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. Since then more and more polymeric materials have been introduced for textiles, electronic equipment, upholstery, automobiles and building materials. As a consequence, various treatment processes on the drinking water production location. These reservoirs were possibly influenced by the Moerdijk incident. For precautionary reasons, 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 antipodarum), amphipods (Gammarus) and chironomidae larvae (bloodworms) are exposed to effluents from treatment plants...

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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 mass spectrometry. After cryo-grinding, we extracted samples with a salting-out assisted LLE followed by a purifying dispersive SPE. 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 preoccurring micro-pollutants in aquatic micro-organisms.

MO 205

Solid phase extraction and LC-MS/MS analysis of pharmaceuticals in the Irish aquatic environment

G.L. Mceneff*, W. Schmidt, B. Kelleher*, L. Barron*, B. Paul*, B. Quinn*

I*Irish Centre for Research on Separation Science and Irish Centre for Environmental Toxicology, Dublin, Ireland

2School of Chemical Sciences, Dublin City University, Glasnevin, Dublin, Ireland

Analytical and Environmental Science Division, King's College London, London, United Kingdom

Australian Centre for Research on Separation Science, University of Tasmania, Hobart, Australia

Pharmaceutical and personal care products (PPCPs) are resistant water remediation techniques carried out currently in wastewater treatment plants and are therefore released into receiving waters. In previous studies PPCP presence has been detected in Irish effluents and soils in measurable quantities. In the work presented herein, 24 hours of effluent samples were collected from two large Irish wastewater treatment plants and analysed for a total of six pharmaceutical classes from a range of therapeutic classes including non-steroidal anti-inflammatory drugs, 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:acetonitrile and reconstituted in 0.5mL of mobile phase. Qualitative and quantitative analysis was carried out using reversed phase LC separation and LC/MS/MS. Surface water samples were collected monthly at six sites in four Japanese rivers from December 2010. 2-Phenoxyethanol, Resorcinol, 1-Isopropyl-3-methylphenol and Chlorphenesin 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 all sampling sites. The highest concentration found was for 2-Phenoxyethanol at 1µg/L. Further investigations are necessary to evaluate the ecological effect of high concentration inactivation of PPCPs present in drinking water.

MO 206

Use of LC-Orbitrap accurate mass spectrometry in risk assessment after a chemical incident in the Netherlands

A.P. van Wezel, T. van Leerdom, L. Puijker

KWR Watercycle Research Institute, Nieuwegein, Netherlands

Early 2011, a big accident took place in a chemical storage site in the Moerdijk area. There was an extensive fire up to 5000 meters high, with formation of soot. The column of smoke went in the northeast direction within a large area. On the chemical storage site, many different inorganic, organic solid and liquid chemicals were stored. The fire destroyed 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 drinking water was taken out of production and intake of potable 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 the Moerdijk incident. Therefore, several drinking-water related surface waters were sampled and analyzed using broad-spectrum LC-MS/MS and LC-MS/MS analysis. This high sensitivity, the accurate mass measurement makes 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 designed 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 for 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, alternated brominated flame retardants and Dechlorane compounds in sediments from German Bight and Laizhou Bay, China


1Helmholtz-Zentrum Geesthacht, Geesthacht, Germany

Yantai Institute of Coastal Zone Research, Yantai, China

Guangzhou Institute of Geochemistry, Guangzhou, China

Brominated Flame Retardants (BFRs) have been commercially used since the 1970s. Since then more and more polymeric materials have been introduced for textiles, electronic production, 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 China and Germany 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 collected in the German Wadden Sea, the rivers Elbe and Weser and the German Bight in 2011. 48 Chinese samples were collected in Laihou Bay and discharging rivers in 2011. 

The samples were Soxhlet extracted with dichloromethane and then purified by 10% water deactivated silica before measured by GC-ECD-MS. Quantification of 27 different FRs was obtained using 5 mass labelled internal standards [13C]BBB, [13C]BDE-77, [13C]BDE-138, [13C]syn-DP, [13C]BDE-209. 

The data were obtained in Laihou Bay was Dichlorobenzofuran ethane (DBDE) and BDE-209 in German Bight, respectively. The alternate BFR DBDE showed higher concentrations than BDE-209 in most Laihou Bay except some Chinese river samples. 

Dibromodichloromethylbenzene ether (DETE), Dichlorohane (Dec) 602 and 603 were found in Germany, respectively. 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 

A. Wolf, 1 A. Bergmann, 2 T. Flohr, 1 X. Gao, 3 H. Chen, 4 H. Hollert, 1 T. Schiedek, 1 C. Schüth 1 

1) IWWS Bremen, Germany, 2) Leibniz Institute Mülheim an den Ruhr, Germany, 3) Institute for Environmental Research, RWTH Aachen University, Aachen, Germany, 4) Faculty of Urban Construction & Environmental Engineering, Changsong University, Changsong, China

The Three-Gorges Reservoir (TGR) covers an area of 106,000 km² and is one of the most controversial hydroelectric projects in the world. The project was completed in 2006 and the water level was raised to 175 m in 2009. 

During summer 2011, water, suspended solids and river bed sediments from the TGR area were analysed for inorganic (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.

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 rates estimated 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.

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 (WWTP), 100s of chemicals are yearly discharged to surface water. Although occurring in low concentration, these micropollutants might harm aquatic organisms. Especially mutagenic and endocrine disrupting chemicals are of high concern due to their 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 such as hospitals, resort and chemical industries were analysed in combination. The 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 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 

MO 210 

Target and non-target screening of lake sediments using HR-MS/MS 

A.C. Chiu-Hernández, 1 H. Singer, 1 J. Hollender 1 

1) Eawag/Dübendorf, Switzerland

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 (WWTP), 100s of chemicals are yearly discharged to surface water. Although occurring in low concentration, these micropollutants might harm aquatic organisms. Especially mutagenic and endocrine disrupting chemicals are of high concern due to their 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 such as hospitals, resort and chemical industries were analysed in combination. The 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 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 toxins in Effective Directed Analysis 

E. Simon, J.M. Weiss, T. Hamers, P.G. Leonards, M.H. Lamarre 

Institute for Environmental Studies (IVM) - VI Amsterdam, Amsterdem, Nederland

Technological developments 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 etc.) which 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 databases, or software to derive theoretical compound characteristics.

MO 213
Silicone-based agricultural adjuvants: trace analysis in aquatic environment
A. Wörmann1, F. Brachv, V.T. Grize2, H. Wiese2

1DGWV-Technologiezentrum Wasser, Karlsruhe, Germany
2TU Dresden, Fakultät Forst-, Geo-, Hydrowissenschaft, Institut für Wasserchemie, Dresden, Germany

Past research on the use of emulsifying surfactants has shown that surfactants have been an issue in the last decades and agricultural adjuvants are much less studied yet. Among adjuvants, trisiloxane surfactants are increasing in popularity because of their high efficiency compared to traditional surfactant-based products. 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 did not show any acute toxicity when no data are available on the application. A method for trace analysis of trisiloxane surfactants in aquatic environment was therefore developed and validated. It allows the quantification of two important trisiloxane surfactants in different aquatic 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
C.P. Perazzo1, N.C. Chiveré2, E.S. Saouter1

1Science et Environnement, Geneva, Switzerland
2University of Lausanne, Lausanne, Switzerland

In the last few years many results have been obtained on the use 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. 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 Whenever possible, the hazard was evaluated based on experimental data, with priority for information on REACH dossiers. We also used structure activity relationship approaches and biomonitoring data. It is unclear whether these behavioural responses were initiated via traditional toxic mechanisms or stimulatory effects, but we believe that a better understanding of the mechanisms involved is needed.

MO 215
Potential toxicity of sucralose
A.K.E. Wiklund, M. Breitholtz, K. Adolfsson-Erici

Stockholm University, Stockholm, Sweden

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 last year, the impact of personal care processes 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
A.A. Kools1, E.R. Burger2, H. Kooiman1, E.J. Peters1

1Gronimij (Aquasense), Amsterdam, Nederland
2Gronimij, Amsterdam, Nederland

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 routes 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 the eutrophication 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
B. Huggett

University of North Texas, Denton, United States of America

The potential toxicity of sucralose has been discussed in municipal wastewater effluent and surface waters at concentrations ranging from ng/l to low µg/l. Few chronic toxicological ecotoxicological data are available in the peer reviewed literature with respect to sucralose. To address this data gap, 21 d Daphnia magna, 28 d Amphocysis baya (mud shrimp) and studies 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. Chronic studies resulted in no significant lethal or reproductive toxic effects in Daphnia magna or reproduction of mud 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
P.A. H. Eltrhropel, D.G. Coopert, V. Yargeau

McGill University, Montreal, Canada

The Vibrio fisheri bioluminescence inhibition assay (Microtox®) using an in-house diluent containing 2%, DMAO was used to perform a rapid toxicity screening of 24 commercial and 5 potential green plasticizers. Toxicity data was used to develop a structure activity relationship, creating a data set for a database of the toxic target plasticizers. Results indicated that dibenzoate compounds with lower number of C atoms in their alkyl chains such as 1,4 propandiol dibenzoate (PrDDB) and 1,4-butandiol dibenzoate (BBDB), were more toxic (EC50 = 2-6 µM) than commercial glycol dibenzoates such as DEGDB and DPGDR (EC50 = 13-31 µM); however dibenzoates with higher number of C atoms in their alkyl chains such as 1,5-pentandiol dibenzoate (PBDB) and 1,6-hexandiol dibenzoate (HDDB) had similar toxicities to those commercial compounds. Dibenyl and dihexyl compounds of the dialky1 ethers (fatty alcohols, fatty acids and succinates) were more toxic (EC50 = 2-6 µM) 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-ethylhexyl) phthalate compounds are weakly toxic (EC50 = 130-1292 µM) or non toxic at all to the luminescent bacteria. These results indicate that dibenzoates and dibenzyx compounds with lower number of C atoms in the alkyl chains are more toxic than other plasticizers used in this study.
In the present study, the growth inhibition test of green algae (Pseudokirchneriella subcapitata), the Ceriodaphnia dubia reproduction test, and the zebrafish (Danio rerio) embryo/larval/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 high concentrations of PPCPs were detected in Tokushima compared to Kyoto and Saitama. Surfactants, triclozoan 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.

**MO 242**

**Variability in the outcome of outdoor mesocosms with three-spined Stickleback (Gasterosteus aculeatus) populations in control conditions: data from 20 replications in two annual experiments**


In our annual experiments, we monitored 10 populations per year for 2 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 masse of invertebrates, number, length and sex ratio of fish) were standardized in all compartments. At the end of the experiments, results were correlated with 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 influential 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. Intrannual variability can be reduced through better manipulation of the fish introduced in the beginning of the experiment. We developed an original methodology to test that (see another poster of de Kermoysan 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.

**MO 243**

**Blood concentrations of heavy metals (Ba, Cd, Hg and Pb) and Metalloid (As) in raptor species (Buteo buteo, Milvus migrans and Gyps fulvus) from Portugal**

M.A. Carneiro1, B.J. Colaco2, R. Brandão2, C. Ferreira1, N. Santos1, V. Soeiro2, A. Colaço1, M.J. Pires1, O. Oliveira1, S. Lavin1
1Instituto de Medicina e Biologia Ambiental, Centro de Recuperação do Parque Biológico de Gaia, Gaia, Portugal
2Universidade de Trás-os-Montes e Alto Douro, Vila real, Portugal
3Instituto de Ecologia, Recuperação e Vigilância de Animais Selvagens, Gouveia, Portugal
4Centro de Recuperação e Investigação de Animais Selvagens da Ria Formosa, Olhão, Portugal
5Centro de Recuperação de Fauna Selvagem do Parque Nacional da Peneda do Gerês, Braga, Portugal
6Universidade de Trás-os-Montes e Alto Douro, Vila real, Portugal
7Targetador Subaquático, Gaia, Portugal
8Servicio de Ecotaxofauna de Fauna Selvage, Universidade Autónoma 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 area and as top predators they would be expected that they may 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 (between March and October) and 32 black kites (Milvus migrans), 53 griffon vultures (Gyps fulvus) and concentrations of toxic elements were measured by inductively coupled plasma mass spectrometry (ICP-MS), after blood wet digestion.

Results obtained showed the presence of various toxic elements in all samples analyzed. Cd concentrations were the lowest in the three species and no statistically significant differences were found between species (ANOVA, F = 1.278, p = 0.289). However, Pb was found between species for As (p = 0.001), Hg (p = 0.001) and Pb (p < 0.05). Showing that black kite (Milvus migrans), and white kite (Milvus cinerarius) both have Pb concentrations significantly higher than in the other two species.

When comparing between species, there were found statistically differences between Braga and other five districts (p = 0.05), and the animals of Braga are the highest levels of Hg. Results concerning Hg and As obtained in this study suggest that common buzzards are exposed to the highest levels of this toxic metal, when compared with other elements. Black kite and in a lesser extent the buzzard, both have decreased levels of As, probably due to causality with diet, as they are not top predators. Although concentrations of metal elements in the three species would not be directly related to lethal effect, they might constitute a serious environmental factor affecting the survival, and special attention should be given to species in decline.
and reproduction tests were conducted with earthworms. Cocoon production was lower in shotfall soil compared to reference soil and Webster (laboratory standard soil).

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 risks? 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 245
Effects of lead shot ingestion on constitutive immune function of red-legged partridges
N. Vallverdú, M.E. Ortiz-Santaliestra, J.M. Pérez de la Lastra, R. Mateo
IREC, Ciudad real, Spain
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 constitutes 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: 42.6% vs. 36.2% in thiram and 34.1% in maneb, whereas maneb at low 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 effect was even more dramatic, reducing the chick mortality by a factor of 3. The two fungicides are thought to have 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 unsuitable 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 collected partridges provided by hunters.

MO 246
Consequences of metal adaptation on sensitivity to future climate change stressors in Daphnia pulex populations
J. Rodríguez-Estival, P. Álvarez-Lloret, A.B. Rodríguez-Navařo, R. Mateo
Departamento de Biología, Universidad de Oviedo, Oviedo, Spain
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 are contaminated with this particle. We present a preliminary study based on different tests to assess changes in innate immune response of red partridge (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 all the Pb doses, blood sample collection 0.5 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, and total peroxidase activity were affected by the exposure to Pb. Our results highlight the necessity to analyze several parameters to assess the immunotoxicity of a substance. Nonetheless, other tests and 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.

ETOPP - Global climate change: implications for environmental toxicology and chemistry
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
S. Moe, J. Balbus, W.H. Clements,1 A. Fritz, 1T. Gouin, 1R. Helt, 4C. Hickey, 1M. Hooper, 1W.G. Landis, 1R.G. Stahl, Jr.1
1National Institute of Health, Bethesda, maryland, United States of America
2US Geological Survey, Columbia, missouri, United States of America
3US Fish & Wildlife Service, Arlington, virginia, United States of America
4Norwegian Institute for Water Research (NIVA), Oslo, Norway
5Department of Geography, University of Oviedo, Oviedo, Spain
6Univerist, Sharnbrook, United Kingdom
7US Geological Survey, Columbia, missouri, United States of America
Western Washington University, Bellingham, washington, United States of America
This is an invited contribution and is a summary from a SETAC international 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 threats to 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. baseline / reference conditions for estimating resource injury and restoration / rehabilitation will continually shift due to GCC and represent significant challenges to practitioners.
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 cadmium and copper on the development of early life stages of Zebrafish**


*University of Aveiro, Aveiro, Portugal*

In vitro testing of biofuels - Verifying the environmental friendliness of biofuels in comparison with fossil fuels

S. Heges', K. Bluhm', T.B. Seiler', A. Schaffer', H. Hollert'

*Institute for Environmental Research, RWTH Aachen University, Aachen, Germany*

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 effects on the structure and function of these ecosystems. 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 two different temperatures (15, 20 and 25°C). 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 anomalies was performed. The European large river system - the Minho River estuary - is predicted to occur in coastal freshwater ecosystems. However, as many of these coastal freshwater lagoons are expected to be affected by nutrient contamination and the metal contamination and its impact on the molluscan community of the Minho River estuary. 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 Europe, the consumption of biodiesel (the Union states of America, on the other hand, is predicted to increase the amount of biofuels up to 30% until 2030. While the negative effect on the global climate due to increased 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 the present study, we want to investigate the use of in vitro bioassays for the (eco)toxicological investigations for the basis of the three potential biofuels: ethyl levulinate, 2-methyltetrahydrofuran and 2-methylfurant. 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 endpoints that could 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 bioassays and ecotoxicological endpoints. Acute Cytoxicity was determined by means of the Neutral Red Retention assay and ah-receptor mediated endpoints as well as a long-term assay like activity, was detected using the mechanism-specific 7-Ethoxy-0-resorufin-ethylmethylether (EROD) assay, both using cells from the permanent cell line RTL-W1 (Oncorhynchus mykiss) as a test organism. Furthermore, genotoxicity was determined by means of the micronucleus assay using the cell line V79 (Chinese hamster) (ISO14427-2). Besides the direct dosing approach as well as more or a less 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?

