hypostomus emarginatus* and *Hypostomus plecostomus*. The analysis attested to the occurrence of histopathological lesions of different degrees of importance in liver and gills, as proliferation of melanomacrophages, necrosis, aneurysm and neoplasm. The liver damage most observed was the proliferation of melanomacrophages, present in 100% of the total sample and being suggested their use as bioindicators. Among the most serious neoplastic foci were observed in individuals of the genus *Hypostomus*. The highest average lesion index (Index= 9.4) was observed in area associated with livestock activities. River Descoberto showed a mean lesion index equal to 8.3 to the liver and 5.3 to gills. The highest lesion index was associated with the microbiological conditions of the water especially with the presence of cyanobacteria 400cells/mlwater, the high pH(>9) and BOD (35mg/L) values above the standard established by the National Environment Council - CONAMA. Among the preventive measures for the good health of fish include: improving the water quality of tributary; controlling activities around the reservoir and the use of bioindicator. Financing: Corumbá IV Concessões; FINATEC.

WE 397

Embryo toxicity of 17a-methyltestosterone used to induce male monosex in freshwater aquaculture

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The 17 α -methyltestosterone (MT) is used in worldwide to induce sexual reversion in tilapia aquaculture. The use of MT in aquaculture differs from each country; in big fish producers such as China the use of MT is banned but in others like Thailand its still used. Nowadays, in Brazil the MT is illegally used in aquaculture since MT products are not regulated by national authorities for these purposes. MT is well known by its androgenic effects on different fish species however the toxicological adverse effects of this synthetic hormone on early life stages fish species are not reported yet in the current literature. The present work aims to assess the effects of MT (commercial product used in Brazilian aquaculture from Bioactive - 90%) (cMT) and active ingredient (aMT) from Sigma Aldrich - 99.9% on zebrafish embryos. In order to archive this objective several embryos assays were performed based on the OECD draft guideline on Fish embryo Toxicity (FET) Test using the same concentrations for cMT and aMT, 5, 7, 9, 11 and 13 mg/l. The FET test was extended up to 6 days and during this period lethal (mortality) and sub lethal (development) endpoints were evaluated. A second test were performed using the same sub lethal range of concentrations (0.004, 0.02, 0.14, 0.83, and 5 mg/l) of each compound to verify alterations in biomarkers activity, namely cholinesterase, glutathione S-transferases and lactate dehydrogenase levels. Our results showed high toxicity of aMT at the first day of exposure (24h-LC50 11.34 mg/l) but exposure of 4 days both cMT (96h-LC50 10.30 mg/l) and aMT (96h-LC50 10.85 mg/l) showed similar values of toxicity. Furthermore, a similar pattern of developmental alteration such as delay in head developed, skin ulcerations, and cardiac edema were found for cMT and aMT in exposures over than 9 mg/l. Also the hatching rate was inhibited at the day 3 in the 7mg/l exposure treatment, considering both MT presentations. A consistent inhibition of ChE and LDH were found at 5 mg/l to both compounds tested (cMT and aMT). The inhibition of ChE could cause a disruption of the nervous system function and the LDH inhibition suggests an action of aMT and cMT on energetic metabolism pathways of exposed embryos. In general, our results have shown similar effects between both presentations of tested MT. In conclusion, MT is highly toxic for zebrafish embryos causing enzymatic changes, delayed hatching and embryonic malformations.

WE 398

Theoretical study of the interaction of herbicide glyphosate and its degradation product Ampa (Aminomethylphosphonic Acid) on kaolinite

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Glyphosate is a nonselective herbicide action, which acts against weeds. It acts by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase that acts in the synthesis of the plant's essential amino acids essential to the plant. This work brings a study of the glyphosate interactions of glyphosate and (his) its degradation product AMPA (aminomethylphosphonic acid) with kaolinite. Computational chemistry's tools were used to perform the theoretical analysis, molecular dynamics and quantum mechanics (DFT). The results suggest that glyphosate interacts strongly with clay minerals (energy of interaction 18kcal/mol), going with the shortest distance between the P = O bond and the hydrogens of the octahedral surface of kaolinite.

WE 399

Effects of ametryn sugarcane herbicide on early-life stages and adults of a tropical fish (Danio rerio)

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Ametryn is one of the widely used herbicides applied on sugarcane crops in Brazil to control weeds that can compromise their productivity. Ametryn belongs to the group of triazines, aromatic herbicides with high water solubility, which can enter in aquatic environment through runoff or atmospheric deposition and have potential to contaminate aquifers. In temperate climate, risk assessment of triazines showed no significant risks to aquatic organisms, but for tropical waters information is absent. The objective of this study was to evaluate the toxicity of ametryn in acute exposure of zebrafish and quantify biochemical markers (GST, CAT, LDH and CHE) as indicators of exposure to this herbicide. The test with embryos was conducted following the OECD's protocol for the Fish Embryo Toxicity (FET) Test, 2006, and for adults, the protocol adopted was OECD TG 203, 1992. To determine the toxicity of ametryn for embryos we used the following treatments: 0 (control), 0 + (solvent control), 10.0, 16.8, 28.3, 47.6 and 80.0 mg L⁻¹ of ametryn. For adults, the treatments were 0, 0 +, 2, 4, 6, 12 and 20 mg L⁻¹ of ametryn. Finally, to determine the biomarkers' activity on early-life stages we choose lower concentrations of ametryn to avoid mortality. The treatments used were 0, 0 +, 5.0, 10.0, 7.17, 10.10, 14.20 and 19.99 mg L⁻¹ of ametryn. The lethal concentration for 50% of the population in 96h of exposure (LC_{50-96h}) was calculated with the help of ToxCalc spreadsheet developed in Microsoft Excel software. For data analysis of enzyme activity, we used the One-Way ANOVA test, of SigmaPlot 11.0. LC_{50-96h} for the zebrafish early-life stages was 48.46 ± 2.20 mg L⁻¹, while for adult fish it was 7.65 ± 1.91 mg L⁻¹. Regarding biomarkers, we observed an increase in GST and inhibition of CHE activity in both larvae and adult fish exposed to ametryn. Furthermore, CAT and LDH activities were not significantly affected by ametryn exposure. The parameters evaluated in this study allowed a better understanding of the mode of action and toxicity of ametryn to *Danio rerio*. Currently, a link between endpoints and the fitness of individuals is still missing but would be a prerequisite for a sound sub organismal endpoint to be considered in risk assessment. Therefore, we conclude that biomarkers provide useful information to assess the risk associated with exposure of fish to ametryn, at sublethal levels.

WE 400

Toxicity and accumulation of cadmium and lead to the tropical snail *Archachatina papyracea*

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Snails are important link in the transfer of contaminants especially metals in the food chain, yet few studies have examined the toxicity and accumulation of metals in snails. In this contribution, the toxicity and accumulation of two non-essential metals (cadmium and lead) to the tropical snail *Archachatina papyracea* were assessed. As far as we know, this is the first study assessing metal toxicity to this tropical snail species. Specimens of the snail *A. papyracea* were exposed in a natural soil collected from Ile-Ife, Nigeria and spiked with varying concentrations of Cd and Pb over a 28-day period. Survival and weight change of snails were monitored weekly while tissue accumulation was assessed at the end of the 28-day period. Survival was a more sensitive endpoint than weight change of snails. The LC50 (effect concentration at which 50% of snails died) value (with confidence interval, CI) for Cd was 87 (75-100) mg/kg while the EC50 (effect concentration at which there was 50% change in an effect parameter) for weight change could not be computed since no substantial mean weight change difference was observed between the snails in control and all other concentration of Cd. For Pb, LC50 value (with CI) was 2617 (1840-3720) mg/kg while the EC50 value (with CI) for weight change was higher at 4159 (3033-5702) mg/kg. Therefore, Cd was a factor of about 30 more toxic than Pb, consistent with findings on relative toxicity of Cd and Pb to other soil organisms including earthworms, springtails and mites. Although not included initially as an endpoint, egg production in the snails decreased with increasing Cd and Pb concentrations in the substrate. Metal analysis of the foot and visceral mass of surviving snails showed progressive accumulation of Cd and Pb as concentration increased showing the tendency to use body residue of *A. papyracea* as an indicator of metal pollution. It further suggests the role of this snail species in above ground metal transfer in the food chain, and highlights potential danger for human consumption.

WE 401

Biomagnification of mercury through a subtropical marine food web

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To assess the efficiency of mercury trophic transfer in coastal waters off Southwest Florida, total mercury (THg) and nitrogen stable isotopes (δ¹⁵N) were determined in 320 tissue samples from 57 species of invertebrate (17 species) and finfish (40 species). Species mean THg concentrations (log 10 transformed) were positively correlated with species mean δ¹⁵N (p<0.001, r² = 0.66); having a slope of 0.21. When the δ¹⁵N of the calico scallop (*Argopecten gibbus*), as a primary consumer, was used to convert δ¹⁵N to trophic level (TL), values ranged as high as 4.15 for both Spanish mackerel (*Scomberomorus maculatus*) and crevalle jack (*Caranx hippos*). A food web magnification factor (FWMF) of 5.05 was calculated from the relationship between species mean THg concentrations (log 10 transformed) and TL. The δ¹⁵N-derived slope and FWMF were comparable to the values reported from other regions and ecosystems, but appeared to be slightly higher than values for other marine systems. The basal THg value, calculated as 0.00064 mg/kg, was also higher than other marine systems. These results suggest that the relatively high Hg levels found in SW Florida are not just a result of efficient transfer but also how much methylmercury enters the base of the food web.

WE 402

Sediment toxicity of port areas in equatorial regions: state of Ceará, Brazil

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Ports and harbors are often associated to pollution on coastal areas due to their discharges and the proximity to industrial and urban contamination sources. Thus, they affect the environmental quality, including the sediments and associated biological communities. This study aimed to assess the sediment toxicity and its relationship with contaminants for two ports located at the state of Ceará: Mucuripe and Pecém Ports. Sediment samples were evaluated through two lines-of-evidence: (1) acute toxicity of whole sediment to *Tiburonella viscana* and chronic toxicity of liquid phases to sea urchin *Lytechinus variegatus* embryos (sediment-water interface and elutriates); (2) environmental characteristics: grain size distribution, organic matter (OM), total organic carbon (TOC), total nitrogen, total phosphorus, Al, Fe, Hg, Cd, Cr, Cu, Ni, Pb, Zn, Polycyclic aromatic hydrocarbons (PAHs) and Linear alkyl benzenes (LABs) contents. In Mucuripe, contamination was associated to depositional areas, and toxicity was associated with the contaminants. Contamination was indicative of external anthropic origin. In Pecém, the most external areas sediments were toxic; however, the relationship of toxicity with the contamination could not be well defined, due to interfering factors during the bioassays, such as ammonia and sulfides. However, the occurrence of deposition and enrichment of some contaminants could be detected. This study is the first concerning to sediments toxicity in these areas and the results provide a subsidy for understanding the quality of the sediments along the northeastern Brazilian coast.

WE 403

Sensitivity of freshwater organisms to cadmium and copper

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A study was conducted in the laboratory to determine acute toxicity of heavy metal cadmium (Cd) and copper (Cu) to six species of freshwater organisms i.e. one species of shrimp (*Macrobrachium lanchesteri*), two species of fish (*Rasbora sumatrana* (Seluang) and *Poecilia reticulata* (guppy)), one species of gastropod (*Melanoides tuberculata*), one species of ostracod *Stenocypris major* and one species of midge (larvae) *Chironomus javanus*. All organisms were exposed for a four-day period in the controlled laboratory condition to a range of Cd and Cu for 96 hours. Mortality was assessed and median lethal concentrations (LC50) were calculated. 96 hours LC50 for *M. lanchesteri*, *R. sumatrana*, *P. reticulata* and *M. tuberculata*, *S. major* and *C. javanus* of Cd and Cu were 7, 225.9, 168.1, 1491.7, 13.1 and 59.7 µg/L, and 32.2, 5.6, 37.9, 143.8, 25.2 and 171.3 µg/L, respectively. This study indicated that prawn *M. lanchesteri* and *R. sumatrana* (Seluang fish) was the most sensitive to Cd and Cu, respectively. Copper was more toxic than Cd to the fish and mollusc; and Cd was more toxic to the prawn, ostracod and midge.

WE 404

Combined effect of sublethal Cr(VI) concentrations, eutrophication, and competition with the exotic cladoceran *Daphnia curvirostris* on *Ceriodaphnia dubia* demography under laboratory conditions

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Exotic filter-feeding species may elicit environmental stress on native species in zooplankton communities. *Daphnia curvirostris* is a large-sized cladoceran found widely in Europe and northern Asia, which has also been collected in Canada and the northeastern US. Recently this species was recorded for the first time in the Mexican Plateau (in the "Chimaliapan" wetland) and is possibly also present now in North Mexico. The site where it was collected receives chromium discharges from tanneries and is remarkable for the high population densities observed, which are much higher than those documented for other cladocerans such as *Ceriodaphnia dubia*. Furthermore, since most shallow water bodies in Mexico sustain eutrophication, native filter-feeding zooplankters may be facing the effects induced by several stressors acting concurrently. To evaluate the combined effect of three environmental stressors (variable food concentration, competition, and toxic pollution), a life table study was designed. Three food concentrations (low, intermediate and high), two sublethal Cr(VI) concentrations (6 and 15 µg L⁻¹), and combined breeding with the exotic cladoceran *D. curvirostris* in a factorial experiment were tested, using an endemic strain of *C. dubia*; all experiments were performed at 25 °C. The presence of *D. curvirostris* had a negative effect on population development in *C. dubia*, which may be related to the size difference between the two. The lifespan was comparatively longer in *D. curvirostris* than in *C. dubia*, but the life cycle of both was shortened at high food concentrations (simulated eutrophication conditions). Although the Cr(VI) concentrations used are lower than the limit established by the official Mexican norm, they induced negative effects on all demographic parameters in both cladocerans. Effects induced on *C. dubia* reproduction and longevity at the higher concentration of Cr(VI) were reduced by increased food concentration, while *D. curvirostris* was more tolerant at the intermediate food concentration. Food availability and Cr(VI) concentration elicited significant effects (P<0.05) on the longevity and life cycle of both cladocerans, but a significant interaction between both factors was also observed. In conclusion, *D. curvirostris* exhibited both competition and tolerance in order to ensure its dispersal in polluted tropical environments sustaining eutrophication.

WE 405

Oxydative stress biomarkers and biological indices in the giant clam (*Tridacna maxima*) and the reef fish (*Epinephelus merra*) as tools for the assessment of marine water

quality of Moorea Island

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Lagoon and coral reefs are ecologically sensitive ecosystems threatened by increased anthropogenic activities (urbanization, mass tourism[3DOTS]). The input of chemical pollutants through sewage, fertilizers and other human wastes has been shown to play an important role in reef degradation (Juhász et al, 2010). Since mollusc bivalves and fish are recognized as bioindicators of environmental quality, the giant clam *Tridacna maxima* and the honeycomb grouper reef fish *Epinephelus merra* were used in this study as they represent abundant and economically important species of the French Polynesian Moorea Island reef ecosystem. In this work, biological indices and three oxidative-stress biomarkers: catalase (CAT), glutathione-S-transferases (GST) and thiobarbituric acid reactive substances (TBARs) levels, were measured in the hepatopancreas and the muscle of *T. maxima* and in the liver and the muscle of *E. merra* collected in April 2011 from two sites differing by their level of contamination (the bay of Cook and Vaïare). The bay of Cook located on the north coast of the Island close to a Marine Protected Area was selected as the reference site whereas Vaïare, located in the main harbour on the east coast of Moorea represented the impacted site. No significant differences between sites were observed for Condition Index (CI) and Hepatosomatic Index (HSI) of both species. Concerning *E. merra*, total length and total and liver weights were significantly higher in fish from the reference bay of Cook site compared to the impacted Vaïare site. For both species, significant higher levels of the three biomarkers studied (CAT, GST, TBARs) were observed in the hepatopancreas of clams and in the liver of fish compared to muscle. Significantly higher CAT activity and TBARs levels were observed in the hepatopancreas and GST activity in the muscle of *T. maxima* from the impacted site (Vaïare) compared to the reference site (bay of Cook). In contrast, an inverse pattern was observed for TBARs levels in the muscle of *T. maxima*. Concerning *E. merra*, higher TBARs levels were observed in the liver of fish from Vaïare compared to the bay of Cook. These results clearly showed an oxidative-stress endured by both species (*T. maxima*, *E. merra*) from the impacted Vaïare site. This study demonstrates for the first time that *T. maxima* and *E. merra* are suitable species for coastal reef environmental quality assessment.

WE 406

Influence of the pH on acute toxicity of vinasse to microcrustaceans

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Piracicaba (State of São Paulo) is a major producer of sugarcane in Brazil. Vinasse is generated in the process of alcohol distillation, and is composed of water, nutrients such as nitrate, potassium, high organic matter and acid pH. These characteristics make the vinasse have high pollutant power, which is about one hundred times greater than that of domestic sewage, characterizing it as harmful to the animal and plants. This residue has been used in the fertilization of sugarcane due to its composition rich in nutrients; however, when applied in high concentrations can contaminate aquatic environments through leaching and runoff. The aim of this study was to evaluate the acute toxicity of the sugarcane vinasse on the *D. rerio*. The vinasse used in this study had pH 3.5. Firstly, was realized a preliminary test in order to establish the concentrations to be used in the definitive acute test, which were: 1.562; 2.083; 3.125; 4.167; 6.25; 8.34 %. The experimental design was realized in 2-L aquarium. All concentrations were conducted in two replicates with seven organisms each plus control (water supply). The test duration was 96 hours and after this period the LC50 (24h); LC50 (48h); LC50 (72h) and LC50 (96h) was calculated using the statistic program Trimmed Spearman Karber and were respectively 3.19; 2.82; 2.75 and 2.62 % which corresponds to 31.9; 28.2; 27.5 and 26.2 mL-L⁻¹. We concluded that the vinasse is very toxic to *D. rerio*.

WE 407

Acute toxicity of the vinasse on *Danio rerio*

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Piracicaba (State of São Paulo) is a major producer of sugarcane in Brazil. Vinasse is generated in the process of alcohol distillation, and is composed of water, nutrients such as nitrate, potassium, high organic matter and acid pH. These characteristics make the vinasse have high pollutant power, which is about one hundred times greater than that of domestic sewage, characterizing it as harmful to the fauna and flora. This residue has been used in the fertilization of sugarcane due to its composition rich in nutrients; however, when applied in high concentrations can contaminate aquatic environments through leaching and runoff. The aim of this study was to evaluate the chronic toxicity of vinasse for the *Ceriodaphnia dubia* and *Ceriodaphnia silvestrii* before and after pH correction. All concentrations were worked out in percentage. The vinasse used in the study had 3.5 pH and its correction was made for 6.8. From the EC50 (48h) without pH correction for both species, we used the dilution factor of 1.5 to determine the chronic concentrations. For the test without pH correction, five concentrations were used plus the control with 10 repetitions each, and one organism in each repetition. Therefore, the concentrations were: 0.45; 0.22; 1.15; 0.07; 0.04 for *C. dubia* and *C. silvestrii*. The exposure period was seven days, and every two day the test solutions were renewed. The algae *Pseudokirchneriella subcapitata* was used as food. For testing with pH correction, chronic concentrations were also determined by the EC50 (48h) for both species. *C. dubia* was exposed to: 1.99; 1.32; 0.88; 0.59; 0.39; 0.26; 0.17; 0.11 and *C. silvestrii* to the concentrations: 1.58; 1.05; 0.70; 0.47; 0.31; 0.20; 0.13; 0.08. The experiment design was the same that test without pH correction. The mean values of reproduction in the treatments were compared with the control by the Dunnett's test at $C = 5\%$ and subsequently determined the NOAEC, LOAEC and CV (chronic value). The values of NOAEC, LOAEC and CV to *C. dubia* without pH correction were respectively 0.07; 0.15; 0.11% and to *C. silvestrii* were 0.15; 0.22; 0.185%. After pH correction was found the following values of NOAEC, LOAEC and CV for *C. dubia*: 0.26; 0.39; 0.325 respectively and 1.05; 1.58; 1.315 for *C. silvestrii*. It is observed that after pH correction the chronic value for both species was higher than that when pH was low. Therefore, we concluded that the pH of vinasse influence on chronic toxicity for these microcrustaceans.

WE 408

Structural and functional alterations in aquatic systems after addition of the pesticide Vertimec® 18EC, with emphasis on zooplankton

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Vertimec® 18EC, whose active ingredient is abamectin, is commonly used as an acaricide, insecticide and nematocide on fruit and vegetable crops as well as ornamental plants. Despite its widespread agricultural use, various direct and indirect negative effects are associated with not only its active ingredient, but also with other substances in the product. There are few studies of the toxicity of the commercial formulation to aquatic organisms. The objective of this study was to assess the potential of this product to cause alterations in the zooplankton community in artificial aquatic ecosystems. For this purpose, a field plot was contaminated with Vertimec® 18EC at the concentration recommended for strawberries (0.125 liter of solution m⁻²) and another plot of equal area was left uncontaminated. The two plots were spaced so there would be no contamination from the first to the second. Then heavy rainfall was simulated (19 mm) and the runoff water was collected and transferred to mesocosms. There were five treatments: 1) control mesocosm, receiving no runoff - C; 2) runoff from the uncontaminated plot - UR; 3) runoff from the plot contaminated with Vertimec® 18EC - CR; 4) direct application of Vertimec® 18EC in the mesocosm water - V and 5) samples collected randomly to check if there was any contamination between the mesocosms - RS. The experiment lasted 240 h (10 days), and the mesocosms were contaminated once only, at nominal abamectin concentrations of 4 and 40 µg L⁻¹, respectively, for the CR and V treatments. During this period water samples were collected for physical and chemical analyses, and to evaluate the zooplankton. The results showed that the addition of the contaminated runoff and pesticide directly in the mesocosms caused changes in the structure (composition and density) of the zooplankton community. The effects were acute and chronic, especially for Copepoda and Cladocera species, while Rotifera species were less sensitive to the product's effect. The 10-day period was not enough for the microcrustaceans to become reestablished, even after a single contamination, thus evidencing the high toxicity of Vertimec® 18EC to zooplankton.