F. de Laender1, D. Verschuren1, R. Bindler1, C.R. Jansen1

1 Ghent University, Ghent, Belgium

2 Umeå University, Umeå, Sweden

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) were caused by condensed chemical contaminants (Cu concentrations) on the biodiversity of freshwater ecosystems. Historical trends of copper, biodiversity and land-cover were reconstructed from copper concentrations, counts of fossil diatoms, and percentage of terrestrial pollen - all measured in sediment cores - respectively. Temperature anomalies were calculated using 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 nearest to metal smelters (Cu up to 30 000 μg/l) had consistently higher biodiversity (up to 40 species/l) than the further located lower taxa (Cu < 300 μg/l). The presence 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**

A.S. Seeland, R. Müllers, J. Oehlmann

Goethe University, Frankfurt, Germany

The impacts of climate change are not only observed 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 several 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 showed that aquatic model organisms tend to react more sensitive towards contaminants under changing climate 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 higher mortality rates 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 of 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 need to be paid 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 255**

**How important is chemical toxicity for biodiversity conservation?**

F. de Laender1, D. Verschuren1, R. Bindler1, C.R. Jansen1

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**MO 256**

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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/101212/2008) and FEDER COMPETE funds (FCOMP-01-0124-FEDER-008556). C. Oliveira had a PhD grant from FCT (SFRH/BD/84423/2010) funded by national funds of the MCTES and FEDER European funds (POPH-QREN-Typeology 4.2.).

MO 256

Algal toxicity tests are useful tools for the assessment of aquatic toxicity and have been widely employed for environmental and regulatory purposes. However, the use of single species tests can lead to underestimation of the risks associated with a toxicant, as many environmental factors can influence the toxicity of a substance. Therefore, it is important to consider the effects of multiple stressors on the toxicity of a substance.

Marine medaka (Oryzias melastigma) are representative fish species commonly used in toxicology research due to their rapid reproduction and sensitivity to environmental stressors. Previous studies have shown that the toxicity of DDT to larvae of the marine medaka Oryzias melastigma is increased at temperatures higher than 20°C.

The aim of this study was to further elucidate the temperature-dependent toxicity of DDT to larvae of Oryzias melastigma. The study was carried out at three different static temperatures (12, 16, and 20°C) and a non-static temperature scenario with temperature fluctuations of ±2°C 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 used full life cycle exposure, with daily measurements of survival and reproduction during the entire lifespan. To our knowledge, this is the first study to compare the effects of static and fluctuating temperature on toxicity of DDT.

ET12P - Soil ecotoxicology

The use of natural biocides in agriculture is a safe approach for soil ecosystem. Case study of non-target bioactive compounds produced by rhizosphere soil Streptomyces sioyaensis TM 822

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/101212/2008) and FEDER COMPETE funds (FCOMP-01-0124-FEDER-008556). C. Oliveira had a PhD grant from FCT (SFRH/BD/84423/2010) funded by national funds of the MCTES and FEDER European funds (POPH-QREN-Typeology 4.2.).

MO 257

The influence of fluctuating temperature on the effect of copper toxicity in Caenorhabditis elegans

N.J. Norhave, D. Spurgeon, C. Svedsen, N. Cederberg

University of Copenhagen, Frederiksberg c, Denmark

The nematode Caenorhabditis elegans is widely used in ecotoxicological research due to its unique scientific and educational qualities. In this study, we aimed to assess the sensitivity of C. elegans embryos to zinc pyrithione stress at three different static temperatures (12, 16, and 20°C) and a non-static temperature scenario with temperature fluctuations of ±2°C 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 used full life cycle exposure, with daily measurements of survival and reproduction during the entire lifespan. To our knowledge, this is the first study to compare the effects 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

J. Li, P.T.Y. Leung, K.M.Y. Leung

ET12P - Soil ecotoxicology

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

N. Nakaew, R.O. Oral

Ege University, Faculty of Fisheries, Izmir, Turkey

The present study investigated the effects of the antifouling biocide zinc pyrithione on the marine medaka Sphaerechinus granularis. The study was carried out at three different static temperatures (12, 16, and 20°C) and a non-static temperature scenario with temperature fluctuations of ±2°C 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 used full life cycle exposure, with daily measurements of survival and reproduction during the entire lifespan. To our knowledge, this is the first study to compare the effects of static and fluctuating temperature on toxicity of copper.

MO 259

Temperature-dependent toxicity of DDT to larvae of the marine medaka Oryzias melastigma: pollutant responses at thermal extremes

N.J. Norhave, D. Spurgeon, C. Svedsen, N. Cederberg

University of Copenhagen, Frederiksberg c, Denmark

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MO 260

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

N. Nakaew, R.O. Oral

Ege University, Faculty of Fisheries, Izmir, Turkey

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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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metadata

ET12P - Soil ecotoxicology

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MO 262

Protemic response of larvae of the marine medaka Oryzias melastigma upon cadmium exposure at thermal extremes

E.T. Leung, A.J. Li, K.M.Y. Leung

The University of Hong Kong, Hong kong, Hongkong

The present study investigated the effects of the antifouling biocide zinc pyrithione on the marine medaka Sphaerechinus granularis. The study was carried out at three different static temperatures (12, 16, and 20°C) and a non-static temperature scenario with temperature fluctuations of ±2°C 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 used full life cycle exposure, with daily measurements of survival and reproduction during the entire lifespan. To our knowledge, this is the first study to compare the effects of static and fluctuating temperature on toxicity of copper.

MO 263

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Reproduction was impaired starting at medium salinity (2 g NaCl/Kg soil) in *F. candida* and at low salinity (1 g NaCl/Kg soil) in *E. crypticus*, and ceased completely at high increasing from low to medium salinities.

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/emigration/immigration of animals is hindered. Thus within TME to a constrained to a small scale, potentially selecting organisms e.g. restricted spatial requirements.

For the plants’ exposures a dose-response relationship was observed, showing a delay and decrease on seed germination in both plant species.
MO 268

Using the comet assay to determine species sensitivity of earthworms to copper
A. Reinecke, F. Fournier, A.J. Reinecke
Stellenbosch University, South Africa

The endpoints normally used to construct Species Sensitivity Distributions (SSDs), comparing the various sensitivities of species to toxicants, are predominantly 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 alternative media and substrates. The results show that using DNA assessment 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 were used to indicate that a biomarker, the comet assay, can be reliable and useable data from which to infer species sensitivity between species.

MO 269

Determining chemical effects on earthworms at the population level through the use of energy budget and individual based modelling
A.S. W. Di Marzio, M.E. Hodson, P. Fourie1, T. Alvarez1
1University of Reading, Reading, United Kingdom

Syngenta Ltd., Bracknell, United Kingdom

ECO-Risk Solutions, Norwich, United Kingdom

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 thresholds are not passed, field tests 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 processes, depends on a combination of environment-related 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 environmental conditions. This enables understanding 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.

MO 270

Ecotoxicity of phenanthrene and its nitrogen-containing analogues to the earthworm Eisenia fetida in soil
N. Anwar, M. N. Yassins1, M. J. Parida1
1University of the Punjab, Lahore, Pakistan

Lancaster University, United Kingdom, Lancaster, United Kingdom

Major sources of these homocyclic and N-heterocyclic polycyclic aromatic hydrocarbons (PAHs) are found in the environment being discharged from petrogenic, pyrogenic and biogenically sources. Eisenia fetida is the most versatile earthworm species and a model organism in ecotoxicology. We investigated the ecotoxicity of nitrogen heterocyclic compounds in its nitrogen heterocyclic compounds on E. 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 values were compared to other studies in the literature and our results differed significantly. The results of this study showed that the effects of nitrogen heterocyclic compounds in soil may be additive and may also have high solubilities to soil organisms as they are also very water-soluble. These findings may 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
C. Courti1, M.D. Woldchawari1, D.H. Oughton1, K.E. Tollefsen3
1Norwegian University of Life Sciences, Aas, Norway
Norwegian Institute of Water Research, Oslo, Norway

Bioartificial Tissue Equivalence Earthworm (BITE): ATIE/wwf

The aim of this study was to assess the acute and chronic toxic effect on the earthworm Eisenia fetida of two transformation products of amines, dimethylnitramine (DMNA) and 2-nitramine (nitr) (NAE) and their degradates. The effect of these compounds in soil was studied using terrestrial organisms. Acute toxicity tests were performed using E. fetida. The acute toxicity was determined using EC10 and LOEC values. These showed that DMNA was the most toxic and P. excaturus 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 272

New earthworm endpoints based on coelomocytes
C. D. Di Marzio1, W.D. Di Marzio1, M.E. Sáenz1, A.D. M. Sáez1
CONICET - UNLu, Luin, Argentina

New earthworm endpoints based on coelomocytes
W.D. Di Marzio1, M. E. Sáenz1, M. S. Sáez1

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. The selection of these endpoints is dependent on the choice of ecotoxicological systems. The type of cuttings: from the drier and from centrifuge, with artificial OECD soil (10% organic matter content). The barium doses tested were: 300, 900, 1500, 2100 and 2700 mg Ba/kg soil. The lowest in 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 273

Effects of the use of onshore drilling cuttings as soil amendment on reproduction of Eisenia andrei
M.O. Bianchi1, H.M. Machado1, M.E.F. Correia1, M.B. Amaro Sobrinho2
1Institute of Environmental Engineering, Federal University of Campinas - UNICAMP, Brazil

Brazilian Federal Rural University of Rio de Janeiro, Seropédica- RJ, Brazil

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 voluminous and therefore, the contamination depends on the geological features of the land. The drilling cuttings are treated to recover the fluid, using different equipment, as an alternative to other cuttings. A common drilling fluid in Brazil contains barium sulfate (BaSO4), what requires an appropriate disposal or safe 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 copper that can act in a synergic harmful way to soil organisms. The objective of this is to study the potential toxic effects of this residue to soil non-target organisms. The results demonstrated that the waste from the cuttings drier (EC 50 = 114 mg Ba/kg) had a more deleterious effect to E. andrei reproduction than the waste originated in centrifuge (EC 50 = 797 mg Ba/kg). A more detailed ecotoxicological study has 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.

Mo 265

Use of the comet assay in the assessment of the genotoxicity of cuttings to earthworms
A. Reinecke, F. Fournier, A.J. Reinecke
Stellenbosch University, South Africa

The endpoints normally used to construct Species Sensitivity Distributions (SSDs), comparing the various sensitivities of species to toxicants, are predominantly 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 alternative media and substrates. The results show that using DNA assessment 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 were used to indicate that a biomarker, the comet assay, can be reliable and useable data from which to infer species sensitivity between species.
MO 274

Validation of the Czech approach to dredged sediments ecotoxicity assessment

1, M. Sanka, 1, R. Vacha, 2, J. Priessnitz, 2, J. Lana, 2, P. Skulcová, 1, L. Skulcová, 1, R. Bauda, 1, J. Lana, 1, L. Skulcová, 1, L. Skulcová, 1, J. Lana, 1, L. Skulcová, 1, R. Bauda, 1, J. Lana, 1

Research Centre for Toxic Compounds in the Environment (RECETOX), Brno, Czech Republic

Research is focused on the actual environmental impact - a dredged sediment assessment 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 (practical ones) and to compare bioassays of eluates with the soil bioassays.

MO 275

Selection of relevant ecotoxicity tests to assess the effects of biosolids used in agriculture

1, P. Skulcová, 1, I. Namerová, 1, L. Chancerelle, 1, P. Pandel, 1, P. Bauda

INERIS, Verneuil-en-halatte, France

In recent years, in line with existing environmental standards, 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 plant samples. The objective of this work is to establish a 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 chronic endpoints are the following:

- algal growth inhibition of Pseudokirchneriella subcapitata (NF EN ISO 8692)
- inhibition of 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 (ISO 17512-1 and ISO 11126-2)
- avoidance and reproduction inhibition of the earthworm Eisenia fetida (ISO 17512-2 and ISO 11126-2)

All of these bioassays were conducted using their respective standardized protocol, except for C. elegans soil tests which were carried out using the method detailed in another work. 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 LUFAs) were mixed with biosolids at defined doses (application rate, and e.g. 2 times, 5 times, 10 times, 20 times the

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Interreg: the ‘ReOrgRisk’ Project: environmental risk assessment of the use of organic residues as soil amendments

1, N. Manier, 1, L. Chancerelle, 1, P. Bauda, 1, J. Lana, 1, L. Skulcová, 1, L. Skulcová, 1, R. Bauda, 1, J. Lana, 1

Research Centre for Toxic Compounds in the Environment (RECETOX), Brno, Czech Republic

The objective of this work is to establish a battery to assess the effects of biosolids, by comparing their respective sensitivities towards these materials.

Research is focused on the actual environmental impact - a dredged sediment assessment 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 (practical ones) and to compare bioassays of eluates with the soil bioassays.
This study was funded by Spanish projects CTM2010-19779-C02-01/02 and RTA2010-00004.

MO 279

Effects assessment of biosolids soil amendments on terrestrial organisms

G. Carbonell1, M.A. Martínez2, M. Torrijos3, E.M. Beltrán1, A. de la Torre1, I. Navarro1, M.P. García1, C. del Rio1, J.L. Pareja1, J. Pro1, M.A. Porcel2, C. Fernandez3

1INA, Madrid, Spain
2Ciemat, Madrid, Spain
3CIEMAT, Madrid, Spain

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 them with organic matter and nutrients, improving the quality of the soil, but may, along the time, 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 risk. The results were used to support the development of guidelines and standards and 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.

This study was funded by Spanish projects CTM2010-19779-C02-01/02 and RTA2010-00004.

MO 280

Analytical and ecotoxicological characterization of biosolids collected from Spanish wastewater and municipal solid waste treatment plants

C. Martinez1, M.A. Martínez2, E.M. Beltrán3, M. Torrijos4, I. Navarro1, A. de la Torre1, P. García1, G. Carbonell3

1INA, Madrid, Spain
2Ciemat, Madrid, Spain
3CIEMAT, Madrid, Spain
4INIA, Madrid, Spain

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 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 Wasteable, 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 and to specify previous step by step an integrated microbiocenosis 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.

This study was funded by Spanish projects RTA2010-00004 and CTM2010-19779-C02-01/02.

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Ecotoxicological fate and effects of German river sediments

S. Gartiser1, C. Hafner2, C. Hercher1, M. Garcia-Kaeuper1, S. Peddinghaus1, S.H. Keiter1, H. Holler1

1Hydrosol GmbH, Freiburg, Germany
2RWTH Aachen University, Aachen, Germany

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 vertebrete-based sediment contact assays to investigate teratogenic, mutagenic, toxic, endocrine disrupting, xenobiotics 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 (Altrip and Ehrenbreitstein) and one from the outer Verin 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 vertebrete-based methods in the contamination level of the study sediments. The vertebrete-based assays applied to assess toxicity of sediment solubilize (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 2079, 2005), the Luminescent Bacteria test (ISO 11346, 1997), the Ames test (ISO 16240, 2005) and the umut 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 Anti-bacterium 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 1.7-5.9 mg L-1 (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 umut 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 EC50 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 soil ecotoxicology standards in Brazil: challenges and perspectives

L.S. Siuino

SoloTox Consultoria, Rio de janeiro, Brazil

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 [Normal X3:1-250, 1984 using earthworm (Eisenia fetida) based on AFNOR (ASSOCIATION FRANÇAISE DE NORMALIZATION). This method was applied for 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 created the Special Committee of Ecotoxicological Analysis of ABNT [Brazilian Association of Technical Standards], an organization member of ISO - International Organization for Standardization for responsible of 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 raising of academic and research activities with Germany and Portugal. So, in 2006 ABNT 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 earthworm, 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

Description of soil screening levels (SSLs) for Uranium, Copper and Cadmium using a natural reference soil

A.L. Caetano1, C. Gavina1, F.P. Carvalho2, J.P. Sousa1, F.J. Gonçalves1, E. Ferreira Da Silva1, R. Pereira1

1Departamento de Biologia e CESAM, Universidade do Aveiro., Aveiro, Portugal
2Instituto de Tecnologia e Nuclear (ITN), Sacavém, Portugal

1MAR-CMA, Departamento de Ciências da Vida, Universidade de Coimbra., Coimbra, Portugal
2Departamento de Geociências, Universidade de Aveiro, GeoBioTec Research Center, Aveiro, Portugal

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 must be based on specific soils could not be appropriate for site-specific assessment. Hence, a Portuguese natural soil (PTSS1), 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 physico-chemical properties (pH, EC, H2O, MeHg, PCBs and polynuclear aromatic hydrocarbons). Enzymes (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. Since differences in sensitivity among the species were remarkable 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.

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Environmental risk assessment in the São Domingos Mine area (Portugal). Tier I: screening phase

J.L. Pareja1, C. del Rio2, J. Pro3, C. Fernandez3

1IHC, Madrid, Spain
2CIEMAT, Madrid, Spain
3CIEMAT, Madrid, Spain

Application of risk assessment of uranium in a site contaminated with metals, a man made source of risk for the human health and the environment. The levels of uranium and other metals were found to exceed the standards in some areas, where a detailed assessment is required. For this purpose a soil screening assessment was performed according to the guidelines developed by IAH (UK) and by the US EPA (TRI-98). The project includes a first tier screening assessment based on land use (urban, agricultural, industrial) and soil properties and the determination of toxic concentrations for specific species. The results of the screening assessment are presented and evaluated in order to identify the areas of risk and to support the decision-making.

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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 (metals analysis and calculation of toxic pressures) and the ecotoxicological line of evidence (Vibrio fischeri solid-phase test, Folsomia candida, Acrithosaurus atra and Enchytraeus fetida). 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, pH, exchangeable cations, organic matter content, water holding capacity, and P quotas) to group the soils. Four groups of soils were defined indicating the need to use a multi-reference approach. To compare with the latter three additional soil samples were collected (at 13 to 20 km from de CA), to be used as reference material, together with a soil from one of the transect points. Total analytical results and expert estimations 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 reference site. The objective 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).

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) 1, C. S. Campiche 2, F. S. Campiche, C. Bandow 3, M. E. T. Nunes 1, M. O. Bianchi 1, M. O. Bianchi 1, M. E. F. Correia 1, A. Haddioui 4, M. Ksibi 2, R. Pereira 2, J. P. Sousa 2

Contaminated soils from North Africa have been introduced in the Mediterranean region, and several spills related to the industry’s development have been reported. Industrial phosphogypsum production is widespread in North Africa, including phosphorus-rich Tunisia. Moderate to high levels of contaminants such as heavy metals and organics have been observed in Tunisian phosphogypsum. In this study, the survival and reproduction of an enchytraeid, Folsomia candida, and predatory mites, Folsomia candida and Lensia n. sp., was evaluated using red mud from a phosphogypsum plant at the Farsak Sfax site, Tunisia. The site is located close to the central railway line, which is the inactivated railway line that was used for ore transport across the area.

The study aimed to assess the potential toxic effects of the red mud on soil non-target organisms. Five different treatments were evaluated using different concentrations of red mud: 0, 5, 10, 20, and 30% of total soil dry weight (w:w basis). The inactivation of the railway line 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. 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. 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. 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. 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. 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. 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. 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. 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. 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. 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. 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. 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. 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.
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 resilience (last pesticide applications occurred in August) was used during fall, spring and summer in 20 apple orchards with different management strategies using six pitfalls in each orchard. In spring, orchards through a high reproductive potential. The main challenge for field tests with micro-arthropods is the agroecosystem where 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 of pesticide applications on the soils'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 design. The seasonal dynamics of soil micro-arthropod populations in agricultural ecosystems was investigated. The species richness decreased with increasing SAB concentration, with the Shannon-diversity index decreasing from 1.5 to 0.2. 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 292 Effects of different land uses on soil microarthropod biodiversity in a Mediterranean urban context
L. Santoro1, J. Cotere, G. Maistro2
1University of Napoli Federico II, Napoli, Italy
2Université de Lorraine - UMR INPL/INRA 1120, Nancy, France
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 investigated 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 types (natural, residential, industrial and agricultural), were collected in Naples and surroundings (Southern Italy). The sampling of macrofaunal soil arthropods was performed by means of pitfall traps, bulk density, water and organic matter content, content of nutrients, metals, PAHs. To evaluate the arthropod biodiversity, the organisms were extracted using Maclayden method. Abundance of nMites (identified at the sub-order level) and abundance and distribution of Collembola (identified at the species level) were evaluated. 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. The Agrobacterium strain AM1 was used to infect seeds. Arthropods were sampled during fall, spring and summer in 20 apple orchards with different management strategies using six pitfall traps 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 (nospo, homeostasis) or positive response (carpet mites) 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. The aim of this study was to compare different sampling schemes and their potential for the evaluation of micro-arthropod communities in field trials. Sampling resilience (last pesticide applications occurred in August) was used during fall, spring and summer in 20 apple orchards with different management strategies using six pitfalls in each orchard. In spring, orchards through a high reproductive potential. The main challenge for field tests with micro-arthropods is the agroecosystem where 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 of pesticide applications on the soils'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 design. The seasonal dynamics of soil micro-arthropod populations in agricultural ecosystems was investigated. The species richness decreased with increasing SAB concentration, with the Shannon-diversity index decreasing from 1.5 to 0.2. 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 291 Spatial distribution of micro-arthropods in agricultural ecosystems
S. Knaebe
Eurofins AgroScience Services EcoChem GmbH, Niefern-oeschelbronn, Germany
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 a structure as far as 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 290 Seasonal dynamics of soil micro-arthropod populations in agricultural ecosystems
S. Knaebe
Eurofins AgroScience Services EcoChem GmbH, Niefern-oeschelbronn, Germany
The seasonal dynamics of the functional protection goals for soil 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 design. The main challenge for field tests with micro-arthropods is the agroecosystem where 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 of pesticide applications on the soils'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 design. The seasonal dynamics of soil micro-arthropod populations in agricultural ecosystems was investigated. The species richness decreased with increasing SAB concentration, with the Shannon-diversity index decreasing from 1.5 to 0.2. 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 289 What to do when there are too many specimens: two strategies for sub-sampling in Off-crop studies for non-target arthropods
C.J. Mazzia1, M. Hedde1, M. Richert2, Y. Capowiez2
1University, Avignon, France
2INRA, Versailles/france
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 nematode application (IPM vs conventional). Arthropods were sampled during fall, spring and summer in 20 apple orchards with different management strategies using six pitfall traps 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 (nospo, homeostasis) or positive response (carpet mites) 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. The Agrobacterium strain AM1 was used to infect seeds. Arthropods were sampled during fall, spring and summer in 20 apple orchards with different management strategies using six pitfall traps 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 (nospo, homeostasis) or positive response (carpet mites) 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).

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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. 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 in soils amended with compost and with a mineral fertilized soil. Subsequently, several plants of rosemary (Rosmarinus officinalis) were planted on these soils. The impact of authorized fungicides used in agriculture and forestry on the ECM fungi Pisolithus arhizus, Hebeloma crustuliniforme, Lyophyllum and Cenococcum geophilum was evaluated. The efficiency of the extractor is verified with Collembola species (Folsomia candida and Heteromurus nitidus) in different soil types and soil humorig regiments. At least 80% of F. candida and 96% of H. nitidus were recovered from soil cores.

An evaluation of interactive toxic effects of binary metal mixtures to the bioluminescent fungus Gerronema viridilucens was done. The interaction of authorized fungicides used in agriculture and forestry on the ECM fungi Pisolithus arhizus, Hebeloma crustuliniforme, Lyophyllum and Cenococcum geophilum was evaluated. The efficiency of the extractor is verified with Collembola species (Folsomia candida and Heteromurus nitidus) in different soil types and soil humorig regiments. At least 80% of F. candida and 96% of H. nitidus were recovered from soil cores.

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Advantages of fungicides on ectomycorrhizal mycelium growth - impact of quinoxone, boscalid, tebuconazole and azoxystrobin. The impact of authorized fungicides used in agriculture and forestry on the ECM fungus Pisolitrus arbus, Hebeloma crustuliniforme, Lyophyllum and Cenococcum geophilum was investigated. In a first step active ingredients in quinoxone, 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 microbial dilutions were performed 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 determining the radial growth of mycelium corrected by results from the control group. In a subsequent study the lowest inhibition wherein the lowest inhibition of mycelial growth was found for quinoxone. For Pisolitrus arbus 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 cultures medium.

Selective removal of high molecular weight PAHs in crude oil and tar contaminated soils using biodiesel

T.O. Ortizke, D.M. Jones

Newcastle University, Newcastle upon Tyne, United Kingdom

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 microcosms. 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 92% depletion of benzo(a)pyrene in the biodiesel amended samples after 60 days. However, the depletion 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, respectively.

Further work to investigate this process and also 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

C. Schurig 1, R. Smittenberg 1, J. Berger 1, F. Kraft 1, S.K. Woche 1, M.O. Göbel 1, H.J. Heipieper 1, A. Milterm 2, M. Kätner 1

1 UFZ for Environment, Research, Leipzig, Germany
2 Stockholm University, Stockholm, Sweden
3 Max Planck Institute for Developmental Biology, Tübingen, Germany

Leibniz Universität Hannover, Hannover, Germany

Recently, stocks of soil organic matter (SOM) have been shown to have decreased in European soils and also worldwide, which compromises soil fertility and enhances emissions of carbon dioxide and other, even worse greenhouse gases, to the atmosphere. However, the general structure of SOM, and thereby the mechanisms behind its genesis and loss, remains unclear. In this framework, microbial biomass is generally regarded to be of low importance for SOM formation. In particular on fresh soils, 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 colonisation of previously clean and sterile activated carbon surfaces after incubation in groundwater. Hence, we hypothesized that, at least in the initial stages of formation, bacteria and their fragments may play an important role in particulate SOM formation leading to 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 X-ray diffractometry. The initially barren mineral surfaces were first rapidly covered with microbial residues as soil age increases. Moreover, this data comes well to growing CN-ratios, water contact angles and fatty acid contents in earlier dated samples.

MO 304

Bioavailability and effects of cadmium, zinc and copper on microorganisms, community respiration and activity of soils in SE-Georgia

H. Kaplan 1, S. Ratering 2, T. Hanauer 2, P. Felix-Henningsen 3, S. Schnell 1

1 Justus Liebig University Giessen, Germany
2 Justus Liebig University, Institute of Soil Science and Soil Conservation, Giessen, Germany
3 Justus Liebig University, Institute of Applied Microbiology, Giessen, Germany

The study area is located in SW-Georgia, USA and characterized by a subtropical climate with mild, humid winters 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 nonferrous metal mine. For this reason, the irrigation in the Mashavera valley are highly contaminated with trace metals. Aim of the study was to test the use of soil microbial parameters for the evaluation of the habitat function of soil contaminated with trace metals.

Overall 51 soil samples were collected of the Mashavera river in March 2010. Habitat quality was evaluated by determination of pH-value, organic matter, soil texture and total and bioavailable (aqua regia, EDTA-, NH4NO3-extraction) trace metal concentrations. Total contents ranged between 284 mg kg^-1 and 1193 mg kg^-1 for Cd, Cu between 303 mg kg^-1 and 975 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 were also measured. 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 were 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 are 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 are 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.

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.
Predictions of Kd based on Log Kow have been attempted, but these are not very reliable, especially for ionic 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 was 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
C. Possberg, B. Schmidt, A. Schaefter
RWTH Aachen University, Aachen, Germany

- 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 fauna under real conditions” under real conditions investigations were performed. The aim of the project was to present the results of the experiments and to test the concept of using ‘in house’ prepared cartridges of sludge at different sludge/silica ratios.

**MO 309**
Understanding the relationship between extraction technique and bioavailability
C.V. Eadsforth1, M. Galay Burgos1, M.S. Hof1, D.J. Letinski1, H. Noble1, R. Oliver1, M. Telscher1
Shell International Ltd., Chester, United Kingdom

- 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 310**
Antimicrobials in sewage sludge compost: fate and residue analyses after 2 years
E. Haiba1, M. Lillenberg1, J. Falla2
1Tallinn University of Technology, Tartu, Estonia
2University of Bremen, Bremen, Germany

- The sewage sludge was mixed with different materials as tree bark, peat, oil shale ash, litter and sawdust. The antimicrobial decomposition rate dependance 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. We will optimize the sewage sludge composting technology to produce safe agricultural fertilizer.

**MO 311**
Degradation of pharmaceuticals in sewage sludge compost
E. Haiba1, M. Lillenberg1
1Tallinn University of Technology, Tartu, Estonia

- Plant uptake of some commonly used pharmaceuticals L. Nei1, M. Lillenberg1, E. Haiba1
2Tallinn University of Technology, Tartu, Estonia

- Estonian University of Life Sciences, Tartu, Estonia

- 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.

- Snell, remarkable amounts of pharmaceuticals enter the soil via fertilizer with sewage sludge. As long as such a disposal practice is widely applied, a better knowledge on the fate of pharmaceuticals in sewage sludge treatment is necessary.

- Only a relatively small number of investigations have been published on the mobility and bioavailability of pharmaceuticals in the environment.

- In the current study uptake of ciprofloxacin, norfloxcacin, ofloxcacin, sulfamethoxine and sulfamethoxazole was demonstrated in the case of lettuce, potato, carrot and wheat. Measureable residues of the pharmaceuticals and antibiotic-related compounds were also measured in food plants.

- 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 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 312**
Prospects of maniscanthus x giganteus for the rhizoremediation of PAH contaminated soils
1Université de Montpellier, Montpellier, France
2Université de Poitiers, France
3Université de Lyon, Villeurbanne, France
4Univeristé de Metz, Metz, France
5Université de Metz, France

- Phytoremediation of large area using crop cultivation appears as an attractive strategy to allow for long-term and economically viable site rehabilitation programs.

- The aim of this study was to characterize the phytoremediation potential of Maniscanthus x giganteus PAH contaminated soils. For this purpose, three factors affecting bioaccumulation, 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 microbial diversity (total and degrading microflora enumeration, 16S-rDNA analysis) and PAH distribution. 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 or complexed iron: what to use for soil remediation?
J. Filler, E. Lesnkov, I. Burdeind, U. Uebers
University of Bremen, Germany

- 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 nutrient-poor 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 unconcentrated amounts in soil. The present study aimed to evaluate the effect of nano-iron 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
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 objective is to study the changes in PAH and oxy-PAH levels during the remediation process (1) study the availability of the compounds over time using methanol extraction and (3) study if the toxicity in the soil is reduced in proportion to the putative reduction of the PAHs, using the H4IE-loc dioxin bioassay, based on rat hepatoma cell lines. 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 the presence of PAHs and oxy PAHs found in PAH contaminated soils, the degradation of these compounds, formation of oxy PAHs and changes in availability during a biological remediation.