WE 409

Use of pesticides at Bom Repouso, Brazil, and soil risk assessment

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The district of Bom Repouso, Minas Gerais State, Brazil has their economy based on agriculture, with potatoes and strawberry as the main crops. The widespread use of pesticide in these cultures could contribute for environmental poisoning and risks. Hence this study aimed to characterize the agricultural activities in the municipality and to assess the risk for soil. Using structured questionnaires, data were compiled through interviews with farmers and their families, focused on pesticide use. It was observed an intensive and inappropriate use of pesticides and application of products highly hazardous to human health and the environment. Besides that, rainfall and topographic features of the region can contribute to increased risks. For abamectin, the pesticide most cited by farmers, the risk for soil was estimated with the toxicity exposure ratio (TER) by dividing the LC50 (14 days) for earthworms, obtained through standard ecotoxicological tests adapted to tropical conditions by the respective Predicted Environmental Concentration (PEC), considering soil properties, pesticide chemical properties and application rates. The PEC was calculated using the FOCUS model. In three scenarios that considered actual rates of application of the product, based on information provided by farmers, potential chronic risk to soil organisms was identified. The data obtained in this study are important for future ecological risk assessments in tropical regions.

WE 410

In situ experiments to evaluate the effects of xenobiotics in the phytoplankton community

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The increase in world population and increasing demand for food have motivated the use of large amounts of pesticides, resulting in a direct or indirect contamination of aquatic ecosystems. This contamination occurs through runoff from agricultural areas or by direct application of the product near water resources. The present study was undertaken to precisely assess the direct and indirect effects of pesticide Vertimec®, whose active ingredient is abamectin, on the phytoplankton community (composition, density and biomass) from mesocosm experiments, lasting 10 days. To conduct the experiment, a portion of soil was contaminated with the pesticide Vertimec® and then rain was simulated with the water being drained superficially (runoff), collected and transferred to experimental tanks in four treatments: control tank (C); control of runoff, with

runoff water from the uncontaminated soil (UNR); runoff with runoff soil sprayed with Vertimec® (CR) and direct application of Vertimec® (V). 48 hours past the onset of the experiment, there was significant reduction in phytoplankton density in all treatments, and this reduction of 65.7% in C; 90.5% in UNR; 92.13% in CR and 59.1% in V. It was found that the largest differences occurred in UNR and CR, which can demonstrate the physical effect (turbidity and sedimentation), chemical (nutritional changes and effect of pesticides) or biological (competition and grazing), or the combined effect of all them. Changes in phytoplankton composition were also observed in all treatments, most obviously in reducing the abundance of Bacillariophyta and increased Chlorophyceae. The production of chlorophyll *a* of phytoplankton was lower in treatments with runoff and Vertimec® directly into the water, being more evident in the treatment with direct addition of the contaminant. The green alga *Monoraphidium minutum* was the species with the highest contribution in total abundance of phytoplankton, with more significant participation in the treatment that received the pesticide into the water, indicating a more privileged species (competition for resources, higher growth rate and reduced grazing pressure) in relation to others.

WE 411

Experimental studies (laboratorial and in situ) to evaluate the effects of Vertimec®18 EC on Danio rerio (Cypriniformes, Cyprinidae) in tropical systems

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In spite of the effects of contaminants in natural ecosystems, pesticides have been used in large scale around the world, resulting in considerable abiotic changes and, consequently, alterations in biological processes. Then, the high consumption of pesticides in agriculture have resulted in the development of relevant studies to the assessment of environmental risk contamination. Nevertheless, most of the information about the effects of pesticides in natural ecosystem arises from studies that are carried out in temperate regions. Unfortunately, these results are being used to support the knowledge of the processes that occurs in tropical systems, as well as for the formulation of public policy. Vertimec®18 EC (whose active ingredient is abamectin) is a pesticide that farmers are using as insecticide and acaricide; however, their effects on aquatic ecosystems must be analyzed. To evaluate these effects, laboratorial and *in situ* experiments were conducted with *Danio rerio*, in different treatments (Control: C; direct application of Vertimec: V; uncontaminated runoff: UnC and contaminated runoff: CR). For this purpose, two parcels of soil (8m²) were used: one control and another one contaminated with Vertimec (dose recommended for strawberries cultures). After that, a typical rain in tropical systems was simulated (20-30mm) and the water from the runoff was collected and added in the mesocosms (UnC and CR). Besides, water samples were collected from the mesocosms after the input of runoff to evaluate and compare the results in both experiments. Water and sediment samples were also collected for physical and chemical analysis. The limnological analysis showed the increase of nutrients (nitrogen and phosphorus), suspended solids, turbidity, chlorophyll and conductivity. On the other hand, reduction in dissolved oxygen concentration was observed in UnC and CR after runoff addition. In relation to bioassays, chronic toxicity was registered only in the *in situ* experiment, with considerable effects on the survival and biomass. However, acute toxicity was not observed. The results indicate the importance of the *in situ* experiments to evaluate the real effect of toxicants in aquatic communities (direct and indirect effects). In addition, the effects of commercial formulation of pesticides (Vertimec) were highest in relation to active ingredient (abamectin), indicating differences in chemical, physical and biological processes in response of the contaminant.

WE 412

Hematological biomarkers in tadpoles of Lithobates catesbeianus exposed to agrochemicals mixture in rice crop

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In the rice field, the use of agrochemicals to control pests and rice weeds is recommended to improve yield. However, their use may affect non-target organisms, as the amphibians. Blood is an excellent tool for assessment of the physiological, biochemical and pathological conditions in animals, because it's in contact with organs, tissues and cells and react sensibly to all the changes that take place. The main aim of this study was to evaluate the potential toxicity to tadpoles of *L. catesbeianus* in rice crop irrigated water that receive high loads of pesticides and to determine hematological parameters. The property chosen to realize the tests is a commercial rice crop farm localized in Tremembé city, São Paulo state, Brazil. After 25 days of planting pre-germinated seeds, previously treated with Fipronil, the court was drained for the application of the pesticides: Penoxsulam 240g/L (150ml/ha) + vegetable oil (1L/ha), Bentazom 600g/L (2L/ha) and Permethrin 250g/L (100ml/ha). After 3 days of application, the court was again flooded and the tadpoles were exposed. And after five days about the beginning of the exposition was applied Carbofuran 50g/Kg (500g/ha) with the fertilizer. Screened cages measuring 0.8x0.8x0.4m were fixed inside the rice crops, allowing the tadpoles to access the sediment. In each group it was used 150 tadpoles in the 31 Gosner stage (1960) with medium weight about 7.08 ± 0.49g. Before the beginning of the tests and in the experimental days 3, 7, 10, 14 and 21 it was take 6 animals/group. These animals were anesthetized to blood collection to evaluate hematological parameters: hematócrito (Ht), hemoglobine tax (Hb), counting of erythrocyte's number, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC). After 21 days of exposure, it was verified the survival tax 100% in the control group; 92.7% in the exposure group. The medium values of Ht, Er, MCV and MCHC don't show significant differences: (Ht) 19.37 ± 2.29%, (Er) 21.30 ± 4.74 × 10⁴/mm³, (MCV) 1053.19 ± 247.15 fL and (MCHC) 19.41 ± 3.54%. Hb shows significant difference only in the 14th day, medium values of 4.39 ± 0.70g/100mL to control group and 2.74 ± 0.62g/100mL to exposure group. During the others analyzed days the Hb was kept in normal values of 3.56 ± 0.37g/100mL. The tadpoles of *L. catesbeianus* show resistance to agrochemicals used in rice crops observing the small mortality.

WE 413

DNA Damage in cichlid fish and environmental forensic analysis of the origin of PAHs at a Guatemalan oil field

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<input type='hidden' id='gwProxy'><!--Session data--></input><input type='hidden' id='jsProxy' onclick='jsCall();' />This study focused on several wetlands in Laguna del Tigre National Park (Guatemala) as part of Conservation International's Rapid Assessment Program. Sediment and water samples were collected from a laguna near Xan field, Guatemala's largest oil facility, and 3 other sites for determination of levels of polycyclic aromatic hydrocarbons (PAHs). Cichlid fish (*Thorichthys meeki* and *Vieja synspila*) were collected for determination of DNA strand breakage (by gel electrophoresis) and chromosomal breakage (flow cytometry). PAHs in water and sediment were extracted with methylene chloride and analyzed by GC/MS. Environmental forensic analysis was also carried out using three metrics to draw inferences about the origin (pyrogenic, petrogenic, diagenic) of the PAHs in the sediment. These metrics included a) concentration (mg/kg) of PAHs with 4 or more rings, b) a "pyrogenic index" defined by $\Sigma(\text{all other 3-6 ring PAHs})/\Sigma(\text{alkylated [naphthalenes, phenanthrenes, dibenzothiophenes, fluorenes, and chrysenes]})$, and c) ratio of chrysene/phenanthrene in sediments. Multivariate analyses were also used to analyze all three of these variates together. For *T. meeki* from Xan field, chromosomal breakage and strand breakage was greater than in at least two of the three reference sites. For *V. synspila*, chromosomal breakage and strand breakage were greater in Xan than one of the two reference sites. Patterns of aqueous PAH concentrations, suggests that fish are affected by anthropogenic contaminants. PAHs were elevated at some reference sites, but environmental forensic analysis suggested a pyrogenic or diagenic origin. The environmental forensic analysis also suggested that PAHs near the oil well injection site were primarily of petrogenic origin, and the ratio of petrogenic:pyrogenic PAHs decreased with distance from the oil field. The data are consistent with the hypothesis that oil field brines injected into the ground water caused genotoxicity in fish at Xan field, and it is also possible that pyrogenic PAHs influence levels of DNA damage in reference sites. These analyses represent one of the first efforts to examine genotoxicity in native Mesoamerican cichlids.

WE 414

Effects of trichlorfon on the neotropical freshwater fish tambaqui, *Colossoma macropomum*

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The use of pesticides has been the main strategy to prevent ectoparasites that threaten aquaculture productivity. The non regulated use of such chemicals may lead to damage in aquatic ecosystems that receive the ponds effluents, compromising environmental. The use of chemical products could represent an alternative to reduce the economic losses associated with epidemics. These chemotherapeutic agents actually constitute one of the main environmental problems derived from intensive fish culture. Trichlorfon (O,O-dimethyl O-2,2,2-trichloroethylene) is one of the most used products against fish parasites in aquaculture. In normal conditions of use, it is very quickly hydrolyzed to dichlorvos, which is much more toxic. This study examined biochemical alterations (serum and plasma) in neotropical fish tambaqui, *Colossoma macropomum* (Wt: 49,1 ± 11,0 g) exposed for 96 h to a concentration sublethal trichlorfon (0,125 mg/L, trichlorfon 97g/100g). The experimental design was randomized with three replicates in a semi-static. During the experiment, the fish were not fed. AST, ALT, DLH and Glucose analyzed were spectrophotometrically (enzyme Kit). The cortisol determined with enzyme Linked Immunosorbent assay method. The activity of AST and ALT in the exposed group (313,7 mg/dl and 84,1 mg/dl) was significantly higher compared to control (215,3 mg/dl and 54,7 mg/dl) respectively. DLH exposed group (424,1U/L) was extremely significant compared to control (348,1 U/L). The plasma glucose in the group exposed to the xenobiotic (53,1 mg/dl) showed a significant decrease compared to control (86,1 mg/dl). The concentration elevations of cortisol in the exposed group (26,8 ug/dl) was extremely significant compared to control (15,49 ug/dl). The results suggest that this product, used as "chemotherapeutic", causes changes in the metabolism in fish.

EM01P - Collection and use of monitoring data for environmental risk assessment of chemicals

WE 418

Urban geochemistry of the Berlin Metropolitan Area, Germany

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A third of the population of Germany lives in cities with a population greater than 100,000. Anthropogenic activities are the determining ecological factor in cities and

industrial regions. Berlin is one of the first European megacity to be covered in its entirety by a geochemical survey of topsoil, including large, more or less rural areas. The regional or local geochemistry of an urban area can be evaluated only by comparison with the non-industrial surrounding area.

The distribution of 43 trace and 9 major elements in 3,746 topsoil samples (0 - 20 cm) in Berlin and surroundings was mapped and interpreted. Studies of the Berlin metropolitan area and other urban regions have shown that heavy metals (e.g., Cd, Cu, Cr, Hg, Ni, Pb, Zn and As) are enriched 1.8 to 8.9 times the geochemical background concentrations. In the area around Berlin, large anomalies occur near iron and steel mills and construction material works, as well as in the vicinity of sewage farms. Soils act as long-lasting sink for many pollutants resulting in their accumulation. Regional and local geochemical conditions in an urban area can be only described by comparison with the non-industrial surroundings. Both single-element and multi-element maps (cluster-Q analysis, principal component analysis), and maps showing the geochemical load index for various trace elements were used to evaluate and interpret geochemical data. Study of regional variations and anthropogenic contamination of topsoil by metals and organic compounds is very important for environmental planning and monitoring in urban areas. A geochemical survey of the urban environment provides a reliable database for setting concentration limits for urban and other soils. Sustainable urban development requires combined management and monitoring of urban conditions of soils and chemical fluxes. Geostatistics and multivariate statistical methods provide a comprehensive understanding of the spatial distribution of urban pollution and alteration of soils.

WE 419

Effects of environmental factors on heavy metals residues in soils around the Guanting Reservoir, China

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Topsoil samples from 61 sites around the Guanting Reservoir, China, were measured for Copper, Zn, Cd, Ni, Pb and Cr concentrations. The factors that influence the dynamics of these metals in soils around the watersheds of Beijing reservoirs were examined. Factors, including the exposure time, land use, soil texture, soil taxonomy and soil properties were discussed. Compared with the results determined in the year of 2003, the average concentrations of Cu, Zn, Pb, Cr and Ni were increased, which means the input amounts were much bigger than output amounts. However, the Cd shows opposite results. All of heavy metals concentrations in arable soils and orchard soils were higher than those in uncultivated fallow soils (including several woodland soils). All of heavy metals concentrations in loam soils and light loam soils were higher than those in sandy loam soils and sandy soils. So, land use and soil texture were very important factors affecting heavy metals residues, additional factors such as soil taxonomy and soil properties were also involved in heavy metals retention in soils. The data provided in this study are considered crucial for reservoir remediation, especially since the Guanting Reservoir will serve as one of the main drinking water sources for Beijing in the foreseeable future.

WE 420

Urban geochemistry in Norway - monitoring and developments from 1994-2011

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80 per cent of the Norwegian population lives in cities. The urban environment is affected by a wide range of contaminants. Since 1994 systematic geochemical mapping of soil pollution has been conducted in nine Norwegian cities. High concentration of metals (especially lead) and polycyclic aromatic hydrocarbons (PAH) are found in urban soil in the oldest parts of the cities. The highest PCB concentrations were found in parts of the cities developed during the period 1950 - 1970. Exposure to polluted soil can possibly influence human health. Follow up studies focus on soil pollution in day-care centers and playgrounds, health-based action levels for metals and organic pollutants in Norwegian soils, identification of pollution sources for PCB in the urban environment and dispersion of contaminants from land based sources to the marine environment.

WE 421

Presence of hazardous organic chemicals in recycle tire playground surfaces

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Disposal of used tires has been a major problem in soil waste management. The indestructible nature of discarded tires makes them persist in the environment indefinitely, creating long-lasting piles of toxic, synthetic waste. Today, one of the most valuable applications of used tires is the transformation in recycling products such as rubber mulch and recycled rubber pavers that are used for sidewalks, animal flooring, fitness center flooring, playground surface, sport fields, etc. Rubber mulch is a product that consists of granular rubber particles. It is available in array of lively colors and it is extensively used in playgrounds along the world. The number of this kind of playground flooring is significantly increasing in the last years. This material constitutes a slip resistant, cushioned floor that prevents injuries in sporting activities and playgrounds and it is a visually attractive choice. Nevertheless, and although few investigations of the organic content of tires have been reported, it is well known that rubber tire debris contains toxic compounds such as highly aromatic oils and other reactive additives.

In the present study, the presence of hazardous organic chemicals in recycle tire playground surfaces is investigated. With this purpose, direct material analyses using solvent extraction as well as SPME analysis of the vapor phase above the sample are carried out. Rubber mulch samples were directly collected in twelve different playgrounds. In addition, seven commercial samples of recycled rubber pavers were acquired in a local store of a multinational company. All samples were extracted by ultrasound energy and analyzed by GC-MS. The analysis confirmed the presence of a high number of hazardous substances including PAHs, phthalates, antioxidants such as BHA, BHT, and phenols; benzothiazole and derivatives, among other chemicals. The study evidences the high content on toxic chemicals of these recycled materials. In addition, SPME studies of the vapor phase above the samples confirm the volatilization capability of many of those organic compounds.

From these results, ground rubber applications, especially those relating to play areas and other facilities for children should be a matter of special concern.

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WE 422

Monitoring requirements for assessing water framework directive (WFD) status on a small island

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The Water Framework Directive (WFD) is a holistic approach to managing the water environment in Europe. The WFD, an EU regulation, does not apply to the Channel Islands, but in order to achieve a baseline assessment of water quality status, the island of Jersey has elected to use the WFD methodology to evaluate its waterbodies. Since the WFD is not a statutory requirement for Jersey, WFD data requirements have not been previously addressed.

Given the limited resources available to undertake new monitoring of waterbodies, a risk-based approach was taken to determine the minimum data requirements in order to undertake a robust WFD assessment of water quality status in Jersey, rather than applying the full range of WFD chemical and ecological quality elements.

In a first step, the potential chemical pressures on Jersey's waterbodies were identified and assessed to ascertain which EU priority substances and UK specific pollutants were relevant to the Jersey situation, the locations likely to be receiving contamination, the type of contamination (e.g. toxicity, eutrophication) and the relevant biological receptors. This information was then used to resolve the data requirements for the WFD assessment according to the specific pressures identified. Existing environmental monitoring data for Jersey from a variety of sources were then collated and compared with the WFD data requirements. Data gaps were identified and series of options for new monitoring programmes were designed to potentially fill these gaps in the available information in the most effective and efficient way.

The monitoring options included a screening programme to fully resolve which substances were actually present in Jersey's waterbodies and their sources as well as programmes designed to deliver the missing chemical and ecological data to enable overall WFD status assessments. These ranged from limited physico-chemical and chemical monitoring to determine whether good chemical status was likely to be achieved, to the tiered application of the full complement of WFD monitoring components (chemical and ecological) in which each tier of components was only implemented if the results of the previous tier indicated a requirement for further data.

This presentation outlines fit for purpose and proportionate approaches and procedures to enable the WFD status assessment of Jersey's waterbodies. These approaches could readily be applied to other jurisdictions to deliver similar results.

WE 423

How to compare two rivers? The water quality of Meuse and Rhine (2000-2010) evaluated for the preparation of drinking water

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The Dutch drinking water company Dunea uses water from the river Meuse as source for its drinking water production. Yearly 80 million m³ surface water is abstracted for this purpose to serve over 1.2 million consumers. In case of emergencies on the Meuse, the river Lek (a branch of the river Rhine) could be used as an alternative source. A comparative study on the water quality between the two rivers was carried out to judge their suitability as source for the preparation of drinking water.

Ten years of monitoring data (2000-2010) from the two rivers were analysed and compared. These data included analysis results of inorganic compounds, pesticides, pharmaceuticals, X-ray contrast media, endocrine disruptors and other emerging contaminants. In addition, data on intake stops, limit exceedances and anthropogenic pressure in the catchments were used. The data sets originated from extensive monitoring programs carried out by two drinking water companies, Dunea and Waternet. The monitoring had resulted in a wealth of data since some of the water quality parameters had been monitored up to 52 times a year. A proper comparison was however challenged by the fact that monitoring programs on the two rivers were different and changed over time. To account for these problems, different groups of chemicals were analysed in different ways to enable the best possible comparison of the two rivers. Alternative ways were used to look at problematic compound groups, for example consideration of relative concentrations of compounds in comparison with other compounds, and the frequency of observations above the legal thresholds in comparison with monitoring frequencies.

This exercise resulted in a broad overview of the sorts and numbers of compounds present in each of the rivers and the potential challenges that arise when the rivers are used as source for drinking water abstraction. It was concluded that the water quality of the river Rhine (Lek) - like that of the river Meuse - is sufficient to serve as source

for drinking water preparation. In case of a calamity at the River Meuse, Dunea thus could decide to temporarily use the River Lek instead. The study furthermore provided insight in the possibilities of large sets of monitoring data to evaluate chemical water quality.