MO 315
Ecotoxic problems of erosion, salinization and alkalinisation of soils in Azerbaijan
G.M. Mammadov, Z.S. Mammadova
State Land and Cartography Committee of Azerbaijan Republic, Baki, Azerbaijan
Institute of Soilscience and Agrochemistry of ANAS, Baki, Azerbaijan

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 isostasy land (51% of the land area) and land covered by water. Erosion process and intensively influences to erosion process and gradually changes 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 lands increases. In arable regions of the country more harm gives irrigation erosion. Inappropriation of mountain mass under not follow antierosional activities was grown which 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 th. area, especially in irrigation mountain. Mountain and plain regions of Azerbaijan exposed to irrigational erosion. At the result of wrong irrigation irrigation process of the yield decreases. Research works shows that total lands of territory exposing to erosion is 31444,7 th. ha in our Republic, which means 16% of the total area. Problem of salinization and alkalinisation are the most important problems among the ecoecological problems. Nowadays approximately 37000 th. ha soils from all existing land (8641560 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 mdh. That is why these lands become the most negative point of very important negative dynamics in the territory of our Republic. The 16 priority PAHs, even though often hundreds of 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 316
Ecological characterization of Mountain Shirvan Regions of Azerbaijan
T.G. Gusemda
Institute of Botany of ANAS, Baki, Azerbaijan

The composition of Mountain Shirvan economic and geographic region includes Agus, Ismailli, Gobustan and Shamakha districts. Total area of the region is 63 1000 ha, but 6% of the land area is arable lands. The physical territory is divided into three types: mountain area, the mountains are covered with forest, forest-steppe and steppe. The part of the plains - the summer is some cool, the winter is rather cold and arid. Annual middle temperature of foothill-plain territory is 14,10C (Agus), in upland territory -10,50C. Temperature of the coldest month in Agus is 3,30C, in Shamakha - 0,60C. Annual rainlist is 800 mm (Imayilli) and 379mm in Maraza (Shamakha). Evaporation is 725mm in Agus, 1253mm in Maraza. The rainfall in Agus is 428 thousand mm (69,8%) of general Maraza 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-forest. Composition of land supply: useful lands for agriculture - 376769 ha (67,1%), from them arable - 37,5% (141 thousands hectares), pastures - 2,5% (9,2 thousands hectares), hayland-1,2% (4,6 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, feeding- food. In subalp are meadows: moderate-damp-cereal, mixed-cereal, damp- forb meadow, meadow with clover, meadows with grass and clover, meadows with clover and carpell 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 disturb levels 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 mountain areas
Z.K. Mammadov
Mardakan Arboretum of ANAS, Baki, Azerbaijan

In modern age which the society is developing rapidly and has great technical strength, the relation between nature and human being strengthened and dangerous state is beginning. Even now, 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 is 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 regions which has natural complex and increasing the anthropogenic factors to the cover of soil. This district has according to soil climate conditions or ecological, geographical position to number suitable landscapes. 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 of the North-East slope of Small Caucasus is 433624,86 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 medium degree is 21 %, it is less than the soils which suffer to erosion weakly. 75% soil spreading in the North-East slope of the earth is not full coverd soils, it causes the problem. 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-DDD, 4,4-DD, 4,4-DDT, 2,4-DD, 2,4-DDD, 2,4-DDE, o-Chlordane, γ-Chlordane, Endosulfan I (alpha), Endosulfan II (beta), Endosulfane sulfate, Metolachlor, Metam-sodium, Ethamylacetate, Toxaphene). Because natural population land, human and animal, can be affected on the main concern. So, soils samples were collected in public areas located 25 of those towns. Concentration results indicated that all the organochlorine pesticides analyzed are widely spread in Caucasian valley, with DDTs accounting for the highest contribution to the total pesticides, mainly the 4,4-DDD (70-80%). The highest concentration for 4,4-DDD was 7,000ng g-1, for Endrin aldehyde 4,4-DDD and 2,4-DD and for Endrin aldehyde 8,500ng g-1. For toxaphene, the most pesticide sulfuric distribution pattern showed the major 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 those and maybe other pollutants are being released continuously to the atmosphere as it has been confirmed by our passive samplers in the area.
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 permanent organic pollutants (POPs) that may be transferred from soil to crops and reach the food chain. These findings and their possible impact on marine communities are discussed taking into account the dynamics of the ecosystem and human health.

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.

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.
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 were 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 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, oedema (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 the rotenone and 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
C. Megawley
Louisiana State University Agricultural Center, Baton rouge, Louisiana, United States of America

Plants-parasitic nematodes account for annual losses of 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 organophosphates, carbamate and fumigant nematicides remains the most widely utilized tool. This chemical management tactic is economics-driven in an era hypersensitive to escalating agricultural production costs. Widening public awareness of the negative environmental consequences of over- and misuse 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 nematodes. Research to date had documented significant efficacy of this material against populations of reniform, root-knot, soybean cyst, sting, stubby-root, rice, lesion, lance, spiral, and stubby-root nematodes. Subsequent enhanced formulation resulting from management of these nematodes ranged from 23-60 percent on sugarcane, soybean, cotton and a wide range of vegetables including cucumber, eggplant, bell pepper, cabbage and carrot. Unlike most traditional broad-spectrum nematicides that work by predatory mechanisms, this material has 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
M.H. Haeba
UNIC, Rio branco, Brazil

INTRODUCTION: Modern science has gradually awakened interest in plants of the Amazon. The Croton cajucara, Benth (Euphorbiaceae), known popularly as sacaca (croton) was targeted with four additional bioassays included aspects of dioxin-like activity (AhR-CAFLUX-test), genotoxicity (umuC-test), phytotoxicity (IPAM-test) and toxicity (LD-50-test) of the leaves. These bioassays included aspects of dioxin-like activity (AhR-CAFLUX-test), genotoxicity (umuC-test), phytotoxicity (IPAM-test) and toxicity (LD-50-test) of the leaves. In addition, 300 mg/kg of a 10% aqueous extract of the leaves was used for computerized tomography. In the present study the effects of boiling green tea on Swiss albino mice were investigated. 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. However, blood weights were decreased at 0.4ml comparison to control. RBC, PLI number as well as LdG and Cholesterol, triglyceride, sugar have no different with control group. However, WBC were increased at 0.2ml and 0.4 ml. This study will be to 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
M.C. Oliveira, L.P.M. Barros, M.M. Abreu
UFAC, Rio branco, Brazil

The Sacaca’s study on the treatment of Hypertension, Rio Branco, Acre, Amazonia, Brazil

INTRODUCTION: Modern science has gradually awakened interest in plants of the Amazon. The Croton cajucara, Benth (Euphorbiaceae), known popularly as sacaca is the term used for medical plants with a specific medical function. It is known that the poorest segments of society and older have a higher prevalence of hypertension (SH), and have as the main cause of death. Finally, the use 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 study population was interviewed. All respondents signed a consent form Statistical analysis was performed, 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 those 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 was considered significant for the sacaca. CONCLUSION: We did not observe effects of sacaca in the treatment of SH. 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
1National Research Centre for Environmental Toxicology, University Queensland, Coopers plains, Australia
2Department of Environmental Toxicology, Eawag, Dubendorf, Switzerland
3University of Saskatchewan, Saskatchewan, Canada

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) runoff 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 observed for the different classes of mycotoxins included genotoxicity (umuC-test), phototoxicity (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

SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
MO 334
Occurrence of mycotoxins in Swiss surface waters
J. Schenzel1, T.D. Bucheli1, K. Hungerbühler1
1Agroscope Reckenholz-Tanikon ART, Zurich, Switzerland

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 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 335
Lipid soluble acyl conjugates of hydroxylated polybrominated diphenyl ethers (OH-PBDEs) in blue mussels from the Baltic Sea
D.C. Lindqvist, S. Jensen, L.T. Asplund
Stockholm University, Stockholm, Sweden

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). The conjugation of OH-PBDEs is an important regulatory factor of 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 a potential to work as a detoxification mechanism. 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 conjugates can be conjugated in concentrations of up to 65 ng/g lipids. The conjugates 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.

(1) Malinmäki et al. Chemosphere 2008, 72, 7
(3) Ucan-Marin et al. Toxicol. Sci. 2009, 107
(5) Buusn et al. Waterbod. 2001, 24
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 antigel KLH to investigated immune-related parameters. Although considerable amounts of microcystins were orally administered to rats, levels of MGs in the tissues were below the detection limit (1 ng/g fresh weight; based on the tandem LC/MS/MS). Only traces of the conjugates of microcystins with cystein and glutathion 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 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/kg/day). In conclusion, NAC can be considered a useful chemoprotectant in the prophylaxis of CYN-related oxidative stress induction in fish.

Acknowledgements: the authors wish to thank Junta de Andalucía (P09-AGR-04672) and the Ministerio de Ciencias e Innovación (AGL2009-10026) for the financial support of this study.

MO 341

Properly study of biomarkers in mussel haemolymph after exposure to toxins extracted from Microcystis aeruginosa and Lyngbya sp.

M.C. Oliveira, V.R. Bastos, M.F. Fournier, F.G. Gagné

INRS-IAF, Laval, Canada

Environment Canada, Montréal, Canada

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 hepatotoxic, whereas L. wollei (LYNGTX) produced an analogue to saxitoxin which is a neurotoxin. Selected immune parameters investigating haemolymph integrity and immunomomtheran effects 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, 50/1000μl/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 decreased their viability at the highest concentration whereas haemocytes exposed to LYNGTX showed an increase in their viability at the highest concentration. However, the type of toxin was significantly different for phagocytic activity. Haemocyte exposed to MCYST had a lower phagocytic 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 of L. wollei had increased complement types of toxins. A decrease of the phagocytic index is usually observed response to toxins or in vivo or vitro exposure to toxic concentrations 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

M.N. Oliveira, E.S. Silva, E.C. Bastos, S.C. Prieto

Instituto Federal Fluminense, Cabo frio, Brazil

Universidade do Estado do Rio de Janeiro, Rio de janeiro, Brazil

Universidade de Almeida, Cabo frio, Brazil

Instituto de Estudos do Mar Almirante Paulo Moreira, Arraial do cabo, Brazil

Fundação Ondalando Cruz, Rio de janeiro, Brazil

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 enigmatic activity and biomass of microcystins, we performed a microvivistic 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 assays the total haemolymph and esterases, these 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 were exposed to higher concentrations in vivo (1,5,14,000 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 phosphatase in both extracts, whereas esterases are inhibited only with the Me10. The esterases of L. fortunei are similar to other species of molluscs, as described in their IC50 in the presence of eisenine. The inhibitions caused by the in vitro condition without microcystin present the inhibitions of 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?

J.D. Hochmuth, J. Asselman, K.A.C. de Schamphelaere

Ghent University, Ghent, Belgium

Ghent University (UGent), Ghent, Belgium

University of Seville, Seville, Spain

Universidade do Estado do Rio de Janeiro, Rio de janeiro, Brazil

Instituto Federal Fluminense, Cabo frio, Brazil

Biology Department, Faculty of Sciences, University of Porto, Porto, Portugal

Screening of M. aeruginosa strain NPLJ-4, obtained with 100% methanol (Me100 - extract with microcystin) and 10% (Me10 - without microcystin extract) show that inhibition of phosphatase in both extracts, whereas esterases are inhibited only with the Me10. The esterases of L. fortunei are similar to other species of molluscs, as described in their IC50 in the presence of eisenine. The inhibitions caused by the in vitro condition without microcystin present the inhibitions of the effects of exposure to Microcystis aeruginosa.

MO 344

Phosphatases and Esterases of Limnoperna fortunei (golden mussel) in presence of Microcystis aeruginosa

M.N. Oliveira, E.S. Silva, E.C. Bastos, S.C. Prieto

Instituto Federal Fluminense, Cabo frio, Brazil

Universidade do Estado do Rio de Janeiro, Rio de janeiro, Brazil

Universidade de Almeida, Cabo frio, Brazil

Instituto de Estudos do Mar Almirante Paulo Moreira, Arraial do cabo, Brazil

Fundação Ondalando Cruz, Rio de janeiro, Brazil

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 enigmatic activity and biomass of microcystins, we performed a microvivistic 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 assays the total haemolymph and esterases, these 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 were exposed to higher concentrations in vivo (1,5,14,000 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 phosphatase in both extracts, whereas esterases are inhibited only with the Me10. The esterases of L. fortunei are similar to other species of molluscs, as described in their IC50 in the presence of eisenine. The inhibitions caused by the in vitro condition without microcystin present the inhibitions of the effects of exposure to Microcystis aeruginosa.

MO 345

Physiological effects of a marine algal toxin on a primary consumer: tales of the unexpected

J. Asselman, K.A.C. de Schamphelaere

Ghent University, Gent, Belgium

Ghent University, Gent, Belgium

Current assessment of the 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 (Xin3b and Lin1b). Several cyanobacterial species (Microcystis aeruginosa, Cylindropermopsis raciborskii, Anaabaena sp, Nodularia sp., Oscillatoria sp., Aphzimonon-flosaque) were investigated, ensuring toxins with different Modes of Action (MoA). Semi-static 21-day life-table experiments were performed according an extended second-order two factor central composite design. The effects of 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 the fitted model can be used to make predictions on the magnitude of increase or decrease of Cu toxicity in the presence of cyanobacteria.
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 diarheic 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 effect of OA on the BaP-induced induction of the BaP-induced membrane stability (LMS) of the bauproteins 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 hepatopancreas WW and higher. In the second phase, the mussels were exposed in vivo to toxin producing algae (P. lime). 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/gWW (500 cells/ml) and 740 ng/gWW (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 reduction of the PPase activity this means that these enzymes are sensitive to OA exposure. Secondly, in vitro 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

Oscillatoriales responses produced in line by exposure to repeated doses of cylindrospermopsin from Aphaniizonemon ovalisporeum

R. Guzmán-Guillén1, A. Prieto1, C. Fernández-Blanco1, V.M. Vasconcelos2, A.M. Cameán1
1University of Seville, Seville, Spain
2Centro Interdisciplinar de Investigacion Marinha e Ambiental, CIMAR/CIMAR, Porto, Portugal

Cylindrospermopsin (CYN) is a tricyclic alkaloid, possessing a tricyclic guanidine moiety combined with hydroxymethylacrylic. It is produced by several genera of cyanobacteria, growing worldwide in eutrophic freshwater and it is easily transferred among different trophic levels causing damages to humans, animals and plants. It 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 Aphaniizonemon ovalisporeum (LEGE-X-001). These parameters were: lipid and protein oxidation, and the enzymatic activities of Glutathione-S-transferase (GST), Glutathion Peroxidase (GPx), Catalase (CAT) and Superoxide dismutase (SOD).

In this study, nine groups of Individuals with 8 fish per group were established: 3 control groups, 3 groups exposed by immersion to an A. ovalisporeum culture (10 µg CYN/L), and 3 groups exposed by immersion to lyophilized A. ovalisporeum (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 analysis.

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, in comparison to the lyophilized Aphaniizonemon ovalisporeum.

Acknowledgements

The authors wish to thank the Spanish Ministerio de Ciencia e Innovación (CICYT, AGL2009-10026ALI) and Junta de Andalucía (P09-AGR-4672) for the financial support for this study.

MO 347

Development of a method for determination of CYN in lyophilized Aphaniizonemon ovalisporeum cells by LC/MS/MS: validation and application to real samples

R. Guzmán-Guillén1, A. Prieto1, C. Fernández-Blanco1, L.M. Moreno1, A.G. González2, M.E. Soria1, V.M. Vasconcelos2, A.M. Cameán1
1University of Seville, Seville, Spain
2Mass spectrometry facility, Centro De Investigacion Tecnologica, Seville, Spain

Harmful cyanobacterial blooms are occurring in eutrophic freshwater lakes and reservoirs throughout the world and can present a public safety hazard through consumption of drinking water. There are over 40 species of freshwater cyanobacteria and more than 60 different toxins produced. CYN is the main toxin in CYN-producing cyanobacteria (CYN-producing cyanobacteria) which include both cyclic peptides and alkalds such as cylindrospermopsin (CYN), produced by Aphaniizonemon ovalisporeum 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 for separation of CYN and LC/MS/MS for CYN determination from lyophilized cultures of A. ovalisporeum (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 interferences were obtained (5-15%) 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.

Acknowledgements

The authors wish to thank the Spanish Ministerio de Ciencia e Innovación (CICYT, AGL2009-10026ALI) and Junta de Andalucía (P09-AGR-4672) for the financial support for this study.

MO 348

Screening for cylindrospermopsin, anatoxin-a and saxitoxins in France

M. Dechesne1, F. Fignon1, C. Pagotou1, S. Lenjo1, J. Fastrez1
1Veolia Environnement Recherche & Innovation, Rueil-malmaison, France
2Institut de Limnologie SARL, Rennes, France
3Veolia Eau Direction Technique, Saint maurice, France
4Federal Environmental Agency (UBA), Berlin, Germany

Microcystis 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 Nostoccale 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. A total of 40 microcystins was identified with a maximum level of 168 ng/g wet weight (WW).