WE 424

Pollution by rivers in Central Asia

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We studied water-spreading pollutants and contents of rivers, irrigating canals, drinking water and several kinds of vegetables for last four years. We studied irrigating areas in the following river basins: Ak-Buura, Amu-Darya, Chu, Naryn, Syr-Darya, Vakhsh. These rivers flow across 2-3-4 countries of Central Asian (CA). The pollutants are: a) hydrocarbons aromatic; b) obsolete pesticides (DDT, HCH, Aldrine); c) metals (chromium six valent, mercury); d) uranium (it was determined radioactive background of old tailings situated in river cost). There assessment of human inheritance diseases level and basic health (protein immunity level, c-chromosome test) in the polluted areas. The worst pesticides polluted areas are: Lower Amy-Darya - Aral Sea region (Uzbekistan and Kazakhstan), Lower Syr-Darya (Kyzyl-Ordo area in South Kazakhstan), - which lead to rice pollution. The worst heavy metal pollution are: Ileik river (chromium, North Kazakhstan), and Shakhtanaja (mercury, South-West Kyrgyzstan). But the whole our complex study conclusion is: the most health danger point of Central Asia is Mailuu-Suu in recent time. We created maps of human health pollutant-pressure risks in the most part of Central Asia.

WE 425

Monitoring of substances in marine waters, can regulatory requirements be met?

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In a comprehensive chemical and biological monitoring study (INRAM: Integrated Risk Assessment and Monitoring of micropollutants in the Belgian coastal zone, 2006-2011) data on more than 100 micropollutants were collected from samples taken in Belgian harbours, coastal waters and the Scheldt estuary. A database was subsequently developed summarizing all toxicity and ecotoxicity parameters of these chemicals. Environmental risks of 33 micropollutants were assessed using international environmental protection values.

Highest risks were observed for TBT, PBDEs, PCBs and the PAHs anthracene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, and indeno(1,2,3-cd)pyrene in the water phase compartment and for TBT and PCBs in the sediment compartment. In performing the risk assessments it was noted that established international environmental protection values are often so low (e.g. EQS of TBT is 0.2 ng/L) that they cannot be detected or quantified by most well-equipped laboratories. Therefore, the effectiveness of monitoring programs and hence the overall regulatory implication of these environmental quality standard can be questioned. This study also argues the need to revise the manner in which these values are derived.

WE 426

Sources, transport and fate of PAHs in sediments and superficial waters of a semi-enclosed body of seawater: Linking of compartments

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This work describes a study of the occurrence, levels and temporal evolution of PAHs in a bay characterized by persistent chronic impacts. A total of 40 samples, 20 of sediment and 20 of water, were taken at each of five different stations, in four sampling campaigns. Analyses of pollutants have been made using GC-MS. Results indicate that in a coastal environment subjected to chronic pollution by pyrolytic PAHs, episodes of petrogenic pollution, like oil-spills, can be identified by the combination of different source ratios. Results also indicate that in the study area PAHs are transported from superficial water to sediment. This conclusion is based on the degree of coincidence found in the presence/absence of individual PAHs in both compartments and in their petrogenic/pyrolytic nature; the positive sedimentation rate in the study area together with the performance of the analyses on unfiltered water; and the distribution of sources of PAHs found in the area.

WE 427

Evaluation of tire and road wear particles in the Seine River watershed: a sustainability initiative of the global tire industry

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The Tire Industry Project (TIP), organized as a sector project under the World Business Council for Sustainable Development, has been engaged in a multi-year project to understand the potential for environmental health risk associated with tire and road wear particles (TRWP). In order to understand the potential for exposure to TRWP, a global sampling study was conducted. This presentation provides the results of the study conducted in the Seine River watershed to quantify levels TRWP in air and sediment at locations representative of potential human and ecological exposure. The Seine River watershed was selected from among four watersheds in Europe based on water quality criteria, a representative range of PM10 levels, and size and diversity of the watershed. Sampling sites were selected to represent a wide diversity of human receptors and ecological habitats. For all media, the presence of an identifiable vehicle traffic source was mandatory with additional consideration given to sample point accessibility, availability of historical sampling data, traffic load, population density, and land use. A chemical marker specific to the polymer portion of TRWP was used to quantify tread particles throughout the watershed. Results of the PM10 air samples indicated an average tread concentration of 0.12 µg/m³, contributing 0.62% to the overall PM10 in the watershed. The sediment results indicated a 100% detection frequency and an average concentration of 2200 ppm. The airborne tread concentrations were associated with traffic load and population density, but the trend was not statistically significant. For sediment, a correlation was not observed with traffic load; however, population density category was a significant determinant of tread concentration, as was the total organic content and grain size of the sediment material. The tread marker significantly correlated with a number of other traffic and asphalt associated metals, such as aluminum and cobalt, which further increases confidence in the accuracy of the chemical marker. The results of this study suggest that TRWP are widely dispersed in the environment and can be reliably quantified using the polymer pyrolysis marker.

WE 428

Distribution and source identification of PCB congeners in river sediments using multivariate factor analysis

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In order to assess the level of Polychlorinated biphenyls (PCBs) contamination and identify their sources, surface sediments were collected from selected locations along the Nakdong River, Korea and analyzed for 209 PCB congeners using HRGC/HRMS. The PCBs levels ranged from 0.124 to 79.2 ng/g-dry (coplanar PCBs: 0.295 to 5,720 pg/g-dry), which were similar to those of 3 other major in Korea, but slightly lower than other neighboring countries. In homologue composition, tetra-CBs were most abundant in most samples, but some samples with much higher PCBs concentrations had relatively lower proportions of tetra-CBs and higher proportions of penta- to hepta-CBs. To identify the sources of PCBs in sediment samples, Principal Component Analysis / Absolute Principal Component Scores (PCA/APCS), Positive Matrix Factorization (PMF) and Multiple Linear Regression (MLR) were used with the congener composition of Aroclors (1242, 1248, 1254 and 1260) and the flue gas of waste incinerators (obtained from previous paper) as source profiles. Results revealed that the three models showed similar source apportionments. Most sediment samples with lower PCBs concentrations had higher proportions of incineration derived materials, and some sediment samples with much higher PCBs concentrations had higher proportions of Aroclor 1260. It was regarded to be because many industrial facilities such as landfill leachate treatment facilities were gathered around sampling points with high PCBs concentration and high-chlorinated PCBs are more stable in the elution process of landfill leachate than incineration process. The estimated PCBs concentrations by PCA/APCS, PMF and MLR were very similar to the measured values, with coefficients of determination ranging from 0.77 to 0.99.

WE 429

Water quality in harbors - the PORTONOVO project in Portugal

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Water pollution is one of the major environmental concerns of our times. Aquatic sediments play a significant role on water pollution, as they can behave as a sink for several contaminants but can also release them to surrounding water. The European project "PORTONOVO" aims at developing methodologies to evaluate water quality in harbors in the Northeastern Atlantic, involving different European universities and institutions. In Portugal two main ports were used as case studies in this project: Aveiro (North Western shore of Portugal) and Portimão (South shore of Portugal). In both cases, chemical status and ecological potential were evaluated considering chemical and biological analyses in waters and sediments from several areas in or within the port aquatic system. Different approaches were carried out in both port areas, regarding bioassays, biomarkers, microbial diversity and online biomonitorization, aiming to cross compare the chemical status and the ecological potential of water masses and sediments. Results from the Aveiro port reveal that sediments can be considered as having low contamination when regarding chemical analysis, although bioassays (Microtox) and microbial diversity analysis revealed a different pattern. Strong inhibitions were observed in the luminescence emitted from *Vibrio fischeri* in the bioassays performed and microbial communities present in port areas were significantly different in structure and composition when compared to those present in the reference site. Results from Portimão port reveal that there was a trend of organic and inorganic contaminants in the sediments of the port area. Unlike for the Port of Aveiro, results from bioassays (Microtox, Stress on Stress) performed in the same sediments samples were directly related with those of the sediments. Moreover, some biomarkers namely

Acetylcholinesterase (AChE), superoxide dismutase (SOD) analysed in the gills of *Mytilus galloprovincialis* from the same area revealed that AChE was inversely related with Zn levels in sediments while SOD increased. There was, however, a direct relationship between Cr in the sediments and catalase level in mussels the digestive gland while in the gills lipid peroxidation increases. The results revealed the need to analyze all these descriptors at the same time as proposed by the Water Framework Directive and the Marine Strategic Framework Directive to assess to the good ecological status in Port areas.

WE 430

Trend analysis of ambient lead levels in water, air, sediment and soil samples

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Due to the reduction of diffuse emissions of lead into the environment over the past decades (e.g., restriction of lead addition to fuel), it is expected that ambient lead levels in different environmental compartments (water, air, sediment and soil) have decreased over time. The evolution of lead levels in these compartments, using available monitoring data, was investigated and long-term data series (1980 through 2011) were analysed.

National authorities were contacted to provide monitoring data for the water, air, sediment and soil compartment. Additionally, publicly available datasets were retrieved from the Internet. Several EU countries (among which are Belgium, the Netherlands, Germany, UK, Ireland, France and Sweden) provided good quality data regarding lead concentrations in water and sediment. All datasets were evaluated qualitatively and approached statistically to derive trends in lead concentrations. Air concentrations were taken from the European Monitoring and Evaluation Programme (EMEP), scientifically based and policy driven programme under the convention on long-range transboundary air pollution for international co-operation to solve trans boundary air pollution problems.

The analysis of the collect monitoring data showed that Pb emissions in the environment have generally decreased due National and European emission reduction strategies. Reduction of Pb emissions appeared to have a direct impact on the water (both total and dissolved levels) and air compartments, where a clear decreasing trend was spotted. Pb data in some lakes, however, were not fully in line with these findings, most likely related to the specific nature of a lake environment, where identifying a trend is more complex as this is much more dependent on geo-chemical characteristics of the area combined with human activities.

Based on the retrieved and processed data on terrestrial and sediment compartments, a decreasing trend was also identified.

WE 431

The trend of PRTR data and air toxics monitoring data in Japan

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In Japan, the PRTR Law was implemented in 2000 and the compiled data is available by all the people. This dataset gives us various informations on the status of pollutant emissions. Regarding monitoring data, the air toxics monitoring campaign by Ministry of Environment has started in 1997. This campaign covers all over Japan whose number of monitoring sites is around 300.

The first objective of this study is to clarify the year-by-year trend of PRTR data and air toxics monitoring data through 2000 to 2009. The second objective is to analyze the relationship between PRTR data and monitoring data. This paper, following previous papers by authors (Katatani et al., 2006 & 2007), reports some results of trend analyses and statistical analyses. The analyses were carried out on the prefecture-by-prefecture basis.

The principal results are as follows: 1) Air pollution by toxic chemicals in Japan are generally decreasing except some specific species. It can be considered that they are partly resulted by governmental regulations, and partly resulted by self management by many industries. 2) The consistency between PRTR data and air monitoring data varies slightly year by year.

WE 432

Assessment heavy metals pollution of snow cover using chemical and ecotoxicology methods

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The snow cover accumulates in its composition practically all pollutants emissions in atmosphere. For study are chose 10 crossroads and 20 streets in Kirov (city in Russia Federation 58° 36' 0" N, 49° 39' 0" E). Tests of the snow were collected under stable weather conditions during winter period (December 2010 - march 2011) in 50 sampling points, samples were taken five times at each point.

The studies include pH, salinity, heavy metals (Cu, Cd, Pb, Zn and Ni), bioassay. The bioassays evaluated were 1) *E. coli* (with gene-modified luciferase) - bioluminescence, 30-min. 2) Higher plant *S. alba* - seed germination, growth rough 4-day, 3) *B. taurus* (sperm cells)-mobility, 3 h.

According to the results of the experiment revealed patterns of distribution of heavy metals in the snow. By bioassay evaluated the toxicity of snow. A comparison of data of chemical analysis and bioassay. The Designed method for assessing the spatial distribution of pollutants emissions heavy metals from motor transport in atmosphere in urban environments in snow cover.

WE 433

Contamination status and spatial distribution of persistent chlorinated and brominated organic contaminants in the European eel (*Anguilla anguilla*) in Flanders, Belgium

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Pooled yellow eel (*Anguilla anguilla* (L.)) samples, consisting of 3-10 eels, collected between 2000 and 2009 from 60 locations in Flanders (Belgium) were investigated for their content of lipophilic and persistent contaminants, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs). Eel is a fatty and sedentary fish species, assuring a high accumulation of lipophilic contaminants and providing a representative estimate of contamination patterns within the catching area. European eel stocks are in decline in most of their geographical distribution and their status is considered below safe biological limits. A variety of contaminants have been found to affect the eel and effects were reported on several levels of biological organization, including population level. The aims of the present study were to investigate the current PCB, PBDE and HBCD contamination levels in wild eels throughout a bio-monitoring network in the freshwater system in Flanders, Belgium. The current study expands the knowledge regarding these contaminant concentrations, their patterns, distribution profiles and time trends in aquatic ecosystems. PCBs, PBDEs and HBCDs were detected in all analyzed eel samples and some samples had high concentrations (up to 41600, 1400, 9500 ng/g lipid wt., respectively). CB-153 was the most dominant PCB congener, closely followed by CB-138 and CB-149. Among PBDEs, BDE-47, -100 and -99 were the predominant congeners, similar to the composition reported in the literature. For HBCDs, α -HBCD was predominant followed by ν - and β - isomers in almost all eel samples. The broad range in PCB, PBDE and HBCD concentrations reported in the current study is likely due to the variety in sampling locations, from highly industrialized areas to small rural creeks. PCB levels accounted for the majority of the contamination in most samples. The contribution to the total human exposure through local wild eel consumption was also highly variable. Some eels (16 sites) exceeded largely the new EU consumption threshold for PCBs (300 ng/g ww for the sum 6 indicator PCBs: CB-28, -52, -101, -138, -153 and -180). The current data show an on-going exposure of Flemish eels to PCBs, PBDEs and HBCDs through indirect release from sediments or direct releases from various industries. Therefore concerns are raised regarding the impact of these contaminants on eels and on the human exposure close to industrialized hotspots.

WE 434

Mercury levels in food from the Idrija mercury mine area

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Due to 500 years of mining and smelting activities (1508-1994), the soil in the Idrija region is highly contaminated with mercury. In order to check the present situation regarding the contents of mercury in selected local food samples, levels of total mercury (THg) and methyl mercury (MeHg) were determined in foodstuffs, mushrooms, fish and deer from the Idrija mercury mine area. THg in freeze dried foodstuffs and in freeze dried mushrooms was determined by thermal combustion, amalgamation and atomic absorption spectrometry using a Direct Mercury Analyser (DMA-80). THg in fish was determined by chemical digestion and cold-vapour atomic absorption spectrometry (CVAAS). MeHg in freeze dried foodstuffs was determined by distillation, aqueous phase ethylation and gas chromatography (GC) followed by cold vapour atomic fluorescence detection (CVAFS) using Brooks Rand MERX -Automated Modular Mercury System. MeHg in fish samples was determined by Tetramethyl ammonium Hydroxide (TMAH) extraction followed by aqueous phase ethylation, GC and CVAFS detection using Brooks Rand MERX -Automated Modular Mercury System. Mercury levels in analysed food were not very high but were elevated compared to the levels in food from the non-contaminated areas. However, our study showed that THg is accumulating in mushrooms (X=379 ng/g wet weight, Med=113 ng/g wet weight) and chicory (X=727 ng/g wet weight, Med=173 ng/g wet weight). Based on previous data available, we can conclude that the levels of mercury in food have not diminished significantly during the past 15 years after the closure of mercury mine. Further work will include studying the influence of elevated levels of mercury in foodstuffs on the content of metals such as selenium, cadmium and lead.

WE 435

Polybrominated diphenyl ethers (PBDEs) in Chinese electrical waste recycling areas: human exposure and policy strategies

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Polybrominated diphenyl ethers (PBDEs) are brominated flame retardants (BFRs) widely used in numerous polymer-based commercial and household products. Electrical appliances are thought to be the major source for PBDEs in China. China plays a key role in the electronics industry, producing a significant share of the sector's worldwide output. Similarly, electronic waste (e-waste) has become a major environmental concern in China because of the potential detrimental impacts on the environment caused by the crude processed used in e-waste dismantling and associated release of toxic chemicals such as PBDEs. PBDEs are often exported to China as e-waste, second-hand electronics, and used automobiles. Therefore, e-wastes are an important source of environmental contamination by PBDEs in China.

Human exposure to PBDEs has been of great concern, as the levels in human tissues have been doubling every 2-5 years. Human exposure to environmental contaminants can be monitored through the measurement of external sources of exposures and through internal body burden measurements. The external exposure pathways include diet, inhalation, and dermal absorption. Exposures can also be assessed through internal bodily tissue measurements (i.e., biomonitoring) using biospecimens such as blood, breast milk, and other tissues. These include maternal and fetal blood from the general population in South China, umbilical cord blood in Guiyu, blood serum in Taizhou, Zhejiang Province, blood serum from e-waste workers in Guangdong, breast milk from the general population in South China, and in Taizhou, and hair of residents living at an e-waste site in Taizhou, hair of e-waste recycling workers in Fengjiang, Taizhou, Luqiao, and Wenling. We found that PBDE levels in e-waste recycling sites were significantly higher than those from reference locations.

In this study, we gave a comprehensive report on human exposure to PBDEs in and around Chinese e-waste areas. Based on the result, we put forward current existing problems on PBDE law and policy enforcement in China.

WE 436

Screening of metal levels in human scalp hair from the Portuguese population

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Over the past decades, hair analysis has been widely used in evaluating environmental exposure and nutritional status of human populations, as well as for forensic purposes. Hair and nails have been biological materials that have the advantage of being collected by non-invasive methods, which makes them appropriate for rapid screenings of environmental exposures. Indeed, a few studies in contaminated areas have already demonstrated that hair analysis is an efficient tool to evaluate environmental exposure to contaminants. However, this biological matrix also has several limitations, as it is affected by many confounding factors (e.g. age, gender, hair color). Consequently, the usefulness of using hair samples for characterizing environmental exposures depends on the existence of reference values for contaminants. Despite some contributions from specific geographic regions, the scarceness of information of metal reference values for humans is still large. Bearing this in mind, we initiate the collection of samples to determine reference values for metals in scalp hair of humans within a national context. To do so, we collected hair samples in individuals from remote areas with low industrial pollution pressure. The aim of this work was to determine reference values of metals in the hair of non-exposed individuals in Portugal. An inquiry was applied in parallel to all the subjects who agreed to participate in this study. The inquiry included specific questions concerning dietary habits, sources of food and water, tobacco and alcohol consumption, health condition, and other relevant information. Selected metals were analyzed in the samples by ICP-MS (Al, Mn, U, Cd, Pb, Zn, Ni, Cu, Cr, Sr) and pyrolysis atomic absorption spectrometry with gold amalgamation (Hg). High variability was recorded in all metal analysis for the samples: Hg 0.18-5.7 µg/g dry weight; U and Cd - most samples were below detection limit or below 1 µg/g dry weight. Ubiquitous metals occurred at different scales (µg/g dry weight): Al 2.6-67.1; Mn 0.1-8.8; Cr 0.3-1.7; Zn 71.8-1914; Cu 6.4-160; Pb 0.3-84. Nevertheless, sample heterogeneity was also high in many of the issues addressed in the inquiry: gender (37% and 63%), non smokers (89%) and no alcohol consumers (59%). However, these data are relevant to tackle potential confounding factors in such a large data matrix. We are increasing sample size to produce a more robust assessment of baseline values for contaminants in human scalp hair.

WE 437

Overview of Dioxins, furans and hexachlorobenzene key issues for Mexico

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Over the decade, important advances have been taking place in Mexico with regards to dioxins, furans and hexachlorobenzene towards defining emissions, sources, risks) and identifying what are most important issues and problems to tackle in the short and long term. These advances are part of its commitment as member of the North American Commission for Environmental Cooperation through the Task Force on dioxins, furans and hexachlorobenzene (1) as well as those established as a party of the Stockholm Convention, particularly through the group of experts to review and update the "toolkit".

In this paper, a summary of the activities and achievements in Mexico with regards to dioxins, furans and hexachlorobenzene over the last decade are presented. National emission inventories for PCDD/F are a key element to assess and identify contribution for specific sources and to determine alternatives for reductions and/or elimination. An overview of the process to integrate and update the national emissions inventory for dioxins and furans (2) and the preliminary inventory for HCB will be discussed. Identification of most important sources and alternatives to control or eliminate emissions will be also discussed. The recent emissions factors, experimentally determined for sources present in Mexico, such as the use of wood as fuel for cooking, open burning of garbage and artisanal brick production, have contributed to refine emissions inventories but also to assess the risks associated to these sources(3,4,5). Characterization of sources present in developing countries is important to assess specific needs with regards to risk reduction to these toxics. Currently, base line for ambient concentrations of dioxins and furans are being determined based on the results of the first three years of operation of the Mexican Monitoring Network for dioxins and furans in ambient air (6,7). Finally, a description of the activities done related to risk assessment and air transport modelling will be included. Finally, activities and pending issues for the next years will be discussed in view of the assessment and management of risk with regards to these toxics

LC02P - Developments in life cycle sustainability assessment (LCSA)

WE 441

Conceptual model for Life Cycle sustainability assessment

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Life cycle sustainability assessment (LCSA) is a multi-disciplinary approach to assessing sustainability, thus at the interpretation level it has to deal with information from assessments based on different disciplines and with different levels of aggregation both horizontally and vertically (e.g. the SLCA). A possible way for interpretation of the results from the assessments is the multi-criteria analysis since it can use both quantitative and qualitative indicators. Evaluation methods and tools exist in different disciplines and some of them (e.g. cost-benefit analysis) might be compatible to use in a multi-criteria format. Each of these methods and tools has their own strengths and weaknesses. The purpose of this work is to develop a model in which the best use of the theory and the base for calculations behind these tools is applied. Identifying how the theory behind tools from different disciplines can be used in such a model is quite challenging, but can contribute significantly to the interpretation of results from the different assessments in a single context: the LCSA.