The authors wish to thank the Spanish Ministerio de Ciencia e Innovación (CICYT, AGL2009-10026ALI) and Junta de Andalucía (P09-AGR-4672) for the financial support for this study.

MO 349

Sensitive detection of microcystins using online SPE from environmental waters

R.J. Mousley1, F.J. Tipping2, C.J. Bithell3
1Thermo Fisher Scientific, Sunnyvale, United States of America
2Waterblooms of cyanobacteria (blue-green algae) can produce potent toxins that have become a severe problem for eutrophic aquatic environments. Hepatotoxins are among the 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-coupled 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-coupled on-line SPE method followed by HPLC with UV detection was applied to the determination of three microcystins (-LR, -RR, and -YR) in drinking water. The target-coupled on-line SPE method followed by HPLC with UV detection was applied to the determination of three microcystins (-LR, -RR, and -YR) in drinking water.

Sub-µg/L concentrations of microcystins-LR, -RR, and -YR spiked in water samples were determined, which exceeds the WHO requirement.
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 pathway to toxicosis are scarce. In the last decade, researchers related to organophosphates insectsicides 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 mechanisms 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 (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 showed any statistically significant 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 antioxidant capacity in mice in sub-lethal levels. This fact was proved through the unbalance on the activity of the antioxidant enzymatic defense system.

MO 358
Physical and toxicological properties of phytotoxins - laboratory studies to evaluate the impact of toxic cyanobacteria in agriculture, food quality and human health
A.M. Campos1, D. Guarneri-PezaHenzen2, A. Canamín3, J. Azavedo4, C. Azavedo3, V. Vancasenoc3
1CIMAR, Porto, Portugal
2Area of Toxicology, Faculty of Pharmacy, University of Seville, Seville, Spain
3Faculty of Sciences, University of Porto, Porto, Portugal
4Institute for Pharmacological Research "Mario Negri", Milan, Italy

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 showed any statistically significant difference related to the control during all the treatments. Activity of all the evaluated enzymes was completely recovered after fourteen days.

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MO 361
Environmental impact analysis of 10 active pharmaceutical ingredients with QSARs
C. van der Grinten1, B. Mertens2, D. Caldwell3
1VITO NV, Mol, Belgium
2Janssen Pharmaceutica, Beere, Belgium
3Johnson & Johnson, New brunswick, new jersey, United States of America

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 algae and/or other more sensitive 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. Calculation performed in EPISuite. Model estimations are discussed and compared with measured data.

QSARs 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 external data set.

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 362
Predictive estimative QSAR models: thresholds of acceptance by various external validation criteria and critical inspection of scatter plots
N. Chirico1, E. Papa2, P. Gramatica3
1University of Insubria, Varese, Italy
2Janssen Pharmaceutica, Beerse, Belgium
3VITO NV, Mol, Belgium

The evaluation of linear regression VSAR models performances, both in fitting and external prediction, is of pivotal importance. In the last decade different external validation parameters have been proposed: Q2 (Shi), Q2 (Schuurmann), Q2 (Todeschini), averaged r2 (Roy) and the Golbraikh Tropsha (GT) method. Recently, the concordance correlation coefficient (CCC) has been proposed by our group as an external validation parameter to be used in VSAR studies. In our recent work, published in 2011 on JCIM, we have shown that, for the common used model acceptance thresholds (Q2 >0.6, averaged r2>0.3), 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 most adequate external validation parameter in a precautionary approach, if the aim of VSAR developers is to have the smallest differences among the experimental data and the predictions of the external data set.

In this new study, we have worked 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 r2 values, if functions that 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 VSAR 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 good VSAR 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 VSAR studies, where the data set sizes are often small.

MO 363
Collection screening of QSAR models for REACH
C. Milan1, E. Bentenati1, A. Roncaglioni1, R. Gorolla Diazza2, A. Cassano1
1Institute of Pharmaceutical Research „Mario Negri“, Milan, Italy
2Faculty of Sciences, University of Porto, Porto, Portugal

REACH is the Regulation on Registration, 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 (QSARs) and read-across. Before making an animal experiment the industry should verify if alternative methods exist. However, so far there is a lack of a threshold difference related to the screening study.

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=modellist.

The QSAR models cover endpoints for physico-chemical, environmental, ecotoxicological and toxicological properties. Within ANTARES we are evaluating in details the toxicity of microcystin (MC) and cylindrospermopsin(CYN), which are two trichocyanobacterial toxins, 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 a group of toxic glycopeptides widely distributed in freshwater. This toxin has been directly linked 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, reproduction and oxidative stress induction suggests understanding of the impact 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 Lycopersicum 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.
QSAR classification models of the terrestrial toxicity of (benzo) triazoles: predictive tools for hazard and risk assessment
E. Papa, P.P. Roy, S. Kovarich, S. Cassani, P. Gramatica
University of Insubria, Varese, Italy

Triazoles and benzotriazoles (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 widespread 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 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 model availability. The experimental acute toxicity data used for the proposed models were taken from the literature and the proposed models were trained and validated with the in silico and experimental data for the compounds put forward by the TLM, that was incorporated in the first steps of the development process and was not included in the model building.

The proposed models provided a good statistical performance in fitting and in external prediction with R2, 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 to verify the chemical applicability domain of the models. In conclusion, the proposed models, which were developed and validated, can be used for the prediction of the toxicological profile of new compounds from the same families. 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
University of Coimbra, Coimbra, Portugal

Aqua+ Tech, Geneva, Switzerland

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 of utmost importance. 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 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 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 biofouling 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
F. J. Costa, F. Iacino, C. Janssen, M. Pavan, P. van Sprang, F. Verdonck
1, J. C. Rosa, F. J. M. Gonçalves, D. Hunkeler
University of Aveiro, Aveiro, Portugal

University of Coimbra, Coimbra, Portugal

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 H.C3 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 to toxicologically important hydrocarbons. 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 low-slit 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 25 microgram/ml. Chronic effects were not observed for hydrocarbons with measured water solubility below 5 microgram/liter. To estimate aqueous PNECs and discriminate toxic from non-toxic structures, the equation used in the TLM to extrapolate to chronic narcosis was modified.

The modified TLM was then evaluated using test data from this study and additional chronic effects data reported in the literature. A larger set of hydrocarbon substances over 260 aliphatic compounds with known chronic effects 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 water soluble hydrocarbons in support of risk assessment of complex petroleum substances using the hydrocarbon block method.
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.

For a set of 12 compounds, consisting of 4 aldehydes, 4 ketones, and 4 esters, 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 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 two substituents at the C=O, 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.

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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 toxins. In the present study, we determine the individual toxicity of mono-, bi- and tri-valent metals at concentrations up to 2500 μg/L. While for metals with mono- and divalent cations at concentrations up to 400 μg/L, the toxicity of the free metals were predicted using the QICAR approach. The following parameters were used: ionic radius (r), atomic number (Z), electronegativity (Xm), valence index (Xm2r), first hydrolysis constant (logKOH), softness index (σ), ionization potential (IP), electrochemical potential (ΔEo), reduction potential (ΔE0) 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 QICARs (100 random models and 5,000 iterations to evolution). The best correlation was found with AN, Xmr2 and log[log50 + 1.00 * 0.24(AN) + 0.52(Xm2r) + 1.30(AN) + x2, adj R2 = 0.95, pred R2 = 0.91, LOF = 0.87]. This mathematical expression allowed the prediction of IC50 values for mono-, bi- and trivalent metal, with good accuracy for IC50 with r < 0.15 for monovalent metals. 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.
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 (IS\_L), 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 Cladocera being more sensitive than the most sensitive insects of the order of Odonata. In contrast, 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

**EMCt**

University of Washington, Tacoma, Tacoma, United States of America

**MITOX Consultants, Amsterdam, Nederland**

"Bioresearch and Promotion, Amsterdam, Nederland"

In earlier experiments NTA field studies were found 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 arthropods 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 localizations. The focus of this analysis was as at the population rather than at the community level.

For each study we calculated direct effects on populations sufficiently represented in the samples as the ratio of population densities in treatment and control groups at the beginning or end of exposure. Duration of effect was considered as an additional parameter. Analysis of 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. Finally, in the relevance of the SPEA for terrestrial arthropod communities was discussed from the perspective of using ecological traits for prospective or retrospective risk assessment in terrestrial ecosystems, similar to existing methods used in aquatic systems.

**MO 377**

Application of a lentric SPEAR index to pond mesocosm experiments: why community assembly characteristics and season matter in ERA

**M. Roucaute, A. Aubert, L. Lagadic, T. Caquet**

INRA, Rennes, France

An adapted version of SPEAR metrics for use in lentric experimental systems, LSPEAR (Lentic SPEArCies 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 Daphnia multiplicata were introduced but asellids did not successfully settle. Eight ponds were then introduced 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 unamended 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 PRC, only 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 various factors when the experiment started. During the second experiment, pyrethroids induced significant negative effects on invertebrates, especially crustaceans. This was due to the selection of taxa exhibiting various traits associated with a higher tolerance to stressful environments. It therefore possible to draw 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

**H.S. Kraak**, **E. Mulder**

University of Amsterdam, Amsterdam, Nederland

"RIVM LER, Bilthoven, Nederland"

Detritus processing and decomposition 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 link 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, stingless 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 overlying 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 subletal 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


INRA-Agrocampus Ouest, Rennes cedex, France

"SERVAL, Total Petrochemicals, Paris, France"

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. Macroinvertebrates should be a priori considered as more sensitive and should be used to detect environmental impacts from downstream and that larvae should be less sensitive and should be a priori considered as less sensitive 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).

Out of the 15 mesocosms (length: 40 m, depth: 50 cm) and ponds (volume: 7 m³, depth: 90 cm) were exposed to a dithiocarbamate fungicide, thiabendazole. 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 controls 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 in litter was used as a proxy for hyphomycete biomass in order to assess the effects of the treatments on fungi. A strong effect of exposure to thiabendazole was shown in streams with a decrease in the abundance of various taxa such as gasps, mods and asellids. Midge larvae were indirectly positively favourised by treatments. These structural effects of thiabendazole 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 several taxa and no effects were observed on water chemistry. Reducing data of thiabendazole 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?

**J.D. Stark**

University of Washington, Tacoma, United States of America

"Washington State University, Puyallup, United States of America"

"University of Washington, Gothenburg, Sweden"

"University of Washington, Tacoma, Tacoma, United States of America"

The ability of a species to recover from pesticide exposure 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 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) protocol used in Europe does not account for life history characteristics. 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 was 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

SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
H. Shinn, G. Grenouillet, S. Lek
1IMAR - Instituto do Mar, Coimbra, Portugal
2Laboratoire Evolution et Diversité Biologique, Toulouse, France

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 3-day test in controlled, flow-through conditions. Our hypothesis was that fish swimming activity, the height 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 the suitability 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?

F. de Laender1, F. Taut1, C.R. Janssen2
1Katholieke Universiteit Leuven, Leuven, Belgium
2University of Antwerp, Antwerp, Belgium

Regarding sensitivity of the tests towards nature of pollutant, slight differences could be appreciated only through chemical tests. The species used for testing consisted of phytoplankton/mesozooplankton densities and the associated ecosystem functions to stress occur because functional rates (e.g., photosynthesis rates/ingestion rates) differ among trophic groups in a consistent manner. These variations can be explained by food web ecology but not by direct effects on organisms, 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

R.B. Schafer1, M. Bundschuh1, E. Szecs1, P.C. Von der Ohe1, V. Pettigrove2, R. Schulz1, D. Nugegoda1, B.J. Kefford5
1Environmental Sciences, University Landau, Landau, Germany
2School of Chemistry, University of Adelaide, Adelaide, Australia
3UFF - Helmholtz Centre for Environmental Research, Leipzig, Germany
4Melbourne University, Melbourne, Australia
5University of Auckland, Auckland, New Zealand
6RMIT University, Melbourne, Australia

One challenge for ecologists is to develop reliable and sensitive indicators for environmental and biophysical stressors on freshwater communities that 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, woodsticks) in coarse and fine bags were investigated. Second, we measured stream metabolism that delivers information on ecosystem functions. 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 wood stick breakdown was less sensitive and responded also to exposure to salinity and a combination of both stressors. Hence, 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 may lead to impairment of ecosystem functions service provision and possibly water purification. Hence, future studies should examine the spatial extent of these effects.

MO 384
Effects of SSIs on aquatic predator-prey interactions

M.L. Hedgespeth, J.A. Jonson, O. Berglund
Lund University, Lund, Sweden

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 effects have implications in terms of ecosystem services provided by the organisms. These pharmaceuticals, when ingested, will result in decreased activity and may possibly decrease the efficacy of the predator's functional response. This research will allow for application of ecological models to predict the effects of these substances on the ecosystem functions and services. We found that sertraline, a selective serotonin reuptake inhibitor (SSRI), on predator feeding rates were sensitive to predator changes in the predator's functional response. This research will allow for application of ecological models to predict the effects of changes in predator-prey interactions caused by pharmaceuticals on ecosystem structure and function. The effects of prescribed rates of water per minute is needed to improve the understanding of potential environmental effects of SSIs.

MO 385
The ecosystem services triad: linking stakeholder engagement, biophysical models, and ecological production functions to develop indices of ecosystem services for biodiversity

N. Booth, S. Law, T. Wickwire, J. Ma, J. Salatas
Environmental Sciences University of the United States of America

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

L.G. Goicoechea, P.A.M. Meininger, E.C. Giprián
Tecnalia, Dérbi, Spain

The Ecological Risk Assessment (ERA) methodology currently applied in the Basque Autonomous Community (BAC) is based on Pontentially Affected Fraction (PAF %) based on the concentration 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, persues 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 colcemblana, nematodes, arthropods and enchytraedos were not used in any test. So far, this means that available tests are not applicable to every existing soil function. This is why we have here the need to progress beyond the state of the art in by developing new testing tools and procedures combining empirical data to assess specific reproductivity. 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

H. Bol, G. Grenouillet, S. Lek
1IMAR - Instituto do Mar, Coimbra, Portugal
2Laboratoire Evolution et Diversité Biologique, Toulouse, France

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 3-day test in controlled, flow-through conditions. Our hypothesis was that fish swimming activity, the height 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 the suitability 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.
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 ecosystem services frameworks (ESF). They seek to understand how 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 synergistic management. These 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 in space, each with a different set of science and policy 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 Environment 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-access 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 included in wide-ranging environmental decision making and policy will be discussed.

MO 388

DASEES: a tripartite decision framework to achieve sustainable environment, Economy & society growth and management goals

T.J. Canfield, T. Stockton1, B. Dyson1, M. ten Brink1, K. Black1, P. Bradley1, S. Yee1, W. Fisher2, J. Carigger3, A. Vega3

1USEPA, Ada, United States of America
2Neptune and Company, Los alamos, United States of America
3Laboratory LIEBE - CNRS UMR 7146 - Université de Lorraine, Metz, France

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 areas of key players are in many of the decisions facing societies as they strive to operate in a more sustainable manner. Typically decisions are made with 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 manner. 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 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 developing and delivering effective and user-friendly decision methods and approaches that decision-makers can use to determine, 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 impacts 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 for a Sustainable Environment, Economy and Society (DASEES) tool. 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

B.R. Raskovic, M.M. Markelic, S. M. Spasic, Z. M. Markovic, V.P. Poleksic

1University of Belgrade, Faculty of Agriculture, Belgrade, Serbia
2University of Belgrade, Faculty of Biology, Belgrade, Serbia
3Laboratory LIEBE - CNRS UMR 7146 - Université de Lorraine, Metz, France

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. Copper can cause wide range of toxic effects in 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; 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 longitudinal for microscopic observations. The slides were stained histochemically with PAS (periodic acid-Schiff reaction) and with hematoxylin and eosin, and examined using bright-field 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 (≥2 mg/L), whereas at lower doses only slight degenerative changes were observed. In 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

L. Minguez1, S. Devin2, D.P. Molloy2, F. Guéroud3, L. Giambérini4

1Laboratory LIEBE - CNRS UMR 7146 - Université de Lorraine, Metz, France
2Department of Biological Sciences, State University of New York, Albany, United States of America
3Laboratoire IEB - CNRS UMR 7146 - Université de Lorraine, Metz, France
4Laboratorio di Biodiversità, Dipartimento di Scienze della Terra e della Vita, Università di Roma La Sapienza, Rome, Italy

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-specificity and 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 used 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 provide paradigmatic approaches to the study of interactions 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 micro-parasites (i.e. bacteria and fungi), whereas it prevented those by macro-parasites (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 multiple multivariate analyses and modelling are promising tools to study zebra mussel parasite communities related to pollution.
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
M.V. Silva, J. Isman, C. Baudouin, C. Génicot-Olive
Institut des Sciences Analytiques, Villeurbanne, France

Animotest and human medicine are more and more used for animal treatments and are excreted unchanged. So, these compounds can enter the environment via land application of sewage sludge. This phenomenon could induce a competition for antibiotics to human or animal medicine. Steroids, which are endocrine disruptors are also found in the environment due to animals and 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, selected compounds were selected among the most important hormone classes. The surface of the columns were spiked with 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 experiments 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 pluviosity and the composition of soil (clay, loam, sand and organic matter contents).