WE 442

Development of LCIA methodology for resource consumption considering environment impact on social economy

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Rare metals and noble metals are included in electronic products such as mobile phones, personal computers using liquid crystal devices, semiconductor, motors, and so on. And rare metal are also included in wastes of those products.

However, China limited export ceiling of rare metal last year. As a result secure and steady supply of rare metals for future became difficult. LCIA (life cycle impact assessment) assess environmental impact of manufactures that developed method to assess by economy and energy.

Existing methods (for example LIME1), eco-indicator 99 and ReCiPe2)) proposes method for assessing the impact of resources quantitatively. In this paper, all resource depletion is assessed using the methodologies of social economics. The method that can assess resource depletion of all the metal and rare earth are developed. LIME (user cost) with ReCiPe (surplus cost) are compared to pick out the problem. And, a new method using the concept of shadow price are developed to access the reserve, grade, cost of mining, market price for the rare metals.

1) Itsubo Norihiro and Atsushi Inaba. 2010. LIME2. Tokyo, Japan.

2) Mark Goedkoop, Renout Heijungs, Mark huijbregts, An De Schryver, Jaap struijs and Rosalie van Zelm. 2009. ReCiPe. EU.

WE 443

Beyond environmental assessment tool to design with systemic methodology

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A complex approach on Sustainable Design may provide new perspectives for problems that cannot be easily addressed within a single discipline. On the basis that living systems are open, they continually draw upon external sources of energy and maintain a stable state of low entropy, as the physicist Erwin Schrödinger asserted in 1946; some of the next theories on industrial processes applied that concept also on artificial systems. Material and energy loops are open in order to decrease environmental impacts and resource depletion. The methodology of Systemic Design (SD) looks at making a better use of material and energy flows in order to model industrial production and energy systems after nature. Many industrial ecosystems have come about ad hoc for better business, while others have been facilitated through external actors. However, as these theories and ventures may be innovative for industries, they are still no more than solving problems that arose from environmental pressure and economical revisions. Wonders arise also from the design sector in terms of conceptual and operational tools able to meet the high degree of complexity of the emergencies related to environmental sustainability, also from a social and economic point of view.

Environmental LCA is one of the most internationally recognized methodology for the evaluation of environmental burdens, related to a product or a service during all of the life cycle stages.

Nevertheless the life cycle assessment approach is based on a linear model of a real process and the evaluation of the environmental burdens is a little bit partial. The limitations of current assessment methodologies, regarding the evaluation of impacts in a world where complex problems exist, result in a need for a thinking that goes beyond one of 'deduction' alone.

The Authors are working on the definitions of new features that can improve LCA tool in order to develop it from E-LCA to Sustainability LCA.

Sustainability LCA not only provide the environmental impacts of different materials, but also is able to suggest new use of material and energy flow strictly connected to local resources, biodiversity, human welfare, etc.

The features here presented are a starting point of discussion to define a way in which the E-LCA can evolve in a more complex and useful tool to satisfy these new

WE 444**Communication of LCA results: how to support decision making**A. Bonou¹, S.I. Olsen², F. Walachovic³¹Siemens A/S, Ballerup, Denmark²Dept. of Management Engineering, Technical University of Denmark (DTU), Lyngby, Denmark³Corporate Technology, Sustainable Life Cycle Engineering, Berlin, Germany

In order for an LCA to fulfil its role in effective decision support, the output indicators should address the specific needs of the decision to be supported and should be communicated accordingly. In order to communicate effectively there is therefore a need to define 1) what are the different decision contexts and 2) the types of decision criteria for the different contexts. Bearing in mind that the criteria probably change with each case study the decision maker have to, based on these criteria, weigh a) among different environmental impact categories as well as b) among impacts from all sustainability pillars. Which of the LCA results are more important for the decision maker and how does the technological geographical and temporal contexts of the specific case study affect the decision in question? Can archetypical decision contexts and criteria be identified? What are possible alternatives to weigh among the different environmental impact categories and prioritise the alternative decisions? How to integrate in the decision process different metrics for social and economic impacts and what are the alternative ways for result communication? These questions will be addressed in an attempt to identify sustainability assessment output results that could maximise the applicability of LCA without overwhelming and/or confusing the decision maker. The field of application are existing LCA case studies from the industrial sector.

WE 445**Agent-based Modelling of the agricultural industry sustainability of the Picardie/champagne region (France)**N. Bichraoui¹, B. Guillaume¹, A.N.T.H.O Halog²¹University of Technologies of Troyes, Troyes, France²University of Maine, Orono, United States of America

Attaining industrial sustainability at the local/regional level requires collaborative efforts from participating agents toward common goals including resource conservation, low carbon emission, production efficiency, economic viability, and corporate social responsibility. Our existing socio-technical systems should transition or evolve towards achieving system's sustainability. This study aims to operationalize the idea of system's sustainability by developing an Agent-based model framework for designing an industrial ecology-modeled agricultural biorefinery.

Agricultural product industry and its network are seen as an evolving system of complex interactions between nature, physical structure, market rules, and participants. Participants face risk and volatility as they pursue their goals and make decisions based on limited information and their mental model of how they believe the system operates.

The purpose of simulation modeling such as Agent-based modeling is to generate and explore alternative futures that may develop under different conditions. These simulators can explore various "what if" scenarios under different economic, social goals. They can show the possible evolutionary trajectories of given scenarios under different conditions and geographical context. An industrial eco-park organized around the agricultural product industry is a relevant area of application : On one hand, this task will identify and provide a first contextual analysis of the structuring factors and the main driving forces for the development, along the way, of prospective scenarios (ecological constraints; regulations, economic, cultural and political context; carbon market; technological routes, etc) On the other hand, the goal will be to confront visions resulting from the possibilities of realization of the future with the models studied in task number one. Indeed, the industry due to its current economic challenges and inherent structure offer a variety of opportunities for synergies.

For this model the actors (agents) will be agricultural-based food plants, biorefinery plants, and agricultural. The goal of this agent-based model is to see how they can work in symbiosis such as the conditions for them to allow transfer of flow of material between their plants, the main forces will their sensitivity to regulation of Co2 emissions, and subsidies incentives.

WE 446**Diverging technology rankings in the NEEDS project: analysing the compatibility of the total cost and MCDA approaches**

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In the European project New Energy Externalities Development for Sustainability (NEEDS), two different kinds of approaches were used to rank power generation technologies: the total costs approach, adding private and external costs, and a Multi-Criteria Decision Analysis (MCDA). Both approaches made use of Life Cycle Assessment (LCA) indicators. In the end, they yielded rather different technology rankings that can be explained by differences in concept, quantification and scope.

The aim of this study is to identify to what extent these ranking methods are different as a matter of principle and to what extent the ranking approaches used in NEEDS can be modified so as to cover a similar range of aspects.

The study identified that there is a considerable degree of overlap between the approaches MCDA and total costs. However, crucial differences exist that would prevent the rankings from becoming equivalent despite potential efforts for their harmonization. The following main reasons for this conclusion were identified: (i) MCDA does not require the indicator values to be expressed in monetary terms, leading to the next point; (ii) The consideration of environmental, social and economic aspects in MCDA is not limited to private costs and (technological) external costs as is the case for the total cost approach. In NEEDS, most of the technological externalities that were covered in the MCDA were also covered in the total cost approach. The main exception concerns biodiversity-related externalities, followed by noise-related impacts, visual impacts on landscapes and risks associated with waste storage. Further indicators considered in the NEEDS MCDA are mainly beyond the scope of the total cost approach. If these aspects were important in decision-making, efforts aiming at harmonizing the total cost and MCDA approaches would be rather useless. The two approaches also differ in the way how risk aversion is taken into account. (iii) Individual preferences have a strong influence on the MCDA rankings. This is because they can put higher or lower weights even on "hard indicators" like private costs.

In the end, any analysis seeking to comprehensively estimate external costs will be limited due to the constrained possibilities to monetize externalities, which mostly concern biodiversity or ecosystem services-related externalities. Even though already under way, more research is needed in this domain.

WE 447**Subcategory assessment method (SAM) for S-LCA consumer stakeholder: method and application on a family Italian winery**P.S. Sanchez Ramirez¹, L.P. Petti¹, C.L. Lie Ugaya²¹University G. d'Annunzio, Pescara, Italy²Universidade Tecnológica Federal do Paraná, Curitiba, Brazil

Going from raw data to impact assessment in S-LCA is still a challenge. Over the years, several methods have been presented in the literature. Due to the limits of the methods related to subcategories listed by UNEP and SETAC (2009), the objective of this paper is the proposition of a Subcategory Assessment Method (SAM) to reduce the variability of the evaluation of subcategories in S-LCA studies. A proposal for stakeholder consumers and a case study are presented. SAM includes 5 subcategories (health and safety, feedback mechanism, consumer privacy, transparency, end of life responsibility) for the stakeholder consumers and enables analyzing the organization in a Likert scale: A, B, C and D. Fulfilling Class B means that the organization meets basic requirements which are defined for each subcategory, based on International Agreements. Class A means that the organization shows more proactive behavior than established in basic requirements. Classes C and D are below basic requirements and are differentiated due to generic data (may be information about the country or sector). Afterwards, SAM was applied in a small winery in Italy. The goal of the case study was to analyse the wine consumer's subcategory. The functional unit considered was the production of a bottle of wine ("novello" produced and consumed in the same year). Specific data (data from the site) was collected by interviewing managers and technicians of the wine company and representatives of consumer organizations. A questionnaire was prepared, based on the methodological sheets proposed by UNEP and SETAC (2010), and sent to the wine company and the consumer organizations. The results showed that the company did not reach the basic requirement for most of the subcategories. However, in some cases, simple behavior changes would improve the performance. The case study also showed that it was possible to collect data and to evaluate the company regarding the consumer stakeholders using SAM. The method could also be implemented for the entire product life cycle, notwithstanding, one should be aware that S-LCA is as time and work demanding as (environmental) LCA, because the information changes from company to company, sector to sector and region to region. Future development of SAM could include the remaining subcategories, providing basic requirements for each of them.

WE 448**LCA of 'drop-in' biofuel supply chain: case of UMaine TDO advanced biofuels**

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To date, there has not been a clear definition of the term "drop-in" biofuels. Literature consulted use "drop-in biofuels" interchangeably with the term "advanced biofuels".

This is visible in the naming of research consortiums working on achieving the goals of replacing conventional fuels directly with bio crude, bio gasoline and bio jet fuel. Examples of these situations are seen in the United States and the European Union. The National Advanced Biofuel Consortium (NABC) of the United States (made up of 17 partners from industries, universities and national laboratories) was formed recently to oversee the development of direct replacement biofuels or supplements to existing gasoline, diesel and jet fuel that could be used without making changes to existing fuel distribution networks or engines. The other important example mentioned is the formation of the European Advanced Biofuel Flight path.

With the movement towards ensuring sustainability of the earth's resources gaining ground, Life cycle assessment (LCA) which is gradually being reformed into Life Cycle Sustainability Assessment studies as an impact assessment approach is on the rise. There have been numerous LCA studies in various fields of endeavors and this has even led to the modification of LCA from an environmental profiling tool to a sustainability measurement tool in the form of the UNEP developed Life Cycle Sustainability Assessment (LCSA). This presentation will shed light on the LCA studies that have been conducted on drop-in biofuels over years and will elaborate on the key characteristics of the studies conducted in this field.

The second part of the presentation will deal with the Life Cycle Sustainability Assessment procedure developed by the Research Group for Industrial Ecology, LCA and System Sustainability (IELCASS) at the University of Maine. This procedure which integrates concepts such as system dynamics, agent based modeling, multi criteria decision making analysis, input-output analysis and GIS with LCSA perspective will help us analyze the sustainability of biofuels supply chains.

Initial work being conducted in collaboration with Forest Bioproducts Research Institute has led to an environmental LCA study of the newly developed UMaine TDO “drop-in” biofuel. Preliminary results of this LCA work of the LCSA developed framework will be presented.

WE 449

Life cycle sustainability assessment of ethanol production using the virtual sugarcane biorefinery framework

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The Sugarcane Virtual Biorefinery (VSB) is an innovative framework that integrates computer simulation platforms with economic, social and environmental assessment tools to evaluate technical and sustainability issues of different sugarcane biorefinery systems. In this framework, computer simulation provides technical process data which are used for the sustainability and competitiveness assessment. The assessment is performed considering economic, social and environmental indicators derived from the integration of Economic Engineering, Input-Output Analysis and Life Cycle Assessment. Among other results the VSB showed the importance of integration of 2nd generation (2G) ethanol production to 1st generation (1G) sugarcane mills (either autonomous or annexed plants) and helped to identify and quantify some of the main technological bottlenecks of 2nd generation ethanol production. Results also show that ethanol production cost (1G and 2G) is reduced using current 2G production technology in the integrated process, although profitability might be decreased; production of other high added value products in the biorefinery may help to encourage adoption of 2G ethanol production technologies in sugarcane mills; implementation of strategies for use of industrial residues for energy production, including biogas production through stillage biodigestion and the use of 2G ethanol production residues as fuel in boilers, may allow all bagasse and trash available in the industry to be used as feedstock for 2G ethanol production; agricultural stage has a strong impact on both environmental and economic impacts of ethanol production and improvements on this stage may lead to significant gains for the process; and depending on the way the environmental impacts are allocated for electricity or lignocellulosic material, environmental gains on the integrated 1G and 2G generation ethanol production may exist. These results show that the proposed framework can be used to design, evaluate and optimize future renewable energy systems with regard to its technical and sustainability aspects.

WE 450

Sustainable material flows in wind energy plants

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Due to the increasing use of energy and the recent conscience about taking care of the environment the human being is looking for renewable energies, which allow us to use energy without wasting resources or damage our environment.

Over the five-year period end-2004 to 2009, annual growth rates for cumulative wind power capacity averaged 27 percent. The capacity installed in 2009 is equivalent to nearly a quarter of total global installations, and cumulative capacity has doubled in less than three years. In the EU, wind power is by far the most popular electricity generating technology. For the second year running wind energy had the largest market share: of almost 26 GW installed in the EU in 2009, wind power accounted for 39%. All renewable technologies combined accounted for 61% of new power generating capacity.

Due to the fact that wind energy is renewable, we want to achieve that most materials needed for windmills shall be recyclable from the beginning until the end. As the tower or other components are made of steel, aluminum or recyclable materials most of the whole windmill is recyclable. If it comes to the recycling of rotor blades which consist mainly of fiber glass combined with other materials there is still no feasible solution to separate all materials and often you need a lot of energy for the processing. Therefore, it is necessary to avoid as much of these materials as possible if it isn't included in the product.

The presentation will give an overview on material flows needed for the wind energy generation and what we might have to deal with in 20 or 30 years. Is wind energy really sustainable? What are we going to do with all the waste of used rotor blades for example?

WE 451

Life Cycle management on energy and material resources

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The increasing scarcity of material and fossil resources, the global competition on limited and high valued materials for High Tech products and the coincidence of increasing costs are urgent drivers for improving sustainable production and services. The Social Dimension is challenged through the working conditions of sourcing processes on limited raw materials but also on maintaining competitive production in well-developed countries and involving simultaneously the economic and social development and prosperity of transition and developing countries. This will be considered under S-LCA assessments.

This paper will discuss the use of LCA-Life Cycle Assessment and LCC-Life Cycle Costing for developing ecodesign criteria of Products. The European legislation on 'Energy related Products' requires a Life Cycle Assessment (LCA) -based approach to develop criteria for ecologically sound Products. Re-Use and recycling is of growing importance for limited and high value materials such as noble earths.

For this purpose, LCA -based methodology is being used as a management tool for decision-making.

The selected products are characterized by high market volume of appliances, environmental relevance and potentials for improvement.

They comprise among others Consumer Electronics, Household Appliances and Pumps, Lighting etc. being used in private households, industry and public institutions.

LCA methodology includes: goal and scope definition, inventory analysis, impact assessment, interpretation and improvement analysis including options/proposals for the decision-making process.

Additional Management Systems such as Energy Management follow procedurally the same strategy of a systematic inventory analysis, impact assessment and improvement analysis like LCA. Also recording, monitoring and review are comparable. An important additional component is the active involvement of personnel/co-workers in supporting the responsible implementation and application of procedures to make the sustainable process operational. This combination of sustainable technology and processes and the full application and internalization through the personnel confirms the need of the overall and integrative process (Life Cycle Sustainability Assessment- LCSA =LCA+LCC+S-LCA).

The next step will be the development of a Resource Efficiency and Resources Management System following the same structure as the other well-experienced Management Systems.

WE 452

Environmental analysis of enhanced geothermal systems through life-cycle assessment and induced seismicity risk criteria

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The development of geothermal systems based on enhanced low-temperature reservoirs is arousing attention as a promising alternative for electricity production using renewable energy.

However, promoting the development of this technology is inappropriate without a critical assessment of its impacts on the environment. In fact despite the low emissions during the operation phase, large quantities of energy and materials are required for the construction of the plant. The investigation of the impacts of enhanced geothermal systems (EGS) from a cradle to grave perspective can be effectively performed through the life-cycle assessment (LCA) methodology with a relevant set of environmental criteria adapted to EGS. Such customized assessment is applied to the analysis of ten significant EGS design options in central Europe.

A large variety of interdependent factors, related both to reservoir conditions and technical choices, determines the size of the installation and the final amount of electricity delivered to the grid. Hence, different sets of parameters are defined to identify the relevant scenarios, accounting the influence of the geothermal flow rate and its temperature, the number and the depth of the boreholes, the reinjection strategy, the power supply process at drilling stage and the risk of induced seismicity.

The latter is a key factor that is progressively gaining importance at design stage, especially after the suspension of the project in Basel (Switzerland) and the experiences of current EGS installations. Hence seismicity risk has been identified as a fifth criteria, together with the four end points proposed by the method IMPACT2002+, to assess the environmental performances of EGS along a multi-criteria approach.

Results show that impacts of EGS are comparable to those of other renewable energy technologies and significantly lower than those of conventional power plants. The comparison among the ten scenarios enables to formulate recommendations on the environmental suitability of their design. Finally, the risk of induced seismicity is highlighted as a key discriminating factor, as it increases inversely with the environmental benefit.

WE 453

Comparative life cycle assessment of long-lasting insecticidal nets - focus on human health benefits and impact calculation

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Malaria kills more than one million people each year, most of them in sub-Saharan Africa where malaria is the leading cause of death for children under five. Bayer CropScience Environmental Science (BCS-ES) has performed a comprehensive environmental impact assessment of three different mosquito nets using Life Cycle Assessment (LCA) methodology. Results were used (1) to identify “hot spots” (in terms of key parameters and life cycle stages) over the life cycle of the products assessed, (2) to identify strategies to be explored further for impact reduction and (3) to assess the human health benefits related to mosquito net use and compare them to the adverse potential impacts of net production.

The considered system is referred to the following Functional Unit (FU) “offer an efficient protection of persons against malaria with a mosquito net during a period of time encompassing 30 washes”. In the study, the effectiveness of the LLIN is only guaranteed by the persistence of insecticide (based on World Health Organization Pesticide Evaluation Scheme (WHOPES) evaluation).

Using a mosquito net allows the protection from mosquito bites and therefore from malaria and its consequences in terms of morbidity and mortality. Thus, mosquito nets use phase benefits on human health have been assessed, and compared to the potential life cycle human health impacts of the nets. The impact on human health includes the contribution from traditional impact pathways such as respiratory effects, toxicity impacts, photochemical ozone formation and from climate change.

The most impacting life cycle stage regarding all the impacts is the mosquito net production. The sensitivity analyses also show that the physical resistance and the end user acceptability are parameters that could strongly influence the overall results.

Under the limitation that calculation of averted DALYs thanks to the mosquito net protection is based on rough assumptions, the results reveal that the benefits of using the nets are about five orders of magnitude (factor 10'000) higher than the potential impacts generated on human health due to their production, use and disposal, meaning that the benefits of distributing the mosquito nets exceed the environmental burdens.

WE 454

Sustainability Life Cycle Analysis of best available techniques applied to the ceramic industry

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The aim of this work is the development of a methodology for assessing from an environmental, economic and social perspective, different Best Available Techniques (BATs) proposed for the ceramic sector in the framework of the Directive on Industrial Emissions. Taking as a starting point a baseline scenario whose life cycle inventory data have been collected directly from 20 Spanish ceramic tile facilities, thirteen alternative scenarios have been proposed by incorporating to the baseline scenario different BATs related to energy efficiency, dust emissions removal, abatement of the gaseous compounds and noise reduction.

The proposed methodology is based on a combination of methods to assess the effect that each BAT (or combination of BATs) produces on the baseline scenario: 1) Life Cycle Assessment methodology allows the analysis of the environmental improvement by means of indicators for different impact categories; 2) Eco-efficiency allows to estimate the relationship between economic aspects and environmental impacts and 3) Survey to different stakeholders involved in the ceramic sector allows to consider their degree of knowledge and preferences for each BAT.

LC05P - Monetisation for weighting and aggregation in Life Cycle Impact Assessment and Cost-Benefit- Assessment

WE 457

Methods for monetisation in LCA and their impacts on the value of characterisation factors

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There are currently several methods in use for monetisation of environmental and health impacts within the framework of Life Cycle Assessment. In case studies they often produce different results. The aim of this paper is to demonstrate on the example of several elementary flows the extent to which the results of the characterisations are influenced by the monetisation method applied. Some underlying differences between the currently available monetisation methods will be discussed as well.

Among the monetisation methods that are compared is the EPS 2000 method, the Stepwise 2006 method, the Ecotax method, the ReCiPe method and the EcoSense method. The results have shown that the characterisation results for a single pollutant or resource (expressed as EUR/tonne) vary considerably depending on the monetisation method applied. For some pollutants the obtained characterisation factors are within a narrow range of values (e.g. CO₂) but for others the difference can be up to several orders of magnitude (e.g. Cd and PM_{2.5}).

Despite their use for the same purposes, the five methods covered by this analysis differ in what they try to achieve, in the effects they consider, in the depth of analysis, in the choice of the characterisation models and in the way economic values are derived. All these characteristics of the method will determine the calculation procedure and the monetary value which the monetisation method assigns to various emissions and interventions.

WE 458

Comparison of external costs of impacts on human health with and without equity weighting for selected source regions in the Southern Hemisphere

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Within the life cycle assessment characterisation factors have often been provided for unspecified regions or rather, as global average. The ExternE project series www.ExternE.info suggested that human health damages need to be assessed in a spatially resolved context in order to increase the accuracy of impact assessment results. The assessment of impacts to human health due to air pollution has several spatial and temporal aspects. The spatial aspects include the dispersion and chemical transformation modelling, which is dedicated to derive spatially resolved ambient concentrations of pollutants for individual source regions. This enables the use of spatially resolved information on receptor areas in order to calculate impacts, and it enables to weight these impacts appropriately to area-specific details like population density, age distribution or income. Hence, the actual impact and damage per unit of emission depends on the location and time of the corresponding emission. Within the TM5-FASST dispersion modelling framework, the world is divided into 56 regions and grid cell with a resolution of 1°x1°. Each region serves as a source region and each grid cell serves as a receptor region. Population data and concentration response functions are applied in order to calculate the relevant diseases. This paper presents new and spatially explicit characterization factors (CF) in terms of human health impacts and damage cost factors per unit of emission for various air pollutants in selected source regions in the Southern Hemisphere. The considered air pollutants are ammonia (NH₃), oxides of nitrogen (NO_x), sulphur dioxide (SO₂), and primary particulate matter (PPM) in form of primary particulate matter. The impact assessment is also accounting for secondary inorganic aerosols (SIA) consisting mainly of ammonium nitrate, ammonium sulphate and other nitrates and sulphates. Finally, the application of monetary valuation is used to derive specific damage cost estimates per unit of emission. Monetary valuation of the corresponding impacts is done in two ways, namely a) including equity weighting by using European average monetary values and b) without equity weighting by purchasing power parity adjusted monetary values. The results are compared in order to emphasise the importance of spatially resolved dispersion modelling, and in order to demonstrate the effect of different value systems with regard to the welfare loss.

WE 459

Aggregation of multiple environmental benefits

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Environmental programs and projects often have a range of environmental benefits, be it in terms of improving water and air pollution or multiple attributes of the same impact receptor. The valuation of the benefits of such programmes requires one to aggregate these benefits. Difficulties arise when the environmental benefits are complements or substitutes. Simple summation of benefits would underestimate the true aggregated benefits if the benefits would be complements, while the reverse would be true if the benefits would be substitutes. The small literature that addresses this issue includes examples of both types of relationships. In this paper we develop a tool for aggregating environmental benefits that takes account of potential substitution and complementary effects among environmental domains. The basic idea underlying this tool is that the social value of an environmental improvement in one domain may not be independent of the size of the improvement and of the size of changes in other environmental domains. To account for these possible interdependencies, a simple utility function is developed that describes social utility or welfare as a function of the changes in the three environmental end-points: climate change, human health and ecosystem quality. We want to determine the value of the complete bundle of environmental end-points. The question is: is the value of this bundle equal to the sum of its individual components or is it smaller or larger? We argue that this depends on the composition of the bundle; i.e., on the relative shares of the end-points in the bundle. More specifically, we argue that a "balanced" bundle ("a bit of everything") is likely to be preferred over an "extreme" bundle ("all of one component; nothing of the others"). A Constant Elasticity of Substitution (CES) utility function is calibrated on marginal value estimates from the EU FP6 NEEDS research project and on observable "political" preferences on climate change, air pollution-related human health problems, and ecosystem quality.

WE 460

Integrating life cycle environmental costs in decision making, applied to Belgian residential buildings

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The external environmental cost of residential buildings in Belgium was estimated during a four year project - SuFiQuaD (Sustainability, Financial and Quality evaluation of Dwelling types). The aim was to formulate recommendations to move towards a more sustainable building stock. It was investigated if current decisions based on financial investment costs are in line with decisions based on life cycle environmental costs. Policy recommendations were formulated by identifying the measures in order of priority to reduce the environmental impact of our building stock and by investigating if internalisation of the external environmental cost could enhance sustainable building. The research methodology consisted of several steps. In a first step existing methods of monetary valuation were compared. Based on this comparison, a hybrid approach was proposed combining the Clean Air for Europe (CAFE) method with several other literature sources. The methodology proposed was then implemented - in combination with a life cycle costing analysis - in three types of case studies: optimisation of building elements, optimisation of 16 representative dwellings and evaluation of current policy incentives (subsidies and tax reduction) related to sustainable building.

It could be concluded that assessing the impacts included in the CAFE method is insufficient because it leads to an unfair comparison of building alternatives. Impacts as ecotoxicity, land use and respiratory effects due to inorganic substances should be considered too. The analysis revealed that (current) decisions based on initial financial cost differ substantially from the ones based on life cycle environmental costs. The latter moreover proved to be relatively small compared to the life cycle financial costs of common standard and low-energy buildings. The effect of internalisation of these costs in decision taking would thus be rather small. The measures in order of priority to move towards a more sustainable building stock consist of building location (minimise transport of inhabitants during the use phase), reduction of the heating demand, choice of building materials (production phase), reduction of the impact of electricity use and finally freshwater use. The analysis of current energy related subsidies from both an environmental and financial perspective clarified that these are often too high (e.g. photovoltaic solar panels), but on the other hand some should be increased (e.g. floor insulation).

WE 461

Monetizing environmental impacts through integrated assessment

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This paper describes an approach for an integrated economic and ecological assessment of production processes. It is based on heuristic approaches that are used in classical business theory for e.g. planning of production processes within companies. The result of an LCA provides additional parameters for this heuristic approaches. A measurement of the scarcity of environmental aspects, e.g. an emission cap for CO₂ emissions, is required. Through an iterative application of the method, an optimal allocation of resources is achieved, considering economic, ecological and technical parameters. Scarce resources are assigned a shadow price, which can be a basis for internalization of environmental impacts or be used for comparison with external prices, e.g. prices of emission permits.

WE 462

LCC evaluation of building components including environmental impacts

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Economics in sustainable assessment is a very significant topic. Many times, in the building sector, contractors, owners and designers are at issue over the cost of sustainable projects.

A variety of methods have risen in the two last decades in order to include the social and environmental costs and benefits into economic evaluations.

The aim of this paper is to show the results connected to a study for a new assessment model based on the integration of LCA and LCC, called €CO (Politecnico di Torino, Thiebat, 2009). Different methods, from published international researches, have been applied to evaluate the same building envelopes in order to validate and verify the outcome of €CO model.

Methods here represented and implemented are based either on the monetization of externalities, as the European ExternE Project and the Swedish EPS method, or on scientific studies that take into account environmental and health impacts without weighting them, such as SETAC Environmental LCC and CES Selector, the methodology developed by Prof Mike Ashby (Cambridge University).

Moreover, a further effort to validate the results is the application of the Emission Trading (EU Dir. 2003/87/CE), market-based approach used to control pollution by providing economic incentives for achieving reductions in the emissions of pollutants. In other words, the research tries to shift this approach to the construction market applying the Marginal Abatement Cost Curve (MAC), that is the cost of eliminating an additional unit of pollution.

In conclusion, comparing the results obtained through €CO model with the methods above described, it is possible to affirm that the scale of values is substantially unaltered.

WE 463

Monetisation of indirect externalities of lead emissions from a waste-to-energy plant with focus on trade-offs related to the time horizon choice

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In this study we calculated, via the impact pathway approach (IPA), the indirect external costs of lead emitted from a waste-to-energy facility in Denmark. After emission into atmosphere, lead deposited on topsoil can be uptaken via ingestion. Lead exposure is linked to neurotoxic impacts in children and to reduced lifetime earnings. Indirect costs are expected to be higher than direct ones (via inhalation): lead accumulates in topsoil and ingestion is a major contributor to total daily lead intake. However, indirect impacts occur in the future; their present value is therefore lower when a positive discount rate is applied. Calculating the indirect externalities poses some challenges: a) increased uncertainties due to the additional air-to-soil dispersion modelling; b) need for a long-term perspective to account for lead accumulation in soil; c) impacts and costs to be integrated over future generations. The choice of the time horizon can potentially influence the final results in terms of total costs [€/kg]. We used different models cross-disciplinarily inside the IPA framework: the Operational Meteorological air-quality model (OML), the Simplified Fate and Speciation Model (SFSM), and the Age Dependent Biokinetic Model (ADBM) were used to determine respectively the metals' atmospheric transport, its deposition and accumulation in topsoil, and its bio-accumulation in the human body. Neurotoxic impacts were quantified according to different long-term emission and exposure scenarios; concentration-response functions from updated literature were used, linking blood lead and IQ. The economic model to calculate lifetime earnings has been adapted to monetise neurotoxic impacts on future generations of children. Calculated indirect costs are of the same magnitude as direct costs (previously investigated), both in the case of actual emission rates and in the case of future emission scenarios for lead. Among the various modelling parameters, the social discount rate has the most influential effect: discounting of future impacts is levelling off the differences between indirect and direct costs, so that even if lead is accumulating in soil with a consequent increasing exposure, the present monetary value of future impacts doesn't increase. The use of lead-specific models allows for a detailed quantification of the lead impact pathway and results are in agreement with previous literature.

WE 464

Monetisation of environmental impacts from LCA with the Choice Experiment method: an experimental approach for waste management systems

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Life Cycle Assessment (LCA) in waste management is one of the most applied methods for environmental planning at a local or regional scale. This tool allows comparison of scenarios according to their different impacts. The implementation of a monetisation method on these results can be seen as a possibility to establish a hierarchy of waste management scenarios to help decision-making.

Contrary to classical monetisation methods, which are mainly based on damage or on emissions costs, we propose to monetise environmental impacts from a LCA at a midpoint level. The originality of the method relies on a simultaneous monetisation of different impacts with a unique valuation process. At first the unit monetary values provide a weighting system of impacts. Then they are coupled with previous LCA results of waste management scenarios to establish a hierarchy between them.

Our explanatory approach begins with a LCA implementation by modelling with GaBi software four waste management scenarios: a benchmark scenario (incineration) and three Mechanical Biological Treatment alternatives. Waste management systems are limited to residual waste and biowaste flows and take into account collection, transport, treatment, refusal management and by-products valorisation. Only three midpoint impact categories, which represent issues in this area, are studied: Global Warming Potential (GWP), Human Toxicity Potential (HTP) and Abiotic Depletion Potential (ADP).

Then, we propose to adapt the Choice Experiment method, derived from marketing research, to obtain monetary values from environmental impacts. Choice Experiment (CE) is a stated preferences method which requires to design a survey and then to submit it to a population sample (a test sample of one hundred persons). People are asked to choose waste management scenarios but do not have to directly express their Willingness-To-Pay (WTP). An econometric analysis of the survey results - here a Mixed Logit Model which takes into account individual heterogeneity - provides marginal WTPs for each impact category.

CE is generally applied to real issues. That's why we need to adapt it by bringing additional information about the topic on the one hand and by giving a "pictured description" of the environmental impacts on the other hand. Results stress the existence of an important intra impact dispersion of individual WTPs and important inter impact differences in average WTPs.

WE 465

Assessing the impacts of abiotic resource use by monetisation

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In this study, a new assessment method is proposed to investigate the impacts from resources use, as none of the existing methods is considered mature enough to be recommended in the ILCD handbook. Based on resource functionality, future abundance and the financial consequences of the use of resources, this new approach permits the monetisation of the impacts as a new impact category indicator is expressed with a monetary unit. This allows the consideration that the extraction of a certain amount of abiotic resource forces one of the future users to pay a different amount to extract the same quantity of the same resource as it becomes scarcer. In order to illustrate the approach, it has been applied to metallic resources used in electrical vehicles batteries to compute impacts on natural resources depletion. Results have been compared with the impacts obtained with other LCIA methodologies to put in perspective advantages and drawbacks of each of them and see to what extent results obtained are consistent when applying different approaches showing different degrees of sophistication. Lithium characterisation factors are obtained with the proposed methodology and are around three orders of magnitude greater than the one with ReCiPe, which also models resource depletion by monetisation. Inconsistencies between ReCiPe and other methodologies are then put into light as the main contributors to the impact differ greatly from one methodology to the other.

WEPC1 - Bioavailability and bioaccumulation - impact of environmental, biological and ecological variation

WEPC1-1

Uncertainty of bioaccumulation and biomagnification measurements in natural aquatic food webs: a sensitivity analysis approach

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The potential of a chemical substance to accumulate in aquatic organisms and to increase in concentration with increasing trophic level are criteria that are used to classify substances as being bioaccumulative in the environment. For aquatic organisms, bioaccumulation factors (BAFs) describe the accumulation of contaminants by living organisms relative to the amount stored in the water compartment. Similarly, biota-sediment accumulation factors (BSAFs) describe the accumulation of contaminants by living organisms relative to the amount stored in the sediment compartment. Biomagnification factors (BMFs) describe the increase in concentration of a substance in living organisms that are separated by a single trophic level step on a food chain. Because of biomagnification processes, individual BAF or BSAF values are dependent upon trophic level position of the organism under consideration. Consequently, trophic magnification factors (TMFs), which describe the increase in concentration of a substance in living organisms that occupy successively higher trophic levels within a food web, are used to assess bioaccumulation and biomagnification of chemicals in the environment. This presentation will show how these field metrics for assessment of bioaccumulation and biomagnification (i.e., BAF, BSAF, BMF, and TMF) are interrelated. We will use field data to evaluate various approaches that may be used to calculate bioaccumulation and biomagnification metrics from measured concentrations, normalized concentrations

(for example, to lipid content), concentration ratios, and benchmark compounds that are known to bioaccumulate and biomagnify (for example, polychlorinated biphenyls). Several factors may directly affect the relationship between trophic position and the bioaccumulation and biomagnification of contaminants in aquatic ecosystems. Therefore, the evaluation will include an assessment of the sensitivity of the different approaches to parameters such as trophic level, nitrogen-15 enrichment factors (used to estimate relative trophic level position), omnivorous feeding, and lipid content.

WEPC1-2

From cameo appearances to leading roles in bioaccumulation risk assessment? Enter new cast member: M(etabolism)

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In our earlier Setac papers, we concluded based on a combination of field and laboratory data that the bioconcentration factor (BCF) can be a useful predictor of the trophic magnification factor (TMF) for a range of chemicals with specific chemical characteristics and under specific ecological conditions. One of the important "exceptions" where the BCF does not provide accurate information about the bioaccumulative nature of chemicals in the environment is those chemicals that exhibit a high BCF in laboratory tests, but also biotransform in foodwebs, thus not biomagnifying and consequently retaining a low TMF.

Biotransformation is a key factor in determining bioaccumulation and further trophic magnification. In the PBT assessment much attention is being paid to the persistence of chemicals which is assessed with biodegradation tests by microorganisms. In spite of this, metabolism (M) of chemicals by higher organisms is not taken into account in the PBT assessment. Various examples have shown that metabolites of chemicals are more toxic than the parent compounds. B-assessment should therefore also include M assessment with a connection to toxicity. Screenplays require you to know your characters very well before introducing them, which is why in the risk assessment (RA) 'screenplays' for chemicals, we have not been able to give any leading roles to metabolites, which we would like to call "M" here. They make only cameo appearances in RA. In this presentation we discuss some scenarios where M changes the outcome of the chemical risk story. We do this with examples from field and laboratory data.

WEPC1-3

Variation in clearance rates in Japanese mussel populations: bioaccumulation of organic contaminants or natural variability?

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In order to assess whether an environment is being impacted by anthropogenic contamination, a strategy of measuring the health of a sentinel species coupled with quantifying contaminants accumulated in their tissues, is often applied. There are three main factors that can confound the results: (1) variation in environmental conditions (e.g. salinity, temperature etc.), (2) natural variability between populations and (3) exposure to toxic contaminants that are not bioaccumulated. The coastal waters of Japan, such as the East China Sea, are reported to be among the most impacted by human activities in the world. To assess the effects of organic contaminants on marine communities around the coast of Kyushu Japan, mussels were chosen as sentinel species and their health assessed using the well-accepted clearance rate assay. To account for environmental variability, key parameters were measured. Mussel tissues were extracted and analysed by gas chromatography - mass spectrometry (GC-MS). In addition, comprehensive two dimensional GC-MS was also employed to examine compounds unresolved by conventional GC. To assess if contaminants that were not amenable to bioaccumulation were present in the water, Polar Organic Chemical Integrative Samplers were deployed for four weeks in Nagasaki harbour and at an offshore island (Goto Island) away from sources of contamination. Large variations were observed in mussel clearance rates between sampling stations. Some mussel populations had clearance rates well below those from Goto Island. Only very minor differences were recorded for environmental parameters. Attributing the apparent poor health of the mussels to contamination by organic compounds was however problematical. The issues raised by this study are discussed.

WEPC1-4

Metal bioaccumulation in deposit-feeding polychaete depends on sediment geochemistry

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Current understanding of metal bioaccumulation in deposit-feeding animals is still unresolved. Field observations need to be complemented with laboratory based studies and mathematical modeling. Bioaccumulation of arsenic, cadmium and chromium in deposit-feeding polychaetes collected from two different US estuaries, the Chesapeake Bay and San Francisco Bay, was compared to model predictions. A biokinetic model combining aqueous and dietary uptake terms was modified such that the dietary uptake term considered partitioning of the metals in diverse geochemical fractions as determined with a sequential extraction scheme. Kinetic parameters, including metal assimilation efficiencies and efflux rate constants, that were used in the model to quantify metal uptake in the deposit-feeding polychaete *Nereis succinea* from the aqueous phase and from food were determined experimentally using radiotracer protocols. ⁷³As(+5), ¹⁰⁹Cd and ⁵¹Cr(+3) were used for sediment and pore water labeling. Sediments used in the experiments and polychaetes that were later analyzed for metal concentrations were collected from the same sites by a box corer. Dietary sources were shown to account for >97% of the metal body burden. By incorporating the geochemical fractionation of metals in the model the correlation between field observations and model predictions improved considerably ($r^2 = 0.84 - 0.87$) over a model that considered only the total metal concentrations in sediment ($r^2 = 0.11 - 0.50$). Given the importance of the dietary pathway, we investigated mechanisms of metal assimilation, focusing on As. The percent of As released from particles into gut fluid extracted from *N. succinea* was directly related to, and greater than or equal to AEs. Bovine serum albumin, a previously used mimic for worm gut fluid, was less effective than natural gut fluid in mobilizing metal from ingested sediment. Arsenic associated with algal cells was assimilated more effectively than As bound to goethite particles (72 vs. 2%). 15% of As was released from goethite into gut fluid but only 2% was assimilated by *N. succinea*, suggesting that metal release from particles is necessary but insufficient to explain assimilation. Our results suggest that the likelihood of As assimilation is higher when it is bound to an organic compound potentially of nutritional value in the ingested particles.

WEPC1-5

Are transport proteins relevant for the overall uptake of environmental pollutants?

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With the implementation of the REACH legislation in the EU, thousands of compounds need to be tested regarding their potential to damage human health and the environment. However, due to financial, practical and ethical constraints not all of these compounds can be investigated in the laboratory or field, and models are needed to predict the fate and effects of untested compounds.

Most bioaccumulation models assume that uptake occurs via passive diffusion through a series of water and lipid layer. However, organisms have developed active transport and facilitated diffusion pathways to increase uptake of nutrients during food digestion. A number of drugs are known to act as substrate for some of these proteins.

Attempts have been made to characterize the structural requirements of substrates in order to increase oral availability of drugs. However, even if the drug or other xenobiotic compounds is recognized as a substrate, the overall uptake will still depend on the kinetics of the given substrate-protein combination as well as on the expression level of the protein throughout the GIT.

The aim of this study was to evaluate the potential of environmental pollutants to use transport proteins and how this process influences the overall uptake. Therefore, literature was first reviewed to understand how typical nutrients such as glucose, amino acids, peptides and vitamins are taken up by humans and what are the underlying kinetics. In a second step, literature will be searched to evaluate the substrate specificity of these transport proteins. Conclusions will be drawn whether carrier mediated transport might pose an additional uptake pathway for environmental pollutants and whether it should be included into risk assessment.

WEPC1-6

Dynamic factors - a practical tool to evaluate transfer of contaminant from abiotic to biotic environment

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Environmental, biological and ecosystem variables may affect the transfer of contaminants from the abiotic to the biotic compartment including variation in contaminant concentration, type of contaminant, as well as in physiological parameters like biotransformation ability. Since element bioaccumulation depends on physiological sensitivity on the total contamination of soil we use a new biogeochemical parameter - dynamic bioaccumulation factor - to analyze change in bioaccumulation. Dynamic translocation factor can be used to estimate changes of element translocation in plant. Change of element participation in metabolism can be expressed in dynamic factor of biophilicity.