MO 397

Temperature dependence of transformation and translocation processes of manure-amended sulfadiazine in soil
T. Mueller, K. Pamplaniyi, M. Lamshöft, J. Klasmeei, M. Matthies
University of Osnabrueck, Osnabrueck, Germany

Technical University of Dortmund, Dortmund, Germany

Sulfadiazine (SDZ) is a widely used veterinary antibiotic. In pigs, it is partly metabolized to N-acetylsulfadiazine (AcSDZ) and 4-hydroxy-sulfadiazine (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 hydrolysis 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 to the field scale, the influence of temperature on the presence 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, 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 homogenously mixed with a silt loam soil. The batches were adjusted to 40% maximum water capacity and incubated in the dark for 140 days. Samples were regularly extracted with [1] CaCl₂, [2] methanol/CaCl₂, and with [3] acetonitrile/water (ACN) for the extraction of the solubilised fraction (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 enzymatic 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 14C-SDZ was several times more rapid at 20°C than at 10°C. The same tendency was observed for the slow hydrolysis 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.
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 test under controlled temperature, moisture, light conditions and a field trial. In the batch experiments, antibiotic spiked manure was added to Al material of an agricultural soil (Orthic Luvisol) at a ratio of 1:25 manure/soil (w/w). The antibiotic concentrations amounted 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. Possibly, the denitrification activity (up to 30% inhibition) during the whole incubation time of the mesocosm and field trials. Considering the worldwide applied amounts of veterinary antibiotics, particularly sulfonamides, these results suggest a significant risk with antibiotic manure concerning crop production and fundamental soil functions.

MO 402
Sublethal effects of Ivermectin on dung beetles of the genus Onthophagus (O. taurus, O. nuchicornis)
A. Scheffczyk*, K. Floate†, J.P. Lumaret‡, N. Adler‡, J. Roembke‡‡
*ECT GmbH, Floersheim, Germany
†Lethbridge Research Center, Lethbridge, Alberta, Canada
‡University of Veterinary Medicine, Mössnitz IIL, Montpellier, France
‡‡Federal Environment Agency (UBA), Dessau-Roßlau, Germany

European legislation requires parasites to be tested for excrated residues using species of dung flies (OECD 228) and dung beetles (OECD GD 122). In the latter case, the efficiency of a driller was determined using a drilled specimen of fecal material for insecticidal residues in dung (OECD 2010). Larvae of driller 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 testing using A. constans may not be relevant to tunnelers. Recently an international ring test using acetonate was started using two tunneler species of the genus Onthophagus by the SETAC advisory group DOTTs (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 dung and sub-lethal dung using acetonate. 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 batched 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 dw dung were determined at ECT GmbH. In comparison, EC₅₀ values of 100 - 176 µg a.i./kg dw dung 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
S. Žižek, M. Pogacnik, P. Zidar
University of Ljubljana, Ljubljana, Slovenia

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 medicated feed and manure. Therefore, they are a potential threat to non-target soil invertebrates. In the present study, we were interested in potential behavioural responses of earthworms (Eisenia andreii) 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 woodlice. 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
D. Hennecke*, M. Herrchen*, U. Merrittig-Brüns†, J. Tunker†, S. Jäger†, S. Berkner‡
*Fränkische Umwelt GmbH, Erlenbach, Germany
†Umweltbundesamt, Dessau, Germany
‡Fraunhofer IME, Schmallenberg, Germany

Recently a draft to develop a test method to determine VMP transformation in manure is 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 interlaboratory testing; development of Standard Operating Procedures; preliminary outline for an OECD-testguideline to be introduced into the OECD programme. The methods were divided 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. Refinement of the method 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 formation of NER, CO₂ and CH₄ is possible.

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 UC-glucose as reference substance in comparison to the fumigation method used for aerotbic 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
T.K. Wings*, F. Platzecker†, W. Dott‡
*RWTH Aachen University, Aachen, Germany
†University of Veterinary Medicine, Vienna, Austria
‡Umweltbundesamt, Dessau, Germany

A list of 16 veterinary antibiotics is being applied on a large scale in animal husbandry. With close to 800 t administered in 2005 they form the most avidly applied group of veterinary medicinal products in Germany. Quantities arise because antibiotics are being used not only for acute disease treatment, but are also administrated in low dosages as growth promoters. In consequence, antibiotics are expected to be present in the environment. The aim of this work was therefore to develop an extended method for the use on fatstock manure, due to a dramatically higher antibiotic consumption and correspondingly higher residues that can be expected.

Three antibiotic samples 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 batched beetles were identical. For example, EC₅₀ values of 31.3 (12.2 - 186.9) and 34.1 (13.9 - 85.1) µg a.i./kg dw dung (FW) were determined at ECT GmbH. In comparison, EC₅₀ values of 100 - 176 µg a.i./kg dw dung 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 406
Determination of the veterinary antibiotic erythromycin's potential to bioconcentrate in the aquatic invertebrates, Littorina variegata and Daphnia magna, and evaluation of a surrogate model
J.R. Coult*, A. Jessick†, K.L.D. Cartensen‡, C.R. Seery§, N.L. Cooper†, J.R. Bidwell**, T.B. Moorman†
*Iowa State University, Ames, United States of America
†Oklahoma State University, Stillwater, Oklahoma, United States of America
‡UniResearch AFS National Laboratory for Agriculture and the Environment, Ames, Iowa, United States of America
§University of Wisconsin, Madison, Wisconsin, United States of America

The bioavailability of 14C-radioabeled erythromycin was evaluated in aquatic microcosm systems with and without the addition of pond sediment extraction disks and the aquatic worm, Littorina variegata, and using C8-Empore™ disks as surrogate passive sampling devices for comparison. An aquatic microcosm was also evaluated compared to a beaker experiment to examine the potential of erythromycin to leach out of aquatic organisms. After 14 days of exposure, the worms, daphnids, and disks were extracted, and uptake of 14C-erythromycin residues in tissues was determined. Comparison of relative uptake indicated a steady-state bioconcentration factor of 2.2 for L. variegata 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 normalisation to lipid and carbon content. In the pond sediment-containing microcosms, worms exposed to 0.05 and 0.5 mg a.i./l BCFs of 0.4 and 0.9 BCF were observed. The worms showed a BCF of 0.04 for the beaker experiment. Despite the fact that no significant differences between concentrations were observed, an increased exposure time resulted in lower BCF values at the end of the test period. 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 0.05 ppm with a maximal BCF of 8 for 0.05 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), 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 formation of NER, CO₂ and CH₄ is possible.

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 UC-glucose as reference substance in comparison to the fumigation method used for aerotbic transformation studies), and the influence of storage temperature and storage duration on the test manure.
MO 405 Determination of the antiparasitic pharmaceutical toltrazuril and two metabolites in environmental water, soil and animal manure
J. Olsen, M. Hansen, E. Bjorkland, K.A. Krogh
University of Copenhagen, Faculty of Veterinary Sciences, Copenhagen, Denmark
Toltrazuril is a so-called broad-spectrum antibiotic used for the treatment of 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 was presented.

MO 406 Determination of tetracyclines and sulphonamides in swine wastewater
J.M. Lin
Institute of Environmental Health, Taipei, Taiwan
The potential of antibiotics in swine wastewater provides a target of antibiotic occurrence in aquatic environments. 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 (SAs) are the two main groups of antibiotics used in animal husbandry and as feed additives. This study developed a method to determine three TCs and eight SAs in swine wastewater and river water using deuterium-labeled TC and SAs 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 TCs were 39% - 40% 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 ng/L) and the effluent (450 ng/L) from a piggy farm at Tao Yuan, Taiwan. The results show that tetracyclines and sulphonamides were detected in animal wastewater at trace levels. 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 in aquatic environments.

MO 410 Degradation products of Oxytetracycline in anaerobic digestion and its effects on biogas production and microbial structures
G. Ribera, E. Cernè, E. Ercolani, H. Cohen, B. Ince
1Bogazici University, Istanbul, Turkey
2Istanbul Technical University, Istanbul, Turkey
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 OTC/L. Blank and spiked manure slurries were digested in 120 ml serum bottles at 37±1°C for 5 days. During digestion, biogas production, biogas and volatile fatty acid compositions were also monitored. After 30 days, degradation products of OTC were analyzed by 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- and b-apo-OTC which were present at 1-2% of initial parent concentration. According results, life of OTC was less than 5 days. 190±3 kgVS biogas yield was obtained in the experiment. Similar inhibitions on the biogas production, 61% and 200 mg OTC/L, respectively. OTC 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 from Firmicutes by 82% (33% Clostridiales and 49% Bacillales) and Proteobacteria by 18% (46%) and 3% Actinobacteria. Methanosarcinales (46%) and Methanobacteriales (25%) were larger than on Bacillales which showed that similarity changes of bacterial communities were higher than similarity changes of archaeal communities and changes increased with increasing OTC concentration. Similar analyses of clone libraries with environmental parameters were made by Canonical Correspondence Analysis (CCA). Analyses showed that Bacteroides were affected negatively by increasing OTC concentration while some Clostridiales were affected positively. Inhibitory effect of OTC on Methanocorinales was larger than on Methanobacteriales.

MO 411 Flubenzurons: risks and developmental effects on non-target species, a Norwegian perspective
J. Kolasinska1, A. Drobniewska1, B. Adomas1, A. Piotrowicz-Cieslak1, J. Baturczevschi, G. Nalecz-Jawicki2
1Medical University of Warsaw, Warsaw, Poland
2Medical University of Warsaw, Dept of Environmental Health Sciences, Warszaw, Poland
Flubenzurons are CSIs that are mainly used in aquaculture in Norway and are dibenzuron (DIF) (market name Releuce) and teflubenzuron (TEF) (market name Enderon). 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 is an environmental 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.battagliai. A further 7 day developmental acute naupliar (< 3 hr old) single substance and mixture toxicity testing was conducted with TEF and DIF and the copepod T.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.

MO 412 Assessment of tetracyclines toxicity with two Lemma minor assays
J. Kolaisinska1, A. Drobniewska1, B. Adomas1, A. Piotrowicz-Cieslak1, J. Baturczevschi, G. Nalecz-Jawicki2
1Medical University of Warsaw, Warsaw, Poland
2Medical University of Warsaw, Dept of Environmental Health Sciences, Warszaw, Poland
Tetracyclines are used as antibacterial agents in veterinary medicine including aquacultures. The duckweed Lemma 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 semi-field assay were taken using the Ujak sediment core sampler. In the semi-field 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 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 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.

This work was supported by EU-FEDER and national MCTES funds through a PhD grant from the Portuguese Foundation for the Science and Technology (FCT) to Alexandra Martins (SFRH/BDE/65436/2009).

MO 414

Histological changes in fish Astyanax altiparanae exposed to 17-Alpha-Methyltestosterone

F. Knopperts1, F. Kothenbeutel2, L.M. Guimarães1, L. Mendes3, A. S. de Souza4

1Research Center for Eco-Environmental Sciences, Beijing, China
2Helmholtz Zentrum München, Munich, Germany
3University of Salzburg, Salzburg, Austria
4Research Center for Eco-Environmental Sciences, Beijing, China

42nd SETAC Europe Conference - SETAC Europe 22nd Annual Meeting

MO 413

Effects of temperature and florfenicol on Daphnia magna feeding behaviour

Alexandra Martins1,2, C.G. Huber3, A.C. Borges4, L.G. Wendt5, C.K. Grisolia2

1University of Porto, Porto, Portugal
2ICBAS & CIIMAR, University of Porto, Porto, Portugal
3Helmholtz Zentrum München, Munich, Germany
4University of Salzburg, Salzburg, Austria
5University of Salzburg, Salzburg, Austria

MO 415

Use of antibiotics in tilapia cage aquaculture: a case study in the Tha Chin river (Thailand)

A. Rico1, P. Knopperts2, J. Khancarni3, K. Satapornvanit1, P.J. Van den Brink4

1Wageningen University, Wageningen, Netherlands
2Department of Animal Ecology, YU University, Amsterdam, Amsterdam, Netherlands
3Department of Fishery Biology, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand
4WUR, Alterra, Wageningen, Wageningen, Netherlands

The use of antibiotics to prevent and treat bacterial infections 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.

MO 416

Tetracycline and Fluoroquinolone concentrations in the Istanbul Strait sediments

N. Ferreira1, S.C.D.E. Sousa1, A.C. Borges1, C.L.G. Wendt5, C.K. Grisolia2

1ICBAS & CIIMAR, University of Porto, Porto, Portugal
2Helmholtz Zentrum München, Munich, Germany
3University of Salzburg, Salzburg, Austria
4Research Center for Eco-Environmental Sciences, Beijing, China
5University of Salzburg, Salzburg, Austria

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 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, individuals were euthanized to analyze the histological structure of the organs subject to hormone treatment by conventional histology. In the control group showed normal histological structure. The group fed with feed 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 vacuolization and changes in the architecture of the organ. Kidney ducts had enlarged its lumen, the cuboidal epithelium of the tubules vacuolated and the nuclei 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 a 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 gradual 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 shows, 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 417

On-line SPE-LC-MS for ultra trace analysis of antibiotics in river and ground water

A. Rico1, C. G. Huber2, V. Remenyi3, F. Steinert4, C.G. Hube5

1Wageningen University, Wageningen, Netherlands
2Thermo Fisher Scientific, Germering, Germany
3Saarland University, Saarbrücken, Germany
4University of Salzburg, Salzburg, Austria
5University of Salzburg, Salzburg, Austria

Endocrine disrupting compounds (EDCs) represent a broad range of natural or synthetic chemical substances, mostly of anthropogenic origine. 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 coupled to liquid chromatography with high performance liquid chromatography (HPLC) for the determination of selected compounds 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 macrolide type.
MO 422  Climate impacts of transitioning from coal to natural gas fired electricity generation over time
S. Schwietzke, W.M. Griffin, H.S. Matthews
Carnegie Mellon University, Pittsburgh, United States of America

Transitioning from coal to natural gas 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 measure of the physical processes leading from GHG emissions to climate change and climate impacts. The physical processes include the generation of radiative forcing (RF) and the atmospheric lifetimes of different greenhouse gases, which are the timing between a GHG emission and climate impacts.

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 (0.0003-0.0007 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
J.A. de Almeida Neto 1, H.L. Marandá 2, S. Robira 1, Nascimento 1

1, Universidade Estadual de Santa Cruz, Ilhéus, Brazil
2, Federal University of Bahia, Salvador, Brazil

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 Carbon Footprint assessments 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. Compared to the cultivation of land-crops, microalgae offer a better potential for increasing biodiesel productivity and improving the capacity for CO₂ sequestration. This study considered two distinct scenarios in a pilot-scale production of microalgae cultivation for biodiesel production. The first scenario is a typical biodiesel fuel, while the second is biodiesel from microalgae. In this study, microalgae do not show such disadvantages.