Dynamic factors, differently from the factors mentioned in the existing literature, highlight changes in processes rather than changes in element quantities when conditions of the environment change. Equations to calculate factors as well as a case study will be presented and discussed in the presentation.

WEPC1-7

Comparison of fish species used for dietary exposure according to the proposed OECD 305 test guideline

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The proposed revision to the OECD 305 Test Guideline, Bioconcentration: Flow-through Fish Test, includes an approach for exposure via the diet for substances that are highly hydrophobic and not suitable for aqueous exposure. To validate the dietary exposure method, an international ring test was performed to determine the interlaboratory variation and reproducibility of the proposed test guideline. In the ring test, 5 different substances including hexachlorobenzene, musk xylene, ortho-terphenyl, methoxychlor, benzo[a]pyrene were exposed concurrently to juvenile rainbow trout. Due to the fast growing life stage of the fish used in the study, the concentrations of the different chemicals in the tissues of the fish were affected by growth dilution in addition to other elimination processes. Subsequently, a non-growing fish species, such as adult zebrafish, has been suggested as a potential alternative test organism to avoid this problem. To assess the suitability of adult zebrafish, a similar dietary exposure test was performed using the same 5 substances as that tested previously. The test involved exposing adult female zebrafish to spiked commercial fish food for a period of 13 days, followed by a 2 week depuration period with clean fish food. During the depuration phase, fish were sampled at different time intervals for chemical analysis and from these data a depuration rate constant and biomagnification factor was calculated. These data were then compared with data obtained from the original ring test using rainbow trout. The results from the tests with the two different species of fish will be presented in this poster along with recommendations for the suitability of adult zebra fish as an appropriate alternative test organism for the dietary exposure method.

WEPC1-8

A ring test of the draft OECD 305 bioconcentration test guideline dietary exposure method

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The potential for chemical substances to bioaccumulate in organisms is a key element in chemical environmental risk assessment. A commonly used approach to assess this is the determination of bioaccumulation potential in aquatic species such as fish. One of the most commonly used tests is the OECD 305 Test Guideline, Bioconcentration in Fish. This Test Guideline was last reviewed in 1996; it is currently under revision, with a major update to include a method for dietary exposure, which is suitable for highly hydrophobic substances that are not amenable to aqueous exposure.

To validate the dietary exposure method, ring testing has been undertaken to demonstrate reproducibility of results, and provide information on interlaboratory variation.

Ten laboratories worldwide conducted dietary bioaccumulation studies, using rainbow trout. Five test substances were chosen to represent a range of potential for biotransformation: hexachlorobenzene, musk xylene, ortho-terphenyl, methoxychlor, benzo[a]pyrene. The substances were spiked to commercial fish food, on which fish were fed daily during a set exposure regime at two different feed rates, followed by depuration on substance-free food. Chemical analysis of fish tissue during depuration, along with basic substance and organism data, allows for calculation of a fish growth rate constant, a substance depuration rate constant, assimilation efficiency, and biomagnification factor. Results from the ring test are described, including information on feeding rate, corrections for fish growth during the test, reproducibility and interlaboratory variation.

WEPC2 - Environmental OMICs: a global answer to environmental questions

WEPC2-1

Evaluation of chronic sublethal effects of the pharmaceuticals gemfibrozil and diclofenac on the marine mussel (*Mytilus* spp.) using a proteomic approach

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Over recent years the presence of human and veterinary pharmaceuticals in the aquatic environment has become an environmental problem. A major source of these novel pollutants is through the release of waste water effluents, both treated and untreated, into the aquatic environment. The lipid regulator gemfibrozil and the non-steroidal anti-inflammatory drug diclofenac are commonly found throughout the western world including in the Irish aquatic environment. Concentrations are ranging from high ng/L to low µg/L. The potential toxicity of these compounds on non-target animals, like shellfish, remains largely unknown.

In this study, the marine mussel (*Mytilus* spp.) was exposed to environmentally relevant and elevated concentrations (1 & 1000 µg/L, respectively) for 14 days. Chronic sublethal effects will be investigated by looking at the protein expression signatures using two-dimensional gel electrophoresis (2DE) and a suite of biomarkers comprising glutathione transferase, lipid peroxidation and DNA damage. Additionally it will be examined whether blue mussels have the potential to recover from an exposure. Therefore mussels were left to recover for an additional seven days after the treatment.

First results of the 2DE are showing that both drugs have the ability to induce a specific response in blue mussels after 14 days of exposure.

This study contributes to the understanding of the biological effects of pharmaceuticals in non-target organisms, such as the widespread blue mussels.

WEPC2-2

Protein expression profiles in European eel (*Anguilla anguilla*) exposed to perfluorooctane sulfonate; combination of in vitro, in vivo and in situ approaches

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Since the 1980s, the stocks of European eel have been declining in most of their geographical distribution area and they are now considered below safe limits for population survival. Many factors can be attributed to this decline such as pollution by xenobiotics like PFOS. The aim of this research project was to develop a multi-biomarker approach to set up an evaluation and monitoring tool for assessing the health state of eels. The strategy consists in assessing protein expression profiles (PEPs) in post-nuclear fraction of peripheral blood mononuclear cells (PBMC) exposed in vitro to PFOS, before in vivo and in situ validations of biomarkers. The first step of this approach was to find out PEPs of in vitro PFOS exposure. For that, we used PBMC culture from eel to test the in vitro toxicity of this compound. Exposure time and two sub-lethal concentrations were chosen to avoid cell mortality (48h exposure at 10 µg/L and 1 mg/L). PBMC were isolated from blood by centrifugation over a Ficoll/Hypaque gradient. After in vitro contaminations, the post-nuclear fraction was isolated. Two-dimensional differential in-gel electrophoresis (2D-DIGE) was performed on 24cm, pH 4-7 IPG strips and the results were analysed with DeCyder software. The identified differentially expressed proteins have been categorized into diverse functional classes, related to cell structure, protein folding (e.g. protein disulfide isomerase) and signal transduction (e.g. 14-3-3 protein epsilon) for instance with some proteins never found in proteomic studies. These results permitted us to define PEP for in vitro PFOS exposure. The second step of the approach was in vivo contaminations of eels in order to validate this profile. For that, yellow eels were exposed to environmental PFOS concentrations (10 and 0.1 µg/L) during 28 days. As before, PBMC and their post-nuclear fractions were isolated; proteomics analysis was made. Common protein spots that were affected by PFOS exposure as well in the in vitro and the in vivo experiments served to form the PFOS PEP. Finally, eel samplings in 3 Flemish rivers presenting various PFOS pollution degrees were realized in order to validate the previous results.

WEPC2-3

Response of the European flounder *Platichthys flesus* to experimental and in situ contaminations: a proteomic approach

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Estuaries are important areas highly susceptible to anthropogenic degradation such as pollution. Estuarine species have then to cope not only with environmental constraints inherent to estuaries habitats, but also with the presence of contaminants, the occurrence of hypoxic events or with waters warming. The European flounder *Platichthys flesus* is considered as a sentinel species in estuarine water quality monitoring. This study aims at identifying potential biomarkers of the response to contamination by understanding the molecular mechanisms allowing the European flounder to cope with these different stress factors, both in environmental and experimental conditions. A proteomics approach using 2-dimensional electrophoresis followed by MALDI TOF-TOF mass spectrometry allowed us to identify differentially expressed proteins in flounder livers, and then to understand the mechanisms and the pathways implied in flounder response to its environment constraint.

First, farm flounders were experimentally contaminated during 30 days with a PAH/PCB cocktail designed to be 10-times more concentrated than Seine sediments, which allowed us to characterize the response of *P. flesus* to this specific type of contaminants and especially the detoxification mechanisms implying BHMT. Then, Flounders were fished in contrasted estuaries along the European Atlantic coast. The liver proteomic patterns of Flounders from the Seine (France), the Canche (France) and the Tamar (UK) were compared to characterize the differential protein expression between these sites, in relation to the contamination patterns of each estuary. The Seine and the Tamar are indeed highly contaminated estuaries, by contrast with the Canche. Finally, fishes from the Mondego (Portugal-at the southern limit of the repartition area), the Vilaine (France-known to exhibit severe hypoxic events in summer) and from The Seine (France-known to be highly polluted) were gathered in common garden experiments. They were subjected to the same PAH/PCB cocktail already used on the farm fishes. These experiments allowed us better understanding the acclimation mechanisms of these fishes to a new contamination, in function of their origin and the environmental constraints they were used to encounter.

WEPC2-4

Constructing estrogen-sensitive gene networks in the liver of *Fundulus heteroclitus* for characterizing adverse effects of estrogenic pollutants

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Studies using freshwater fish models have shown that exposure to low concentrations (<20 ng/L) of 17 α -ethinylestradiol (EE2) result in a reduction or shutdown of egg production. Remarkably, the mummichog (*Fundulus heteroclitus*) is able to produce eggs at >3000 ng/L EE2. Although individual level effects in mummichog are not pronounced, there are physiological and molecular responses. The objective of this study was to characterize genomic responses and gene networks in mummichog after EE2

exposure to gain mechanistic insight into how mummichog are able to produce eggs normally during high EE2 exposures. Mummichog were exposed to 50 or 250 ng/L EE2 and female endpoints were assessed at different levels of biological organization. There were no differences in gonad morphology, development, GSI or LSI, but condition factor was significantly higher in fish exposed to 250 ng/L EE2. There were no differences in estradiol levels, although testosterone decreased with increasing exposure concentrations. Gene expression (real-time PCR) focused on genes involved in lipid mobilization/transport and steroidogenesis, two pathways important for egg production that are regulated by EE2. No target genes in the steroidogenic pathway significantly differed with treatment, however vtg1 and vtg2 mRNA levels significantly increased, which suggests a physiological response to the estrogenic exposure. Microarray expression profiling in the liver identified 305 differentially expressed genes in both treatments after FDR correction (5%). Gene set enrichment analysis (GSEA) revealed genes involved in processes such as steroid biosynthesis and fatty acid binding were significantly affected by EE2. Sub-network enrichment analysis (SNEA) identified proteins of 21 genes post-translationally modified by Cdc2 (component of maturation-promoting factor), a protein found in all stages of oocyte development, triggers oocyte maturation, and plays a role in liver regeneration. There was evidence to suggest that the mummichog liver is under generalized stress from EE2 exposure. For example, genes for monooxygenase activity and inflammatory response were significantly altered and pathways to protect or repair tissue were activated. These data are comparable to other freshwater species and suggest that there are conserved EE2-mediated pathways in mummichog liver. This study identifies novel gene networks for cell pathways underlying the mechanisms of action of EE2 for egg production.

WEPC2-5

Transcriptional screening of effects of mixtures of POPs in burbot (*Lota lota*) from two Norwegian lakes, using suppression subtractive hybridization and 454 FLX pyrosequencing

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Lake Mjøsa is the largest freshwater repository in Norway, receiving runoff from a wide surrounding region of urban country. As a result of industrial activity, large quantities of persistent organic pollutants (POPs) have been discharged into Lake Mjøsa during the last century. Because of this, organisms in this lake contain high levels of toxic chemicals, i.e. polychlorinated biphenyls (PCBs), brominated flame retardants (BFR), dichlorodiphenyltrichloroethane (DDT) and its metabolites, and mercury (Hg). Very high levels of BFRs have been found in top predators such as brown trout (*Salmo trutta*) and burbot (*Lota lota*) from this lake. The levels of PCBs, DDTs and PBDEs in burbot from Lake Mjøsa (study population) exceed the corresponding levels in burbot from Lake Losna (reference) by a factor of approximately 10, 15 and 300, respectively. Transcriptomic profiling is widely used as a toxicogenomic discovery tool for identifying the molecular basis of biological functions underlying responses to toxicants, revealing potential mechanisms of toxicity. Even though microarrays remain the primary technology for transcriptomic profiling, an emerging alternative is to use next-generation sequencing to directly sequence and quantify transcripts from experimental samples. Compared to microarrays, next-generation sequencing technologies offer several advantages in functional genomics research, including a much wider dynamic range of detection.

We used shotgun and suppression subtraction hybridization (SSH) cDNA libraries followed by 454 FLX sequencing (957 303 reads sequenced in total) and RT-qPCR to study the effects of POPs in burbot from Lake Mjøsa. The gene list of putatively higher or lower expressed genes in liver of burbot from lake Mjøsa compared to liver of burbot from Lake Losna, generated from the SSH cDNA libraries, suggest that mechanisms associated with cancer, lipid metabolism and vitamin and mineral metabolism are differentially regulated in the two populations (Ingenuity Pathway Analysis (IPA) top networks). According to the IPA analyses, the top toxicity list ranking was "Negative/Positive acute phase response proteins", "FXR/RXR activation", "NRF2-mediated oxidative stress response" and "Glutathione depletion - CYP induction and reactive metabolites". Overall, the study shows that next-generation sequencing may be a valuable supplement or alternative to microarray technology in toxicogenomic discovery of environmental samples.

WEPC2-6

Expanding the SWIFT periphyton community test towards metagenomic endpoints

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The SWIFT periphyton test is a simple, high-capacity test for assessing effects of toxicants on natural communities. Periphyton (i.e. attached microbial communities) was developed over 7 to 9 days on glass substratum submerged at sea and then transferred to a controlled indoor environment for 96 hours incubation with toxicants. The SWIFT methodology normally employs analysis of photosynthetic pigment profiles as a measure of changes of community structure and physiology. In addition to this endpoint, we here present methodology to expand the SWIFT test to metagenomic or community genetic endpoints. In two SWIFT experiments periphyton communities were exposed to the antimicrobials triclosan and ciprofloxacin respectively. The toxic effect of these compounds on pigment profiles were compared to their effect on genetic structure of the communities, using Temperature Gradient Gel Electrophoresis (TGGE) methodology. The communities in the SWIFT tests are also compared to communities sampled at several sites and on several occasions in the nearby coastal marine environment. Thus, this study also compares the typical genetic and pigment diversity in SWIFT tests to that of environmental samples. Moreover, it shows that it is possible to expand high-capacity toxicity testing on periphyton communities to include also effects on the genetic structure or metagenomic composition of communities.

WEPC2-7

Metagenomics in ecotoxicology - a new ecologically relevant research field that integrates high-throughput sequencing techniques with community ecotoxicology

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The development of high-throughput sequencing technologies has revolutionized biology and especially the field of microbial ecology, since it has enabled scientists to study the whole genetic composition of natural microbial communities. This research area, named metagenomics, is of utmost importance for community ecotoxicology. Among other things, it might enable the simultaneous study of how toxicants affect community structure and community functions. It has been estimated that only 1% of the microbial species could be studied before the DNA sequencing era, using culturing techniques. It is thus obvious that metagenomics dramatically can increase our ability to detect ecologically important effects of toxicants. Metagenomic approaches can, for example, identify sensitive and tolerant taxa, help in linking specific toxicant exposures to specific biological effects and aid in the determination of mechanism of action of toxicants. However, sequencing platforms like 454 and Illumina produce enormous amounts of sequence data and therefore there is a need for high-throughput, efficient bioinformatic approaches in this field. Here, we present a framework for the use of metagenomics and bioinformatic analyses in ecotoxicology. We provide conceptual ideas on how to exploit the full potential of DNA/RNA sequencing platforms and bioinformatic pipelines that take advantage of different software, databases and the newest online server services. We also exemplify these ideas with analyses of ecotoxicologically relevant sequence datasets.

WEPC2-8

Large scale gene expression profiling on female eelpout (*Zoarces viviparus*) and developing larvae capture around the Baltic Sea area

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The eelpout (*Zoarces viviparus*) inhabits the coastal waters of Northern Europe and has been part of the environmental monitoring of the Swedish coastal areas for several years. In the search for new biomarkers and understanding the molecular mechanism behind physiological changes we now combine classical biomonitoring of the eelpout with large scale gene expression profiling. As the eelpout lives relatively stationary we can link gene expression data and observed physiological responses to the environmental situation where the fish was caught. Furthermore, the viviparity of the eelpout gives the unique opportunity to associate the effects of pollutants to individual reproductive performance, including the development of embryos and fry.

We have earlier sequenced the eelpout liver transcriptome and the sequence data has been used to design and construct an oligonucleotide microarray. This eelpout microarray contains several categories of genes that are of particular interest for ecotoxicological research, including different cytochrome P450 variants, heat shock proteins and genes related to oxidative stress, as well as several known biomarkers. The eelpout microarray platform has been successfully used in our monitoring study performed on eelpout captured in Göteborg harbour, a large port situated at Swedish west coast. Among the up-regulated gene in the harbor, we found genes involved in apoptosis and among the down regulated genes we found genes part of the innate immune system.

We will here present results from a much large gene expression study including 16 sites around the Baltic Sea area involving 160 individual eelpout samples. Results will also include gene expression analysis on developing larvae from 50 individual larvae. Gene expression oligonucleotide microarray is an important tool in the search for new biomarkers and for the understanding of molecular mechanisms resulting from exposures to toxicants. It is therefore an important complement to traditional environmental monitoring. These results are part of an EU-financed project (www.science.balcofish.gu.se).

WEPC3 - Pesticide fate and ecotoxicology

WEPC3-1

The impact of pesticides on the benthic macroinvertebrates in a rural lake

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Human activities at the landscape scale are a threat to the ecological integrity of many aquatic ecosystems. It is often difficult to isolate the effects of pollutants from other causative factors, and to identify the primary pollutants causing ecosystem stress. Additionally, pollutants may not be homogeneously dispersed, with potentially varying effects on biota living within the same water body; therefore there is a need to have appropriate strategies for management in order to maintain the ecological integrity of the whole

ecosystem. The study was undertaken at Lake Hawthorn in northwestern Victoria, Australia. Results from previous surveys of the lake showed that sediments were impacted by a number of pesticides which were also toxic to macroinvertebrates. Pesticides were not dispersed homogeneously throughout the lake. The aims of the study were to a) determine the number of samples that should be collected to ensure that there is an adequate assessment of contaminants present in sediment and water and b) determine if the composition and health of benthic macroinvertebrates are affected by the presence of pesticides. The lake was divided into six sampling locations and between three and five sites selected at each location, where sediment and surface water was collected and a range of chemicals were measured (including metals, a range of pesticides and nutrients). Benthic macroinvertebrates were sampled in a subset of sites at each location for species abundance and diversity and also for changes at the sub-organism level on a number of biochemical biomarkers. Sediments were also assessed for toxic effects on survival, growth and emergence of *Chironomus tepperi* using a laboratory-based bioassay and impacts on indigenous macroinvertebrates using field-based microcosms. There were elevated concentrations of copper and total petroleum hydrocarbons in sediment at one location within the lake, an urban inlet drain. There were also effects on *C. tepperi* survival at this location. Furthermore, there were no in situ chironomids collected at this location. Biomarker analyses on the chironomid *Tanytarsus semibarbitarsus* collected *in situ* showed that chironomids are responding differently within the lake. Our results show that the pollutants present in Lake Hawthorn vary spatially and that they are toxic to aquatic life. Results on sampling strategically and the usefulness of a multiple lines of evidence approach for aquatic biomonitoring will be discussed.

WEPC3-2

Understanding the legacy of aged p,p'-DDT and p,p'-DDE residues in New Zealand horticultural soils

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The long-term use of p,p'-DDT to control insect pests on crops in New Zealand has resulted in elevated concentrations of p,p'-DDT and p,p'-DDE in some horticultural soils. Concentrations of residual p,p'-DDT and its degradation product p,p'-DDE in New Zealand orchard soils can exceed 10 mg kg⁻¹. Previous studies have shown that p,p'-DDT and p,p'-DDE residues in New Zealand orchard soils remain available for uptake by edible plants and earthworms despite four decades of aging. Desorption using Tenax has been proposed as a surrogate measure of the bioavailability of hydrophobic organic contaminants (HOCs) in lieu of time consuming and expensive bioassays. There has been limited assessment of Tenax as a surrogate measure of the bioavailability of aged p,p'-DDT and p,p'-DDE residues in soils. We have previously reported significant correlations between earthworm tissue concentrations and the concentrations of p,p'-DDT and p,p'-DDE desorbed from orchard soils in a 24 hour extraction with Tenax. A long-term tenax desorption experiment with five orchard soils was undertaken to determine the kinetics of p,p'-DDT and p,p'-DDE release from the soils and to estimate the bioavailable fraction. The results were modelled using a three compartment kinetic model that describes rapid, slow and very slow desorbing fractions of hydrophobic organic contaminants. Over 162 days up to 53% of p,p'-DDT and 43% of p,p'-DDE were released from the soils. There was a significant correlation for p,p'-DDT between the total amount desorbed by Tenax over the duration of the desorption experiment and the amount oxidised by persulphate oxidation, a surrogate measure of the bioavailability of HOCs for microbial degradation. Values for the rapidly released fraction, Fr represented 8 to 19% and 10 to 21% of the total p,p'-DDT and p,p'-DDE residues respectively. The rapidly desorbing fractions of p,p'-DDT and p,p'-DDE were significantly correlated with the fraction desorbed over 24 hours indicating that 24 hours may be a suitable extraction period for estimating the bioavailability of aged p,p'-DDT and p,p'-DDE residues in these soils.

WEPC3-3

Impacts of sediment-bound synthetic pyrethroids on non-target aquatic macroinvertebrates

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When in the water column, synthetic pyrethroids have a high acute toxicity to many aquatic macroinvertebrates. However, they rapidly bind to sediment, where they potentially threaten macroinvertebrates through chronic exposure. The effects of such exposure, which may include impacts on life cycle parameters, are less well understood. In addition, there is a need for biomonitoring tools to identify synthetic pyrethroid contamination, in particular subcellular biomarkers that can be linked to organism and community level effects. We aimed to identify and link the effects of two synthetic pyrethroids at these three levels of organization. Laboratory toxicity tests and field microcosms were used to examine the effects of sediment-bound bifenthrin and permethrin on survival and life cycle parameters of aquatic macroinvertebrates. This dual-pronged approach benefits from both the highly controlled approach of lab exposures, and the more environmentally relevant approach of the microcosms, which includes natural environmental conditions and community interaction. This work also incorporates the identification of biochemical biomarkers produced by exposed macroinvertebrates, in order to develop biomarkers that indicate effects at the whole organism and community levels. In the laboratory toxicity tests, clean field-collected sediment was spiked with either bifenthrin or permethrin at concentrations of 12.5, 25 and 50 µg/kg. Second instar *Chironomus tepperi* larvae were placed in beakers containing spiked sediment. At 50 µg/kg of bifenthrin, the number of larvae surviving after five days was lower than that of the controls, however the body length of surviving larvae was not affected. Two weeks of exposure to bifenthrin did not affect the total number or sex ratio of emerged adults. Results for permethrin will be discussed. In the microcosm experiments, clean field-collected sediment was spiked with a range of concentrations of either permethrin or bifenthrin from 8.9 µg/kg to 500 µg/kg. This sediment was placed in wetland microcosms, where colonization by macroinvertebrates was permitted. Emerged adults were collected over several weeks and identified to species level. The chironomid *Paratanytarsus grimmii* showed significantly reduced emergence at concentrations of permethrin above 35.5 µg/kg. Results for bifenthrin will be discussed, as will the links between subcellular biomarkers and effects on whole organism parameters and community composition.

WEPC3-4

Biochemical effects of bifenthrin on chironomids larvae under field and laboratory conditions

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Bifenthrin is a third generation synthetic pyrethroid pesticide that is widely used in Australia and is frequently found in freshwater sediments in relatively low concentrations. However, it is highly toxic to non-target species such as native chironomid larvae. Our main goal was to compare the effects of bifenthrin on chironomid larvae under different exposure conditions. *Chironomus tepperi* larvae (5 days old) were exposed to sub-lethal sediment concentrations of bifenthrin for 5 days. A field microcosms exposure with bifenthrin-spiked sediments (using the same concentrations as the laboratory exposure) was carried out for 50 days to allow for colonization and development of chironomid larvae. At the end of both experiments, larvae were collected and glutathione-S-transferase, glutathione reductase and glutathione peroxidase were measured. Field based microcosms were also conducted to evaluate the impact of sediments spiked with bifenthrin on macroinvertebrate assemblages. No significant effects were detected on survival or enzyme activity in *C. tepperi* larvae following laboratory exposure. Results provide evidence that short-term exposures to sublethal concentrations of bifenthrin did not cause significant effects of *C. tepperi* larvae. The microcosm method showed also to be a valuable tool in the risk assessment of contaminants and effects of pollutants on aquatic invertebrates.

WEPC3-5

What is persistence? Seeking for a practical interpretation for regulatory practice

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Hazard based POP, PBT and vPvB cut-off criteria have been introduced in the regulation 1107/2009 for plant protection products. The persistence of a given substance is defined in this regulation via the comparison of its half-lives in different environmental compartments to specific triggers for those compartments. This definition is rather simple. However, considerable challenges are associated with the practical interpretation of persistence (P) as a hazard based cut-off criterion for the data rich cases of pesticides which may result in unjustified P classifications.

Using a low level of information is not appropriate to establish a scientifically justified conclusion on the long-term behaviour of substances in the environment if higher tier data exist that allow a weight of evidence assessment. A reductionistic and rigid interpretation of persistence neglects a huge amount of data which is available on the partitioning and fate of substances in the environment.

A practical interpretation of persistence will be presented, in which the definition of persistence will be examined with respect to the interactions in the environment as well as within the context of agricultural practice.

It is common interest of regulators and notifiers to perform the classification of substances in a manner that reflects latest scientific achievements.

WEPC3-6

Occurrence of pesticide associated non-extractable 14C-residues in physical and chemical fractions in soils

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This study aimed to investigate the fate and behaviour of non-extractable 14C-residues from three commonly used pesticides (isoproturon, diazinon and cypermethrin) in soil, with particular focus on fractionation of the soil using physical and chemical techniques. Non-extractable 14C-residues were formed following the incubation of the 14C-pesticide in two soils over a period of 24 months. A significant amount of 14C-activity was lost during the incubation period. Soils containing the remaining non-extractable 14C-residues were separated into three physical fractions: sediment (SED, >20 µm), microaggregate (MA, 20-2 µm) and colloid (COL, 2-0.05 µm) phases. The non extractable 14C-fractions were also separated chemically according to their association with soil organic carbon: fulvic acid (FA), humic acid (HA) and humin (HM). Enrichment factors for the organic carbon and the 14C- residues were higher in the MA and COL fractions than that of the SED fraction. Greater association and enrichment of the fulvic acid fraction of the organic carbon in the soil was observed. Non-extractable 14C-residues at the FA fraction diminished, while in the HA fraction increased with decreasing the soil solid fraction size. An appreciable amount of non extractable 14C-residues was located in the HM fraction, but this was less than the amount recovered in the humic substances. Knowledge of the long term fate of non extractable 14C-residues on the soil fractions is important in order to assess any risk associated with them. Combination of chemical and physical fractionation schemes like the ones practiced in this study could help towards better understanding of the fate and behaviour of pesticide non extractable residues.

WEPC4-1

Combining natural competitors and larvicides to improve mosquito control in Cameroon and Kenya

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Currently a tremendous number of infections and deaths in Africa are caused by malaria and other mosquito-borne diseases. Since 2005 the potential of using larvicides and natural competitors to reduce mosquito larvae, as well as the amount of chemicals applied has been successfully demonstrated in the floodplains of river Elbe in Germany and in outdoor ponds in Cameroon. To test the feasibility of this strategy on a larger scale, extended field trials are currently conducted in different habitat types and regions (Nyabondo plateau in western Kenya and the surrounding area of Yaoundé in Cameroon). Preliminary results indicate that the success of the use of natural competitors and larvicides is dependent on the mosquito species targeted and the density of mosquitoes present in the aquatic habitats. Combining traditional measures and those suggested by the novel approach promises to enhance the capability of African communities and their national programs to improve the sustainable control of malaria and other mosquito-borne diseases.

WEPC4-2

Fate of polychlorinated biphenyls (PCBs) in the surface soil of a subtropical forest

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Forest is regarded as a global sink of semi-volatile organic compounds (SOCs) because of its large organic surface area and high organic matter content. Many researches had focused on the atmospheric deposition and air-vegetation exchange processes of SOCs to the forest canopy. However, behaviours of these SOCs in the forest ecosystem were less studied. Therefore, a new Exchange Meter (EM) has been developed to measure the air-surface exchange, leaching and other loss fluxes of polychlorinated biphenyls (PCBs) in the litter that covers the surface layer of the forest soil. The experiment was conducted in a typical subtropical forest-Chinese Dinghu Mountain Biosphere Reserve for every 2 month from June 2010 to June 2011. This study developed a new method for directly measuring the exchange fluxes of SOCs in the interface of soil and air, which led to a better understanding of the environmental fate of PCBs in the surface soil of subtropical forest. The flux estimates indicated that lighter PCB congeners had a stronger tendency to move from soil to air than heavier congeners. Low molecular weight PCB compounds were easy to release from superficial soil via leaching. High molecular weight PCB compounds were more stable in the litter in winter. This study provided evidence that the soil may now be a source of some lighter weight PCBs to the atmosphere.

WEPC4-3

Weed invasion at the rehabilitated Mary Kathleen uranium mine site, northern Australia: toxicity risk to grazing animals

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Caliotropis procera (also known as Sodom apple) is a xerophytic shrub or small tree species, which is native to tropical and subtropical Africa, Asia and the Middle East. *C. procera* is now naturalized in Australia, Central and South America, the Caribbean islands, Indonesia, and many Pacific islands. The plant is alleged to be toxic to humans, cattle, sheep, rabbits and rats. The purpose of this study was to appraise the uptake of elements by *C. procera* at the rehabilitated Mary Kathleen uranium mine site, northern Australia. Since rehabilitation in the 1980s, *C. procera* has colonized all rehabilitated mine site domains and the plant now represents a widespread and abundant pest. Biogeochemical analyses indicate that *C. procera* acquires natural enrichments of Ca, K, Mg and S in its tissue on mine as well as background soils. The concentrations of these elements exceed their maximum tolerable concentrations in the diet of cattle. Considering the chemical toxicity threat to grazing animals due to the presence of steroidal heart poisons and the elevated Ca, K, Mg and S contents in the plant's biomass, control of the weed will be required using appropriate management techniques. At Mary Kathleen, neglect of rehabilitated mined lands by statutory authorities has led to the development of unsustainable vegetation covers over disturbed ground and mine waste repositories in the long term.

WEPC4-4

Effect of Tributyltin (TBT) on testosterone metabolism and imposex expression in *Tympanotonus fuscatus* var *radula* in Warri Harbour, Nigeria.T. Ogbomida¹, L. Ezemonye², I.N.E. Onwurah³, I. Tongo²¹National Centre for Energy and Environment, Benin city, Nigeria²University of Benin, Benin city, Nigeria³University of Nigeria, Nsukka, Nsukka, Nigeria

Tributyltin (TBT) is a biocide that has been widely used in anti-fouling paints for ships. In order to assess the status of TBT pollution in Warri Harbours, imposex was studied in the Nigerian periwinkle, *Tympanotonus fuscatus* var. *radula* an important shellfish in the Niger Delta. The survey results showed that TBT concentration and imposex development were generally low in areas with high intensity of boat and vessel traffic. *T. fuscatus* var *radula* was then exposed to sublethal concentrations (0.1, 1.0, 10 and 20 µg/L) of TBT to determine the effect on testosterone levels. Bioassay test was conducted using OECD Guideline 218 direct sediment toxicity protocol with particular reference to steroid hormones quantification. Serum analysis of the Periwinkles after 30 days exposed to varying TBT concentrations showed increased free testosterone levels with increasing concentration and exposure duration. The result indicated that TBT concentrations elevated free testosterone levels in *T. fuscatus* var *radula* and may lead to imposex expressions. Therefore constant monitoring and assessment of TBT is necessary for protecting sensitive benthic invertebrates representing major proportion of the diet of many species in the Niger Delta ecological zone.

WEPC4-5

Direct and indirect effects of pesticides on aquatic communities: an experimental bioassay in tropical systems (São Paulo, Brazil)

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In recent decades there has been an expansion of agriculture to support the increase in demand for food in Brazil. On the other hand, pesticides are being used for farmers and as a consequence, ecosystems have been changing rapidly, resulting in reduced biodiversity and functional processes in terrestrial ecosystems. However, pesticides may also directly or indirectly affect the aquatic ecosystems, producing undesirable effects on their biological and functional properties. To evaluate these effects, experimental bioassays were conducted with Vertimec®18EC during 10 days, in different treatments (Control: C; direct application of Vertimec: V; uncontaminated runoff: UnC and contaminated runoff: CR). The results indicated the increase of the turbidity (physical effect), nitrogen and phosphorus (chemical effect) in CR and UnC after runoff addition; with reduction in dissolved oxygen after 3 h. In relation to phytoplankton, density reduction was observed in C (65%), UnC (90.6%) and CR (92.0%), but less effect was observed in V (3.7%). The diversity was smaller in V (0.7 bits/ind) in relation to C, UnC e CR treatments (above 1.7 bits/ind) after T240h. Chlorophyceae was more representative in V and UnC (75%), besides Bacillariophyceae in C and CR (40%). The chlorophyll concentrations were similar in T0 (below 10 µg/L) but were highest in C (32 µg/L) and UnC (15 µg/L) in T240h, with considerable differences in phaeophytin concentrations (C>UnC>V>CR). Rotifera was important in T0h and T240h (65%). Density reduction (from 58 to 100%) was observed in Protozoa (C,V), Rotifera (C, UnC, RC, V) and Copepoda (RC, V); nevertheless, the densities of Protozoa (RC), Copepoda and Cladocera (C, UnC) increased in T240h. Different effects were observed in the periphytic community. The highest density occurred in V (303 ind/cm²) in contrast to C (110 ind/cm²); however, the results weren't the same in relation to biomass (C>UnC>RC>C). The diversity values were highest in C and UnC (2.32 and 2.99 bit/ind, respectively), and the major contribution was related to Bacillariophyceae in C, UnC, CR and V. Also, some differences in numbers and dominant species were observed between the treatments (C>UnC>V>CR). The results indicate that Vertimec®18EC and the runoff were responsible for the modification in the communities' structure. Besides, direct and indirect effects were observed, which are associated with biological processes (competition, predation and grazing) and abiotic alterations.

WEPC4-6

The impact of multiple contaminants on the gills of two ecologically distinct fish living in tropical polluted waters

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The contamination of aquatic systems by inputs from agricultural, industrial and domestic residues in tropical and subtropical environments is increasing in the last years. The gills of fish are multifunctional organs involved in gas exchange, ion- and osmoregulation, acid-base balance and nitrogen excretion. The gill cells respond rapidly to various chemicals in order to overcome physiological impairment or tissue damage and, it may have a negative effect on the overall gill function, enhancing fish susceptibility and potentially causing fish mortality. This study investigated the gill mitochondria-rich cells (MRCs) variables and the Na⁺/K⁺-ATPase (NKA) activity in two fish species, *Astyanax fasciatus* and *Pimelodus maculatus* collected in 5 sites (FU10, FU20, FU30, FU40, FU50) of the reservoir of Furnas Hydroelectric Power Station in order to evaluate their potential as biomarkers of water quality by integrating chemical, biochemical and morphological analyses. Water analyses revealed aluminum, iron and zinc as well as organochlorine (aldrin/dieldrin, endosulfan, heptachlor/heptachlor epoxide and metolachlor) contamination, excepting in FU10. Copper, chrome, iron and zinc were detected in the gills of both species and aldrin/dieldrin, endosulfan and heptachlor/heptachlor epoxide were detected in the gill of fish from all sites, except from FU10. *P. maculatus* exhibited greater MRC density than *A. fasciatus*. Fish collected in FU20, FU30 and FU50 showed numerous alterations in the surface architecture of PVCs cells. The emerged MRC density and the MRC fractional area of fish from FU20, FU30, FU40 and FU50 were lower than the reference site (FU10) in the winter and exhibit some variability between sites in the summer. The NKA specific activity was lower in *P. maculatus* than in *A. fasciatus* in most sites. Organochlorine contamination in FU20 and FU50 showed inhibitory effects on MRCs variables and NKA activity especially for *P. maculatus* and in FU30 the signs of physiological alterations were associated to the contaminants, especially metals. Multivariate analysis demonstrated good association between the biological responses of both species and the environmental contamination indicating that MRCs variables and NKA activity could be useful biomarker to indicate warning changes in the environment.

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WEPC4-7

The ecotoxicology of agroindustrial farming in Southern Amazon

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The pressing demands for food, fiber and biofuels that are imposed by a precipitously growing human population turn industrial agriculture into an essential productive activity. These demands will only be met by expansion and intensification of agriculture in the tropics, threatening a very significant fraction of global biodiversity. One important driver of environmental degradation in agricultural landscapes is contamination by plant protection and soil amendment products. We herein provide a quantitative analysis of agrochemical use and freshwater biodiversity patterns along a gradient of environmental degradation (rainforest < pasture < plantation) in the frontier of soybean expansion in southern Amazon. Forty-one active ingredients were applied over a 4 month soy cycle at our study site. Focusing in 3400 ha of soybean fields, over 10 tons of 13 herbicidal AIs, 4,5 tons of 7 fungicidal AIs, and 1 ton of 6 insecticidal AIs were applied. Two fungicides (carbendazim and thiophanate-methyl), 6 herbicides (glyphosate, 2,4-D, clomazone, paraquat dichloride, diquat dibromide, imazethapyr ammonium salt) and 0 insecticides were applied at doses > 100 g/ha. Among them, 4 are moderately hazardous, 3 slightly hazardous and 2 unlikely to be hazardous to human health. One is probable (thiophanate-methyl) and 2 possible carcinogens (carbendazim, 2,4-D); one is toxic to development or reproduction (thiophanate-methyl) and 3 are suspected endocrine disruptors (paraquat dichloride, carbendazim, 2,4-D). Two are PAN Bad Actors (paraquat dichloride and diquat dibromide) and one is a Dirty Dozen (paraquat dichloride). Regarding potential for aquatic contamination and hazard, 7 AIs are potential groundwater contaminants, 7 are of known chronic aquatic toxicity, and 3 are of high or very high acute aquatic toxicity. We observed a strong signal of land use on freshwater communities, with a decrease in diversity as one moves from forest to pasture to soybean fields. Biodiversity patterns comparing soybean fields before, during and after sowing are consistent with negative effects of land management, possibly pesticide application. To understand the relative contribution of pesticides (among other management practices) to biodiversity loss, further experimental studies exposing a subset of these communities to realistic combinations of agrochemicals and physico-chemical modulating factors are underway.

WEPC4-8

Aquatic ecotoxicology of pesticides in the tropics: status and research needs

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The modernization of agricultural practices in developing countries located in the tropical zone has led to an increasing use of pesticides over the past decades. Subsequently, pesticides have been detected in water, sediment and biota of edge-of-field waterways in many tropical agroecosystems. However, ecotoxicological research into the fate and side-effects of agrochemicals in aquatic ecosystems surrounding agricultural fields has often been discussed to be focused almost exclusively on temperate countries. In 1997, Lacher and Goldstein published a review in Environmental Toxicology and Chemistry concerning the status and research needs related with tropical ecotoxicology. The present study presents a brief overview of what progress has been made since then and discusses priority research needs that still need to be tackled. For instance, field studies in tropical farms on pesticide fate in the enclosed and surrounding waterways are recommended. Future tropical effect assessment studies should evaluate whether specific tropical taxa, not represented by the current standard test species in use, are at risk. If so, tropical model ecosystem studies evaluating pesticide concentration ranges need to be conducted to validate whether selected surrogate indigenous test species are representative for local tropical freshwater ecosystems.

WEPC5 - Environmental characterisation, fate/detection, exposure, effect and risk of nanomaterials

WEPC5-1

Adsorption of nanoparticles on to air bubble and soil surfaces in porous media

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Nanoparticles released into the environment would accumulate on the soil layers and infiltrate into the subsurface. Soil layers consist of unsaturated soil and saturated groundwater zones. Fate and transport of nanoparticles infiltrating through the subsurface into the groundwater have not sufficiently researched. This study investigated interactions between nanoparticles and air, and nanoparticles and soil particles. This study used a soil column and a 2-dimensional porous medium to elucidate adsorption of nanoparticles to air bubbles and soil surfaces. Each suspended nanoparticles of fullerene and zinc oxide were separately injected into the partially saturated porous mediums and their fate and transport were investigated as time elapsed. Effects of air and organic matter contents in porous media on fate and transport of nanoparticles were investigated. Air bubbles present in the soil pores significantly affected adsorption and transport of nanoparticles. As expected, soils having high organic matter contents showed the high adsorption tendency of nanoparticles to soil. Fullerene nanoparticles of Carbon-based material were more adsorbed to soil than zinc oxide nanoparticles.

WEPC5-2

Separation and detection of Silver and Gold nanoparticles via A4F/ICP-MS on line coupled

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Since the use of engineered nanomaterial is gaining importance in nowadays market, the need of information on the safety implications arising from the use of nanotechnology-based products has been recognised by many international authorities. The development of an efficient regulation in this field is directly connected with a proper risk assessment. So far, a wide range of analytical approaches (including microscopy, chromatography and spectroscopy) are available to characterise and determine the main nanoparticles (NPs) parameters in real matrices. However, data concerning human and environmental exposure to nanoparticles are still far from being exhaustive. Among all the techniques, Flow Field Flow Fractionation (hereafter A4F) is widely recognised to be a very effective and versatile technique to separate, characterise, and quantify the size distribution of NPs within a wide hydrodynamic range, and it has been successfully applied to separate NPs in complex matrices with different origins (e.g. food, environment, biological matrices). The coupling between A4F and ICP-MS (Inductively Coupled Plasma Mass Spectrometry), which allows the detection of most of the chemical elements, even at very low concentrations, may allow, on one side, the separation and the detection of lower concentration of NPs in the samples, if compared to the limits achieved with the UV detector A4F is normally equipped with and, on the other one, to simultaneously detect NPs with a different chemical composition. In detail, since Ag and Au NPs find different applications in several fields (e.g. catalysis, biology, pharmaceutical and medical diagnosis) their potential impact on the environment has to be carefully investigated. This work aims to develop a proper analytical method for the separation and chemical characterisation of Au and Ag NPs with different sizes by the on-line coupling of A4F and ICP-MS. Furthermore, some applications will be tested on other kind of matrices and NPs in order to spread the range of nano-doped products of commercial use.

The on-line coupling focuses on the optimisation of the most influent instrumental parameters that allow to achieve a good separation. In particular, concerning A4F the cross and membrane flows are subject to investigation, whilst concerning ICP-MS dwell time and the number of reading/replicates.