Life Cycle Energy and Carbon Footprint assessments allow for 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 potential positive effects on the energy and carbon footprints of biodiesel from microalgae cultivation.
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 feedback 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
Y. Ono, K. Horiguchi, N. Isubo
Tokyo University, Yokohama, Japan

Water footprint (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 process optimization to innovation the organizational level and water management. 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 and water 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
C.P. Penal, V. Carmona

Research centre for mining and metallurgy, Santiago, Chile

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) and grey water use (shower, toilet, laundry and garden process). This will be necessary for the application of water assessment methods in a business context. A number of water assessment 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 429
Closing methodological gaps of water footprint: scope and aggregation issues
S. Plotser1, A. Suh2

1UC Santa Barbara / ETH Zurich, Zurich, Switzerland
2UC Santa Barbara, Santa Barbara, United States of America

While 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 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 investigations 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 laudry product
A. Kounina1, H. Franceschini2, I. Munoz2, S. Humbert1, S. Vionnet1

1Quantis, Ecublens, Switzerland
2Unilever, London, United Kingdom

A large number of impact assessment methods have been proposed for measuring the water quantity 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: understand the strengths and limitations of each method; evaluate their informational requirements and identity gaps in the data available for the life cycle of a detergent product; contrast potential impacts related to water quantity and water use at the midpoint and endpoint level and promote the optimization of water efficiency and water management at the organizational 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 from ecoinvent and the Quantis Water Database. The life cycle stages taken into consideration were chemical supply, transportation, 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
G. van Hood1, A. Kounina2, B. Buyle1, S. Humbert1

1Procter & Gamble, Strombeek-bever, Belgium
2Quantis Switzerland, Lausanne, Switzerland

Outline of the paper: 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. Two sets 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 to work, the need 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
S. Gimeno1, D.J. Versteeg2, T.W. Federle1

1Procter & Gamble, Strombeek bever, Belgium
2Procter & Gamble Environmental Stewardship Organisation, Cincinnati oh 45225, United States of America

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 (e.g. 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 constituent 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 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 435
Moving forward: sustainability and the U.S. cleaning products industry
K.L. Donahue1, R.D. Sedlak1
American Cleaning Institute, Washington, United States of America

Since the Beundlant 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 those 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 member manufactures and their ingredient suppliers are being faced with a multitude of different demands and requests for data and information that could support 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 of tools to assess the industry's achievements. A summary 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
Comparison of the chemical footprint of hydraulic fracturing fluids used in natural gas extraction
K. Hitchcock1, P.K. Scott1, K.M. Unice1, J.M. Panko2
ChemRisk, Pittsburgh, United States of America

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 with fracking fluid has began to grow. Biological, chemical and physical 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 437
Application of ERICA index to evaluate soil ecosystem health according to sustainability threshold for liability impact
E.B. Boriani1, D. Baderna2, N.I. Itsubo3
1Aarhus University, Roskilde, Denmark
2Mario Negri Pharmaceutical Research Institute, Milan, Italy
3Tokyo City University, Yokohama, Japan

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 environmental impact of engineered substances 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 study, an updated version of the improved theoretical index was 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, therefore industries able to show this will get an extra value for their products. Furthermore this will lead to awareness by industries of how much their product is 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 is to develop a new methodology that will help industry and regulatory bodies to evaluate the positive effects resulting from mitigation actions and to promote eco-efficiency.


MO 438

Impacts on carbon pools and biodiversity due to land use and land use changes, exemplified with a case study on Norwegian forestry

O.M. Michelsen

Norwegian University of Science and Technology, Trondheim, Norway

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 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 change qualities 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 a 100-year period, are included. In the case study, the different management practices 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. The results motivate to both select a time horizon and allocate impacts to 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

M. Motoshita1, C. Yang, K. Tahara2, A. Inaba1

1National Institute of Advanced Science and Technology, Tsukuba, Japan
2University of Arkansas, Fayetteville, United States of America

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 LULUC is dominant in the case of several countries (27-70% 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 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)

1, D. Nutter1, R. Ulrich1, K. Tahara1, M. Motoshita1, L. Sokka1, S. Soimakallio1, T. Pajula2, J. Hynynen1, S. Launiainen1

1VTT Technical Research Centre of Finland, Vtt, espoo, Finland
2University of Wisconsin, Madison, United States of America

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 impacts of forestry and forest industries, for the benefit of the forest industry. The 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 relevance.

Land use and biodiversity are gaining more interest but are still not as well understood as factors that influence forest management impacts. In the case study, land use and biodiversity are linked to biodiversity and carbon pools impacts discussed in the ReCiPe presentation. The aim of the EVERGREEN project 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 impacts of forestry and forest industries, for the benefit of the forest industry. The 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 relevance.

Life cycle impact assessment (LCIA) method selection: an LCA practitioner’s perspective

V. Nippurira, S.S. Ghonge

Eaton, Pune, India

Life cycle impact assessment (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 among a LCA study. Moreover, according to these categories, these approaches to derive the characterization factors leading to inconsistency. Selection of an appropriate LCIA method by the LCA practitioner is thus, dependent on four 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.


1University of Wisconsin, Madison, United States of America
2University of Arkansas, Fayetteville, United States of America
3University of Wisconsin, Madison, United States of America
4University of Arkansas, Fayetteville, United States of America
5University of Wisconsin, Madison, United States of America
6National Institute of Advanced Science and Technology, Tsukuba, Japan

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 energy use and 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 kilogram 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 consumed. For an average moisture content of 33.0% 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

J. Burek, G. Thoma, D.S. Kim

University of Arkansas, Fayetteville, United States of America

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 from different LCIA methodologies, internal communication was performed. The selected methodologies for this LCA were USEtox and TRACI 2 and two endpoint ReCiPe and IMPACT 2002+ methodologies. The impact category chosen was ecosystem 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% 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 LCA 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...
MO44

IMPACT World+: a new global regionalized life cycle impact assessment method
S.M. Bullé, O. Jolliet, S. Humber, R. Rosenbaum, M. Marquardt
CIRADE - Departement de Geoclimatologie, Faculté de Commerce et de Génie Chimique de Montréal, Montreal, Canada

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 LUCIA, but they are not consistent specifically for the regionalization 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.

MO445

An open source software for Emergy calculation with life cycle inventory databases
A. Marvuglia, B. Bagani, G. Rius, J. Muller, E. Benetto, L. Tiruta-Barba
Public Research Centre Henri Tudor, Esch-sur-alzette, Luxembourg

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 methodology, which prevents it from being a widespread tool.

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 more accurately assessing relevant CFs, but also to regionally assess any geo-referenced emission, and ultimately to determine the uncertainty related to an uncertain input. This could be associated to a specific region of the world. Characterized by the presence of specific regional 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 a consistent model for 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.

MO446

Complementing ecological footprint with risk analysis for the ecodesign of footwear
M. Herva, A. Alvarez, E. Roca
Universidad de Santiago de Compostela, Santiago de compostela, Spain

The ecological footprint (EF) was created in the early nineties and has been gaining in popularity ever since. As a pioneering footprint, its calculation has expanded and now they are not limited to the national or regional 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 that are potentially hazardous. It requires the compliance with a set of algebraic rules that are completely different than those applied in Life Cycle Assessment (LCA) and its direct implementation through automatisation routines in specific software tools.

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.

RA11P - Guidance documents and guidelines for environmental risk assessment (ERA): needs, developments and progress

MO449

Mollusca reproductive toxicity tests - Development and validation of test guidelines

Astrazeneca, Brixham, United Kingdom

For a problem involving only a 7 by 7 matrix the results were also validated using the software Emsim, a free share Emsim emulator 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.

MO450

Mollusca reproductive toxicity tests - Development and validation of test guidelines

Astrazeneca, Brixham, United Kingdom

The selection of fibres produced under sustainable criteria and the reduction of the material's consumption was recommended, since the area requirements would be minimized and the absence of hazardous compounds would ensure safety conditions during the use stage.

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The selection of fibres produced under sustainable criteria and the reduction of the material's consumption was recommended, since the area requirements would be minimized and the absence of hazardous compounds would ensure safety conditions during the use stage.
All laboratories found a concentration-dependent decrease of fecundity in both species under Cd exposure. Values of NOECs and LOECs were generally homogenous 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 approach 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.

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, A. fulica and L. stagnalis in the brood pouch. Because embryo number in the brood pouch can modulate reproductive responses towards a chemical and even mask effects, we assessed sensitivity variations caused by these factors in reproduction tests with the endoparasitic octoylphenol. To this end P. antipodarum was exposed to 1, 3, 10, 30 and 100 µg/L octoylphenol at each temperature scenario and at different times of the year and the resulting effect concentrations were compared.


Acknowledgement - The authors thank the UBA for funding (UBA projects 370861402 and 371165417)

MO 450
Temperature effects on reproduction in Potamopyrgus antipodarum - optimizing a standard test protocol with molluscs
K. Ruppert, K. Bender, A.J. Sieratowicz, U. Schulte-Oehlmann, J. Oehlmann
Johannes Gutenberg University, Mainz, Germany

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 endocrine disrupting compounds are not available. Results from BPA-induced effects on P. antipodarum vary very depending on exposure concentration, 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 approach 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.

Up to now effect studies with mollusc play a minor role in regulatory risk assessment of substances because OECD guidelines for the testing of chemicals have not considered this taxon. Environmental Protection Agency (EPA) and Food and Rural Affairs of the United Kingdom have supported the preparation of a Detailed Review Paper (DRP) on Molluscs Life-cycle Toxicity Testing proposing inter alia the parthenogenetic mudsnail Potamopyrgus antipodarum as standard test organism. Currently, the project aims to optimize and define test and breeding conditions on the outcome of reproduction tests with the potential antipodarum we breed 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 hatchlings. Data were analyzed using standard statistical approaches, as recommended by OECD for the analysis of toxicity test data. Preliminary results generated over survival, growth, and fecundity were regularly monitored. Results for BPA highlighted issues in the main test endpoint. Tested animals came from a single laboratory culture (i.e. the University of Frankfurt for L. stagnalis and the Mainz University for P. antipodarum). 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 exercise.

MO 451
How breeding conditions influence substance effects - the need for a standard test protocol with molluscs
A.J. Sieratowicz, U. Schulte-Oehlmann, A. Wigler, J. Oehlmann
Johann Wolfgang Goethe-University, Frankfurt, Germany

Molluscs are highly sensitive to endocrine disrupting compounds (EDCs) and therefore are of great interest for environmental hazard and risk assessment. The Organisation for Co-operation and Development (OECD) is thus promoting optimization and validation of mollusc-based toxicity tests. Standardised test methods for assessing effects of reprotoxins, and hence a number of EDCs, are currently under development in various mollusc species, including the great pond snail Lymnaea stagnalis. Based upon this ongoing work, we designed and performed a partial life-cycle toxicity test on L. stagnalis and P. antipodarum to investigate the impact of breeding and test conditions on the outcome of reproduction tests with the potential test organism. To this end, P. antipodarum was exposed to 1, 3, 10, 30 and 100 µg/L octoylphenol at each temperature scenario and at different times of the year and the resulting effect concentrations were compared.

MO 452
A partial life-cycle toxicity test to assess the effects of endocrine disruptors in the great pond snail Lymnaea stagnalis
A.E. Bars1, V. Ducrot1, T. Jager2, L. Lagadic1
1 INRA, Rennes, France
2 Vrije Universiteit, Amsterdam, Nederland

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 endocrine disrupting compounds are not available. Results from BPA-induced effects on P. antipodarum vary very depending on exposure concentration, 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 approach 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 453
Environmental risk assessment of a disinfectant for milk extraction systems: comparison of 2 emission scenario documents and EUSES
H.M. Dosogne1, A. Ghekiere1, F.A.M. Verdonck1, L. Uytterhaegen1
1 ARCHE, Gent, Belgium
2 DeLaval, Ghent, Belgium

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 EUSES 2.0 using the Emission Scenario Director (ESD) and 2) EUSES 2.0 using the Emission Scenario Director (ESD) and 2) EUSES 2.0 using the Emission Scenario Director (ESD). 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% of the emission to sewage. 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 CRAs were all below 1, whereas with the ESD of 2011, the CRA was <1 for one active substance, but >1 for the other substance and a refinement of the calculation was necessary. Final 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 public exposure environmental risks of antifouling paint usage with the prevailing BPD?
P. Ytreberg, L. Johansson, B. Eklund
Stockholm University, Stockholm, Sweden

Since 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 antifouling coatings and is regulated in the European Union (EU) by 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 modelling tool MAMPIC. In this model, default scenarios representing an EU harbor and an EU marina is incorporated in the software and used in the process of predicting the fate of biocides. However, 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 obliged 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 leading

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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 marine i.e., from boat hulls, pressure hosing and from the soil at the boat yard. The results shown here indicate that exposure 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

Development of interim guidance for the inclusion of non-extractable residues (NER) in the risk assessment of chemicals

C. van der Willigen, M. Vazquez-Gonzalez
RIVnode, Brussels, Belgium

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 contamination in the surrounding area. Here small granivorous passerine 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 the kernel) is a toxic method of dehusking for the dehusking bacteria due to its low water solubility and/or volatility. Careful consideration of the phylogeny of the chemicals released 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 ready biodegradation comprise of different tests (OECD 301A-F, OECD 310), which differ mainly in vessel size, the aspect ratio of the test vessels, the oxygen uptake rate. To confirm the assumption, reference tests were conducted over several months and the de-husking process was monitored at different time points of the week. The dependence of the oxygen uptake rate on the assumed parameter was confirmed. 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 456

OECD Guideline 209: investigation of validity criteria

DeU-Noack Laboratorien, Sarstedt, Germany

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(GREEXX)[h], the significance of the validity criterion was examined. It is assumed, that parameter like season, sewage flow and weather conditions can influence the oxygen uptake rate. To confirm the assumption, reference tests were conducted over several months and the de-husking process was monitored at different time points of the week. The dependence of the oxygen uptake rate on the assumed parameter was confirmed. 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

Granivorous birds - exposure reduction by de-husking behaviour

J. Gerlach, J. Schabacker, J. D. Ludwig
RIPCOn GmbH, Hirschberg, Germany

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 contamination in the surrounding area. Here small granivorous passerine species need to be considered 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 the kernel) is a toxic method of dehusking for the dehusking bacteria due to its low water solubility and/or volatility. Careful consideration of the phylogeny of the chemicals released 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 ready biodegradation comprise of different tests (OECD 301A-F, OECD 310), which differ mainly in vessel size, the aspect ratio of the test vessels, the oxygen uptake rate. To confirm the assumption, reference tests were conducted over several months and the de-husking process was monitored at different time points of the week. The dependence of the oxygen uptake rate on the assumed parameter was confirmed. 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 458

ECETOC Guidance on identifying endocrine disrupting effects: specificity for environmental species

A. Weyers1, L. Welter2, J. R. Wheeler2
1 Bayer CropScience, Monheim am rhein, Germany
2BaSSF, SE, Crop Protection - Ecotoxicology, D-67117 limburgerhof, Germany

Synopsis

This was recognized that the concept needed an additional 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 proceed with a risk assessment based on the non-endocrine 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 (≤) effect within one study or organism, this effect may be accounted for in a risk assessment by more sensitive non-endocrine effects. The other toxicological endpoints of safety that are taken into account may cover endocrine specific effects. Any risk assessment should proceed with a risk assessment based on the non-endocrine effect.

MO 460

Concentration-response relationship for teratogenic effect of 17β-oestradiol in eelpout Zoarces viviparus

N. Brande-Larsdson, J. Ebsen Morthorst, B. Korsgaard, P. Bjerregaard
University of Southern Denmark, Odense, Denmark

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 vireaparous 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

MO 454

Ready biodegradability testing for Insoluble and volatile substances

S. Fiebig, L. Geisler, N. Mendl, U. Noack
DeU-Noack Laboratorien, Sarstedt, Germany

Under REACH a PBT/PvP 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 biodegradability provide. Often substances fail the criterion for ready biodegradability or an unequivocal assessment is not possible. In these cases 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 ready biodegradation comprise of different tests (OECD301A-F, OECD310), which differ mainly in vessel size, the aspect ratio of the test vessels, the oxygen uptake rate. To confirm the assumption, reference tests were conducted over several months and the de-husking process was monitored at different time points of the week. The dependence of the oxygen uptake rate on the assumed parameter was confirmed. 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.
to octylphenol and 17β-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 184 ngE2/l. The experiment was terminated after 44-48 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 17β-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.