WEPC5-3

Photocatalytic effects of TiO₂ nanoparticles - how do they impact the ecotoxicity of PAHs? A model study with *Caenorhabditis elegans*

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Titanium dioxide nanoparticles (TiO₂ NPs) catalyze the degradation of PAHs under UV irradiation. Because of this TiO₂ NPs are under consideration for the use in the remediation of PAH-polluted soils and wastewaters [1]. The intermediate and end products of this degradation process have been identified for certain examples [2], but little is known about ecotoxicological effects of this mixture of photo modified compounds in the aquatic environment.

In general, photo oxidation of PAHs increases water solubility, most likely connected to an increased bioavailability of the compounds. At the same time modifications of the chemical structure of contaminants caused by photo oxidation alter their intrinsic toxicity and with it their effects on exposed organisms. For this reason, a shift in ecotoxicological effects of PAHs exposed to TiO₂ NPs and UV light is expected.

Independent from photomodification processes acting on the chemical properties of co-contaminants, TiO₂ NPs can affect irradiated organisms directly by photosensitization. Organisms affected by photosensitization are expected to be more vulnerable to co-contaminants in their environment.

This study aims to elucidate the impact of photo catalytic effects of P25, a photocatalytically active TiO₂ NP on the toxicity of phenanthrene as a highly relevant environmental toxicant. The ecotoxicological model used for sediment and aquatic phase testing is *Caenorhabditis elegans*, a soil dwelling nematode. The experimental design was developed to separately evaluate two effects of irradiated TiO₂ NPs:

- 1) photomodification of the co-contaminant phenanthrene
- 2) photosensitization of the test organism *C.elegans*.

A preliminary study was carried out with this same test system to investigate the direct effects of P25 and the impact of simulated sunlight radiation in combined exposure scenarios. P 25 has been shown to significantly affect the reproduction of *C.elegans* in darkness with a mean inhibition of 28% and 46% at concentrations of 10 mg/L and 30 mg/L, respectively. Initial results of combined exposure scenario testing indicate a significant increase of TiO₂ toxicity under simulated sunlight radiation.

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[2] Wen S, Zhao J, Sheng G, Fu J, Peng Pa. 2002. Chemosphere, 46(6):871-877.

WEPC5-4

Ecotoxicological effects of nano zinc oxide to aquatic organisms, in relation to their physicochemical characteristics

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Engineered nanomaterials (NMs), defined as manufactured particles possessing at least one dimension within 1-100 nm range, have different physical and biological properties from bulk materials of the same chemistry due to their increased surface areas. Due to their novel properties, the commercial market for NM-incorporating

products has experienced a massive increase in recent years. For example, sunscreens employing nanosized titanium dioxide (nTiO₂) and zinc oxide (nZnO) as UV-blocking agents are becoming more popular as they are deemed to have enhanced effectiveness and transparency. NMs may be released into the aquatic environment during the product life-cycle, but their ecological impacts on marine ecosystems are presently largely unknown. Therefore, this study primarily aimed to investigate the physicochemical characteristics and in vivo toxicities of nZnO to aquatic organisms under various environmental conditions. nZnO was found to invoke oxidative stress in the rotifer *Brachionus* sp. via generation of reactive oxygen species (ROS). It is also demonstrated to be a possible endocrine disruptor as it altered the transcription of retinoid X receptor (RXR). In a study employing five marine organisms (i.e., microalgae *Thalassiosira pseudonana* and *Skeletonema costatum*, copepod *Tigriopus japonicus*, amphipod *Elasmopus rapax*, and medaka *Oryzias latipes*), crustaceans exhibited the highest sensitivity towards nZnO, and toxicity of nZnO was attributable to metal ion dissolution as well as nanoparticle effects such as aggregation and adsorption of nZnO onto cell walls and exoskeletons. Dissolution of nZnO decreased from 16 mg Zn L⁻¹ at 4°C to 1.4 mg Zn L⁻¹ at 35°C, which probably accounted for the increased growth inhibition for *S. costatum* at 15°C when compared against 25°C. However, physiology and behaviour of the test organisms, such as metabolic depression and dormancy displayed by the amphipod *Melita longidactyla* at lower temperatures, could also influence nZnO uptake and toxicity. *T. japonicus* was exposed to nZnO-incorporated sunscreens to evaluate the effects of commercial forms of nZnO and Zn²⁺ leached into seawater from epidermal surfaces. Their molecular biomarker responses showed that other sunscreen constituents can alter nZnO toxicity, and such results support the need for further research on combined effects of NMs and other common pollutants to aquatic organisms.

WEPC5-5

Do TiO₂ nanoparticles alter heavy metal toxicity? - A factorial approach using *Daphnia magna*

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Nowadays the use of titanium dioxide nanoparticles (nTiO₂) strongly increases. Moreover, nTiO₂ products are widely used outdoors (e.g. paints, sun screens). Hence, it can be expected that nTiO₂ enter surface water bodies via point or non-point sources together with other environmental stressors, e.g. metal ions. This may be of particular importance as nanoparticles can act as carrier for chemical stressors into aquatic organisms, like daphnids. Furthermore, nTiO₂ is widely used as a photocatalyst, since it produces - during irradiation with UV-light - reactive oxygen species, which can be enhanced in presence of metal ions. However, ecotoxicological knowledge considering the combined effects of metal ions and nTiO₂ is limited. Thus, the present study assessed potential implications of nTiO₂ (2 mg/L) on the 96-h acute toxicity of three different elements of the loosely defined subset "heavy metals" for juvenile as well as adult *Daphnia magna*, by applying a 2[GREEKX]2[GREEKX]7 factorial design. Silver (Ag), arsenic (As) and copper (Cu) were chosen, since the metal ion of each element, which is considered to be toxic, is differently charged i.e. Ag⁺, HAsO₄²⁻ and Cu²⁺. This is assumed to influence the interaction with the negatively charged surfaces of nTiO₂ and finally the toxicity of the combined treatments. The presence of nTiO₂ increased Ag toxicity after 72 h of exposure for juveniles, indicated by a 33% lower EC₅₀. In contrast, the toxicity of As as well as Cu was reduced by up to 39% and 85% for juveniles and adults, respectively. These results suggest nTiO₂ as a suitable measure to reduce metal toxicity in distinct cases (e.g. Cu). However, as the underlying processes are not yet fully understood and the detoxifying effects were not apparent for all elements investigated, the application of nTiO₂ should carefully be evaluated in future experiments.

WEPC5-6

Nano-silver ecotoxicological effects in marine mussels

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The ecotoxicological effects of two metallic silver nanoparticles (NPs) (8nm, 50 nm size) were evaluated using a battery of acute and chronic tests in marine mussels within a large range of concentrations spanning from 10 mg /L to 0.001 mg /L. Silver nitrate- which is readable soluble in marine water- was used as reference to compare the effects between colloidal silver and ionic forms. Acute -i.e. mortality, mortality under aerial exposure (stress on stress analysis)- and sub-lethal effects -byssus synthesis- were evaluated in marine mussel (*M. galloprovincialis* Lam) as a function of time and concentration within four days NP exposure in 35 ° Artificial Sea Water (ASW) at 16°C. Bioaccumulation in tissues and silver speciation in sea water were also evaluated by means of ICP-MS analysis of the total and soluble silver fraction (for media only). A battery of biomarkers spanning from molecular to eco-physiological data were included in this survey to get insights into mechanisms of toxicity of silver ions and silver NPs.

WEPC5-7

Effect of Carbon nanoparticles on *Xenopus laevis* development

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The present knowledge about the risk of Carbon nanomaterials (CNMs) requires the developing of new testing methodologies and interpretative tools to gain a more comprehensive picture of their nanotoxicology. Aquatic ecosystems are considered the terminal receptors of nanocontaminants and their realistic accumulation sites. Lethal and teratogenic potentials of commercially available Carbon nanoparticles (CNPs) were investigated by standardized FETAX procedure, and *Xenopus laevis* embryos were screened by advanced confocal and electron microscopy techniques. Morphometric CNP properties were characterized by scanning and transmission electron microscopy, and their hydrodynamic behaviour monitored by dynamic light scattering techniques. PAHs content was evaluated by Soxhlet extraction for 48h using toluene. Embryos were acutely exposed to increasing CNP suspensions (1, 10, 100 and 500mg/L) and after 96h, lethality, malformation rate and growth inhibition were measured. Embryos were then processed for histological and ultrastructural analyses to detect the main affected organs, and to look for specific lesions at the subcellular level. Laser Scanning Microscopy in the reflection mode and EFTEM techniques were used to image and track CNPs into embryo tissues. Only the highest CNP suspension resulted embryolethal for *X. laevis* larvae, while a dose-response was observed in malformed larva percentages with the 100 and 500mg/L groups significantly different from controls (p<0.01 and p<0.001, γ₂ test, respectively). Stomach and mainly gut were the preferential CNP accumulation sites, however, the digestive epithelium remained integer. At this level, the columnar cells forming the absorptive epithelium showed CNP aggregates (200-300nm) into their cytoplasm, but regularly shaped microvilli organized in a well defined brush border. Results have been discussed considering the very little information available on the toxic effects of CNMs in *in vivo* studies in *X. laevis*. Embryotoxic and histological results, combined with reflection and ultrastructural analyses, demonstrated that CNPs are weakly embryotoxic for *X. laevis* larvae, but the presence of CNP aggregates beyond the intestinal lumen, suggested that the effect of these nanoparticles can be transferred to other organs determining possible long-term health consequences later during the development.

WEPC6 - Linking exposure to effects in environmental risk assessment

WEPC6-1

Effect modelling of intermittent discharge of herbicides in watercourses

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In ecotoxicology, effects of agricultural herbicides on non-target organisms, like algae, are often simulated referring to a continuous exposure. But this kind of exposure is very far from the reality. Indeed, exposure models of pesticides in aquatic systems after agricultural applications and after rain events are generally irregular i.e. characterized by short pulses exposure and recovery periods. These pulses exposure, even if they are short, can impair the growth of algae or modify their chlorophyll content. Very little is known about the effects on primary producers of short but high peaks of herbicides fluxes, and on how the algae respond during the recovery period between pulses. The main goal of this study is to develop a model for estimating the effects, and even the risk, on algae population to a repeated herbicide stress. This model will be validated by laboratory experiments applying three typical scenarios of pulses exposure. The scenarios differ from each other by the concentration of the different peaks, the exposure duration and, finally, the recovery period between the pulses. The first one corresponds to three pulses of high herbicides concentrations (EC80) and long recovery periods (around 2/3 of the pulse duration). The second scenario corresponds to three pulses of low herbicides concentrations (EC20) and short recovery periods (around 1/3 of the pulse duration). And the last one is a mix of the 2 previous scenarios. These scenarios are tested on the green algae *Scenedesmus vacuolatus*, which is characteristic of primary producers found in rivers. Two photosynthetic inhibitors herbicides, isoproturon and terbuthylazine, are used as test compounds. As endpoint, the growth of algae will be regularly measured during pulses exposure and recovery periods. The model as well as its validation will be discussed.

WEPC6-2

Can time-weighted average concentrations be used to assess the risks of asulam to *Myriophyllum spicatum* and *Elodea canadensis* under different time-variable exposure regimes?

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We tested the effects of the herbicide asulam on growth of the submerged macrophytes *Myriophyllum spicatum* and *Elodea canadensis* under laboratory conditions using different exposure scenarios. Asulam is a selective postemergent systemic carbamate herbicide which inhibits folate synthesis by inhibiting dihydropteroate synthase. The exposures of each scenario were comparable in the concentration x time factor, viz., the same 21-d time-weighted average (TWA) concentrations but variable in peak exposure concentrations (ranging from 0.1 to 21000 µg ai/L) and exposure periods (1, 3, 7, 14 or 21 days. Endpoints, dry-weight of main shoots, new shoots and new roots and length of new shoots and roots were measured at the end of the experiment (t = 21 days). It appeared that different exposure regimes resulted in similar EC50 values when *M. spicatum* was exposed to similar 21-d TWA concentrations of asulam. Effects on newly formed tissue, especially roots and new shoots, based on short peak were in the same range (EC50: 6-32 µg/L) compared to the effects resulting from relatively longer lasting lower chronic concentrations. For *E. canadensis* the effects were not as clear as for *M. spicatum* due to the high variability in shoot endpoints (length and biomass). The most sensitive measurement endpoints for asulam and *E. canadensis* concerned root formation. In particular the endpoint root length showed little variability so that treatment scenarios could be compared. For *E. canadensis* the EC50 values between exposure scenarios were similar (EC50: 59-80 µg/L).

In conclusion, the data suggest that for asulam and macrophytes the TWA approach can be used in the risk assessment.

WEPC6-3

Lemna TKTD-Model for pesticide risk-assessment

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The first tier pesticide risk assessment for aquatic macrophytes is to date mainly based on endpoints from standard laboratory tests with a *Lemna* species. To detect even slight effects and to make these tests repeatable, they are conducted under optimum growth conditions and static exposure. Both might significantly deviate from the environmental conditions for which the risk assessment is to be performed. Particularly in the case of “non-lethal” endpoints as e.g. reversible growth inhibition it is often an open question in how far effects of an active substance would be different under different conditions with temporally varying exposure and/or slower growth.

We developed a simple growth model simulating the development of dry biomass in dependence of environmental factors as temperature, irradiation and nutrition as well as internal concentration of a growth inhibitor. The internal concentration of the toxicant is calculated from the external exposure with a toxicokinetic (TK) sub-model consisting of a one compartment TK model that includes a mechanistic description of uptake kinetics and partitioning between plant tissue and water. The functional relations between growth rate and environmental factors were taken from the published literature. Toxicological parameters for the toxicodynamic model can be derived from standard test results. The rates of uptake into *Lemna*, however, need to be determined in a specifically designed test. For this purpose the uptake of radio-labeled substance into *Lemna* under static exposure as well as the release from loaded plants into uncontaminated water have been investigated in dependence of time. From the resulting concentration time curves permeability values were derived which make up the essential input parameters for the toxicokinetic sub-model.

It is demonstrated how the model can be applied to assess the risk of pesticide applications considering realistic surface-water exposure patterns.

WEPC6-4

A time-dependent effect model for ecotoxicological effects in *Lemna* species

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The available models for time-dependent effects on aquatic organisms have been recently reviewed and a unified scheme for describing survival has been proposed. However, less recent work has been published on time-dependent effects on gradual endpoints such as growth inhibition of aquatic macrophytes.

The presentation shows a model suitable to describe the time dependence of growth inhibition of *Lemna gibba*. The model allows for the evaluation of the applicability of time-weighted average concentrations in risk assessments as well as for a prediction of the effects of time-variable exposure patterns, within certain limits that are derived from the exposure conditions present in the available experimental data.

WEPC6-5

TKTD modelling of interactions between toxicants and food - a case study for diquat in the pond snail *Lymnaea stagnalis*

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Toxicokinetic-toxicodynamic (TKTD) models are essential in the interpretation of toxicity tests with time-varying exposure. Furthermore, these models are the only possibility to extrapolate between different exposure scenarios. For effects on survival, the recently published General Unified Threshold model for Survival (GUTS) can be applied.

For sub-lethal endpoints such as growth and reproduction, other approaches are needed. At this moment, the only mechanism-based TKTD approaches for these endpoints are derived from the Dynamic Energy Budget (DEB) theory. DEB theory provides a framework that explains how the energy taken up from food is allocated to the major physiological functions at the individual level. Internal concentrations from a TK model can be linked to changes in DEB parameters. Since food can be considered explicitly in a DEB framework, effects of food quality or quantity and potential interactions with toxicants can be considered. Although effects of food level and food source on toxicity have been discussed at length in the literature, their impacts are hardly quantitatively assessed. Food limitation often leads to higher apparent sensitivity (e.g., focusing on the EC_x), which is not necessarily connected to an increased intrinsic sensitivity (i.e., the relationship between the internal concentration and the value of a DEB parameter). Recently, it has been found that the pond snail *Lymnaea stagnalis* is food limited in a part of the life-cycle under standardized test conditions, with severe consequences for the interpretation of toxicity test results. In this study, we use a model based on DEB theory to explain the response of two genetically different populations of the pond snail to pulsed exposure to the herbicide diquat, at four different concentrations. We discuss the interactions between the direct effects of the herbicide on the snails and indirect effects through the food used in the experiments.

WEPC6-6

Applying the general unified threshold model of survival (GUTS) to describe toxic effects of Triphenyltin Hydroxide on the cyclopoid copepod *Mesocyclops leuckarti*

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There is a growing interest towards the testing of non-standard species in the ecological risk assessment (ERA) of pesticides. *Mesocyclops leuckarti* is a good representative species for freshwater cyclopoid copepods, owing to its abundance in European freshwaters, potential sensitivity and ease of laboratory culturing. Being a planktonic species, *M. leuckarti* is susceptible to typical exposure scenarios of pesticides, such as run-off and spray-drift.

We exposed the different life stages of *M. leuckarti* to the organotin compound Triphenyltin Hydroxide (TPT), a non-systemic fungicide, to simulate and interpret its toxic impacts. In this study, nauplii, copepodites and adults (including males and females, 40 individuals per concentration) were exposed to six different concentrations (12.5, 25, 50, 100, 200 µg/l) of TPT for 96 hours. At nine different time points, the mobility of the copepods was checked. The toxicant exposure concentrations in the test medium were quantified by HPLC. Our results show that a majority of individuals die at exposure concentration higher than 100 µg/l. The LC₅₀ of adult copepods and copepodites were calculated to be between 50 and 60 µg/l. There were no significant differences found between the response of males, females and copepodites to TPT.

The generated survival data were analyzed with the “General Unified Threshold Model of Survival” (GUTS), which integrates several toxicokinetic-toxicodynamic models with different assumptions and hypotheses for endpoint survival in one mathematical approach. The model was calibrated with the data set and simulations with several different assumptions (e.g. death mechanism: the concept of individual tolerance versus stochastic death) were run. The simulation with the best fit to measured survival was identified. Also, differences between the simulations were interpreted and predictions for the toxicity of TPT on *M. leuckarti* over longer time periods and with miscellaneous exposure patterns were made. The effect-levels (EC_x) of TPT were calculated with different assumptions (Logit, Probit, Weibull) of the concentration-response relationship and compared to the outcome of the GUTS-model.

WEPC6-7

Integration and calibration of a biodynamic toxicity module for metals in the ecological model AQUATOX

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In their ambition to establish good ecological status in all water bodies in the European Union by 2015, the regulators and water managers are currently facing serious problems in identifying the probable causes of surface water impairment and are consequently limited in their ability to predict the impact of their programs of measures in a quantitative way. In this context, numerous ecological models with different specialization exist and can theoretically be used to support questions concerning watershed management. Among these models, AQUATOX may simulate the combined environmental fate and effects of anthropogenic organic compounds or the impact of nutrients and suspended materials in aquatic ecosystems. AQUATOX is by far the most complete model described in the literature. However, although the model combines aquatic ecosystem, chemical fate and ecotoxicological submodel, it doesn't model metal fate and effects. Nevertheless, metals originating from urban surface runoff and sewer overflows are a common pressure to surface water ecosystems.

To overcome this deficit, the objective of this project is to integrate and calibrate a metal module in the AQUATOX model taking into account bioaccumulation as described with DYMBAM concept and toxic effects of trace metals on food webs. As aquatic insects are often the dominant macro-invertebrate fauna in freshwater ecosystems, a set of species collected in field with different metal sensitivities have been selected to calibrate the DYMBAM model. Toxicity on the macro-invertebrate consortia in the field will be evaluated with tissue residues of metal-tolerant species that correlate with the absence of sensitive species (calibrated monitor approach). The project foresees laboratory investigations on toxicokinetics and lethal and sublethal toxicity on the accumulating biomonitors and selected sensitive species. Validation will be achieved in the field via water and food analysis, tissue residues of biomonitors *Hydropsyche* and *Baetis* as well as macro-invertebrate inventories.

The conceptual model combining bioaccumulation and toxic effects and including parameters defined with laboratory tests and field will be implemented in AQUATOX providing an evaluation tool available that deals with metal pressures in rivers and supporting decisions of water stakeholders.

WEPC6-8

Influence of a variable exposure on *Daphnia magna*, Strauß

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Standard laboratory are generally performed under ‘worst-case’ conditions using most sensitive life stages and maximizing the exposure, e.g. via maintaining constant exposure throughout the entire test duration. Under realistic conditions, however, exposure situations normally differ from those in standard ecotoxicological testing systems since the exposure of water bodies with plant protection products is highly variable due to e.g. different entry routes, uses of the plant protection products and nature of the receiving water bodies. Therefore variable exposure is frequently the more realistic scenario. Also, tests are done with specific - generally most sensitive - life stages; effects on other life stages and thus on the population may differ considerably.

In this investigation the effects of a fungicide and the frequently investigated chemical 3,4-dichloroaniline to *Daphnia magna* are examined in different pulsed exposure scenarios, varying in duration and concentration of pulses. The results of population test and *Daphnia* reproduction tests with pulsed exposure scenarios (referring to OECD guideline 211) including the investigation of the fitness of the F1 generation are compared.

The results of these pulsed exposure scenarios are in a marked contrast to the standard test using a constant exposure scenario. They demonstrate that the impact of a more realistic variable exposure can differ significantly from standard constant exposure studies, particularly for fast acting and rapidly dissipating substances. Additionally, following an initial impact at higher concentrations, a recovery of the populations could be detected after the exposure period.

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 17-alpha-ethinylestradiol, ET06B-3
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