**MO 461**

Use of field studies in different regulatory frameworks

E.M.W. de Jong

RIVM, Bilthoven, Nederland

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

A.J. Lawrence¹, K.C. Brouse², G.K. Frampton³, F.J. Van den Brink⁴

‘Cambridge Environmental Assessments, Cambridge, United Kingdom

Independent Consultant, Devon, United Kingdom

University of Southampton, Southampton, United Kingdom

WUR, Alterra, Wageningen, Nederland

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 Metropolitan (MS) Competent Authorities, in 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. First, this presentation 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

G.J. Heijerck¹, K. Oorts¹, F.A.M. Verdonck¹, E. Smolders²

ARCHE, Gent, Belgium

K.U. Leuven, Leuven, Belgium

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 IDOTS) and of existing national approaches of risk assessment of fertilisers, soil improvers and related products in other EU member states. The application and relevance of the identified relevant methods was further tested and evaluated 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

W.N. Naito¹, Y.H. Hirai², M.M. Murata³, N.M. Miyasaka¹

NITE, Tsukuba, Japan

National Institute of Technology and Evaluation (NITE), Tokyo, Japan

NITE, Tokyo, Japan

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, mobility, and bioaccumulation. It is also necessary to regulate the use of 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

Intrinsiq Materials Ltd, Farnborough, United Kingdom

Department of Mineralogy, Natural History Museum, London, United Kingdom

1, V.E. Forbes

1, F.A.M. Verdonck

2, M.M. Murata

3, N.M. Miyasaka

1, G. Banta

1, J.W. Kim, B.H. Ryu, H.P. Jeon, J.S. Ra, S.H. Kim, J.W. Hwang

MO 465

Status quo and challenges in the EU scheme of environmental risk assessment for nanomaterials


Korea Institute of Science and Technology Europe, Saarbruecken, Germany

Although high potential benefits of engineered nanomaterials (NMs) for overcoming the technological limitations of conventional materials, the unique properties of NMs raise new environmental challenges in understanding, predicting, and managing potential environmental risks. To establish 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) compounds the use of NMs. In order to address the above, REACH regulates controls the use of NMs, hazard 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?

K. Syberg¹, G. Banta¹, V.E. Forbes², F. Khan³, S.N. Luoma⁴, P. Reip⁵, H. Selck⁶, E. Valsami-Jones⁷

Roskilde University, Roskilde, Denmark

University of Nebraska-Lincoln, Lincoln, United States of America

Department of Mineralogy, Natural History Museum, London, United Kingdom

John Muir Institute of the Environment, University of California at Davis, Davis, United States of America

Intrinsiq Materials Ltd, Farnborough, United Kingdom

School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, United Kingdom
The nanotechnology industry is rapidly growing, and the production and use of engineered nanoparticles (ENPs) 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 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 because 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 ERA schemes 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 several metal ENPs. The empirical approach allows us to evaluate the risk assessment frameworks with very specific criteria and thus gives the analysis another dimension compared to an analysis that would have been based solely on theoretical considerations.

MO 464

PBT-Assessment: the need for an agreed guidance across persistences - assessment of persistence as an example


The need for harmonised regulations is needed to ensure the identification of PBT-substances on a common basis. However, there are currently no regulations, but the accompanying guidance differs and does not always give sufficiently detailed information. Also, data availability and risk assessment processes depend on which substances 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 veterinary medicine or a pharmaceutical. Therefore a consolidated guidance across regulations is needed to ensure the identification of PBT-substances on a common basis.

In this study the German Federal Environment Agency persistency 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 465

Stakeholders' view on ecological risk assessment in the EU: what are we trying to protect and why might we be failing?

A.D. Heron, E. Placzek, P. Pernille, V.E. Forbes

We formulated a series of research questions and collected a number of stakeholder views. The topics were related to the current protection goals (indicated by the log Kow > 5) and their 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 risk assessment regulatory bodies and academic ecotoxicologists and ecologists across different jurisdictions and at different levels of governance. The results of the interviews will be presented. There is a consensus amongst the stakeholders that changes are needed.

MO 466

Environmental assessment regulations for active pharmaceutical ingredients: a Canadian contrast

A.G.M. Ratray, A. Beck, R.G. Stringer

Health Canada, Ottawa, Canada

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 the potential for risk. For the purposes of this framework it was decided that 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 examine 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

A.F. Boivin 1, H.L. Larue 1, N.D. Domange 1, V. Poulsen 1

ANSES, Maisons alfort, France

In the spirit of the “One Health” concept, this poster discusses plant protection products (PPPs) and their potential risks to both the environment and human health. REACH, the Water Framework Directive and the Cosmetics Regulation are used as regulatory frameworks for PPPs and their active substances. Water is the common denominator for environmental risk assessment.

MO 471

At which level is an additional factor for EPM within the risk assessment of the benthic environment justified?

S. Hahn, T. Soyka, J. Regelmann, G. Könnecker, O. Licht, A. Bitsch

Umweltbundesamt, Dessau-roßlau, Germany

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 derived based on the equilibrium partitioning method (EPM), as described in the REACH guidance. The EPM is based on the assumption that the 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. ionic 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, BFD and REACH. From these data, a distribution coefficient, sediment adsorption factor values were derived using the EPM. Hereby we applied 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 under the EPM is 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 related to the log Kow. This log Kow > 5 is equivalent to a Kpsusp of > 1410 L/kg. For highly adsorptive, especially ionic substances, however, a decision based exclusively on the adsorption is questionable.

MO 472

Implications of differences in higher tier studies for deriving threshold concentrations

S. Meli 1, M. Meli 1, A. Palmqvist 1

1, M. Meli

University of Nebraska-Lincoln, Lincoln (ne), United States of America

The current protection goals for substances with potential for risk are derived from studies that 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 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 examine 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.
Within prospective risk assessment of PPPs, uncertainties are associated to a number of extrapolations needed from test-systems towards the ecosystem. These uncertainties are mainly due to the lack of acceptable test facilities for natural communities are advised. The regulatory procedures are standardized for a lower tier risk assessment, but they are not at the higher tier because tools and techniques are diverse and substance-specific information need to be included. This can thus lead to differences in endpoints delivered and data interpretation.

To illustrate the variability in data and reasons for varying 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, non-mitotic). 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

**MO 474**
Information systems on environmental quality standards (EQS) and quality criteria (QC)

**MO 475**
Variability in Environmental Quality Standards - how much is there and what are the causes?

**MO 476**
Aquatic Protection 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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**S. Duquesne, S. Matezki, J. Wogram**
Federal Environment Agency, UBA, Dessau, Germany

**MO 473**
The adoption or adaptation of EU predicted No Effect concentrations for use as Canadian water quality guidelines

D.J. Leverett1, S. Roe2, T. Fletcher3, C. Crane3
1WcA-environment, Faringdon, United Kingdom
2Environment Canada, Gatineau, Canada

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 importance. The Council, through its Technical Working Groups (TWGs), develops nationally consistent environmental standards and guidelines. 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. CWQGs for Specific Pollutants are already in place in several MS. It is therefore timely to assess the degree of variability between CWQGs, the causes of that variability, and to understand whether the new EQS Technical Guidance will help reduce the between-MS variability for new EQSs, or for revisions of existing EQSs.

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 for revisions of existing EQSs.

**MO 474**
Information systems on environmental quality standards (EQS) and quality criteria (QC)

D.S. Schudoma1, C.H. Heij2, P.K. Kase3, J.M. Jansen4
1Federal Environment Agency (UBA), Berlin, Germany
2Swiss Centre for Applied Ecotoxicology, Dübendorf, Switzerland
3Expert Centre for Substances, RIVM, Bilthoven, Netherlands
4Environmental Standards for priority substances in the European Union 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 forth.

For this reason, we present 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, risus van stoffen (http://www.rivm.nl/rws/), INERIS Chemical Substance Portal (http://chimie.ineris.fr/en/index.php), and Swiss Centre for Applied Ecotoxicology, Qualitätskriterien (http://www.oekotoxzentrum.ch/qualitaetskriterien) or the UBA ETOX database (http://webetox.uba.de/webETOX) are used, as well as literature, but there are also a lot of differences in handling and content. WHO OHEO 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 (CBSB) 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 is 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?

P. Whitehouse1, M. Junghans2, S. Von Arbi1, I. Werner1
1Environment Agency, Wallingford, United Kingdom
2Swiss Centre for Applied Ecotoxicology, Duesseldorf, Switzerland

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. National EQSs are necessary for substances not covered by EU Directive.

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 can 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 for revisions of existing EQSs.

Our poster compares EQSs developed independently for the same substances. It describes the variability in EQSs, 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 the 14th edition data, including treatment of ‘outsiders’
- Models used to undertake extrapolation (e.g. deterministic vs probabilistic methods)
- Allowance for uncertainty (size of assessment factors)

We consider also 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 Protection for plant protection products. I. A Dutch proposal addressing the requirements of the Plant Protection Product Regulation and the Water Framework Directive

C.E. Smee1, J.T.C. Brock2, G.H.P. Arts3, T.E.M. ten Hulscher3, F.M.W. de Jong4, R. Lantink5, E.W.M. Roex5, P.J.M. van Vliet6
1RIVM, Bilthoven, Netherlands
2Alterra, Wageningen, Nederland
3Alterra WUR, Wageningen, Nederland
4RWS-Waterdienst, Lelystad, Nederland
5Deltares, Utrecht, Nederland
6Cibi, Wageningen, Nederland

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

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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 framework directive 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 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 47

Aquatic effect assessment for plant protection products B. Case studies for the verification of a new procedure in the Netherlands

A. Coors1, J. Römbke1, T. Moser1, R. Schaller2, A. Deutsch3, C. Blasse4

UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany
ECT Oekotoxikologie GmbH, Floersheim, Germany
University of A Coruna, Coruna, Spain
Konkuk University, Seoul, South-Korea (Rep)

Trophic transfer of gold nanoparticles in a soil food chain

Y. J. An, S. W. Kim

Konkuk University, Seoul, South-Korea (Rep)

DNA barcoding tackles the identity crisis in earthworm populations used in ecotoxicology: The case study of South Africa

P. Voua Otomo, M. S. Maboeta, C. C. Bezuidenhout

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

P. Voua Otomo, M. S. Maboeta, C. C. Bezuidenhout

1, J. Römbke1, T. Moser1, R. Schaller2, A. Deutsch3, C. Blasse4

1, J. Römbke1, T. Moser1, R. Schaller2, A. Deutsch3, C. Blasse4

UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany
ECT Oekotoxikologie GmbH, Floersheim, Germany
University of A Coruna, Coruna, Spain

MOPC1-2

Impact of biosolids on soil fauna over a period of several years


ECONET Oeokotoxikologie GmbH, Floersheim, Germany

MOPC1-3

Microdegradation of organic compounds (natural, xenobiotics, and pesticides) and the formation of soil organic matter or biogenic non-extractable residues

M. Kästner, A. Miltner

UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany

MOPC1-4

Trophic transfer of gold nanoparticles in a soil food chain

Y. J. An, S. W. Kim

Konkuk University, Seoul, South-Korea (Rep)

MOPC1-5

DNA barcoding tackles the identity crisis in earthworm populations used in ecotoxicology: The case study of South Africa

P. Voua Otomo, M. S. Maboeta, C. C. Bezuidenhout
North-West University, Potchefstroom, South Africa
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 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, Microtubificoides dubius and at least two other unidentified species. These findings are discussed with an emphasis on their 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
C. Psio1, S. Jojtel1, D. Makowski1
INRA, Versailles cedex, France
Human land use, through intensification of agricultural practices and particularly soil pollution by chemical inputs, has often resulted in loss of biodiversity. Earthworms represent important soil-dwelling species because of their role in soil fertility, 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 diversity 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-tetrachlorodibenzodioxin > benzo[a]pyrene > benzo[k]fluoranthene > benzo[a]pyrene > ZM 189.154 > indeno[1,2,3-cd]pyrene > benzo[a]pyrene > benzo[k]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 MoA 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 MoA warrants additional studies to determine the effects of the interactions occurring and to assess potential impacts on the health of organisms.

MOPC1-7
Soil threats in the Netherlands
M.J.D. Hack1, J.J.H. Van den Akker2, T. Hoogland3, F. de Vries1
1Alterra, Wageningen, Nederland
2WUR, Alterra, Wageningen, Nederland
3Consors for 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 risk assessment of the possibilities 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 ENVAASSO-project and for other threats we used traditional land evaluation methods. Our research resulted in maps showing potential risk areas in the Netherlands (Hack 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
S. Poláková
Central Institute for Supervising and Testing in Agriculture (ÚKÚZ), Brno, Czech Republic
The quality of agricultural soils in the Czech Republic is monitored by the Central Institute for Supervising and Testing in Agriculture (ÚKÚZ) via three programmes. These are “Monitoring of the agricultural soils”, “Monitoring of inputs into the soil” and an administration of the database “Registry of contaminted 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 station. Protection of food chain and preservation of soil quality is the main reason for monitoring of prals 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 contamination in the Czech Republic.

MOPC2 - Endocrine disrupting chemicals: recent developments

MOPC2-1
Combined effects of estrogen receptor antagonists on in vitro vitellogenesis
K.E. Tollefsen1, K. Hylland2, K. Petersen3
1NIVA, Oslo, Norway
2University of Oslo, Oslo, Norway
3INRA, Versailles cedex, France
Some environmental compounds are known to have anti-estrogenic activity and their modes of action (MoA) are believed to include competitive inhibition of 17β-estradiol (E2) binding to the estrogen receptor (ER) or interference with ER-dependent processes through different pathways including increased metabolism of E2 and receptor cross-talk. 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 diversity 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-tetrachlorodibenzodioxin > benzo[a]pyrene > benzo[k]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 MoA 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 MoA warrants additional studies to determine the effects 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
T. Aldenberg1, M. Nendaz2, A. Wenzel3
1Fraunhofer IME, Schmallenberg, Germany
2University of Bern, Bern, Switzerland
3Analytical Laboratory, Luhnstedt, Germany
Identification, assessment and regulation of substances having an impact on the endocrine system of ecotoxicologically 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: 94/649/EEC ). Especially, since for this group a hazard-based approach is proposed by all of the legislation. 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 compounds 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 the standard test battery used 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 concerns have 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 study aims at summarizing existing data and investigating the suitability of these 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.

MOPC-4 Non-aromatizable androgen exposure to mosquitofish (Gambusia spp): links between molecular, physiological and reproductive system changes
K. Anderson
2, D.S. Barber
2, E.R.I.K. Muller
2, L.O.U.I.S
UCSB, Santa barbara, United States of America
Exponent, Alexandria, va, United States of America
Exponent, Alexandria, Va, United States of America
Exponent, Alexandria, Va, United States of America

MOPC-2 Assessment of Nonylphenol for the environment: do the regulatory conclusions differ if we look at endocrine disruption or ‘classical’ endpoints?
T.V. Jufferzohn, E.K. Kajn
German Federal Environment Agency (UBA), Dessau-rolfzua, Germany

MOPC-1 Non-aromatizable androgen exposure to mosquitofish (Gambusia spp): links between molecular, physiological and reproductive system changes
K. Anderson, Y. Ogino, D.S. Barber, I. Taisen
University of Florida, Gainesville, United States of America

MOPC-3 20
17
841.9
595.3
4x
22nd Annual Meeting

motility <1µg/L sowie Arbacia lixula NOECdev anormalities < 0.937 µg/L

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

for future research towards establishing relationships between biomarkers of androgen exposure and reproductive health.

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);

Modeling mechanisms of nanotoxicity at multiple levels of biological organization through dynamic energy budgets
2, E.D.W.A.R McCauley

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

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

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).

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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,

in the 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 17β-trenbolone (TB) for 3, 7, 14, or 21 days. Body morphometrics were assessed and

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.

issues indicated three genes (some homeobox, 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 Fgfr1 in the anal fin, and correlate changes in both anal fin growth and the expression of the egg yolk precursor protein vitellogenin (vtg). Previous studies indicated three genes (some homeobox, muscle segment homeobox C, and fibroblast growth factor 1) as upregulated in juvenile mosquitofish or swordtail anal fins by androgen treatment.

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compute 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.

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 scope of the endocrine disrupting properties in comparison with the systemic toxicological properties. For the PNECwater derivation in the RAC 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 scope of the endocrine disrupting properties in comparison with the systemic toxicological properties. For the PNECwater derivation in the RAC 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.

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MOPC3-3 Non-target analysis and identification of toxicologically significant emerging pollutants

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 approach.

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 (10-100 µM) of SMX metabolites, and analysed via HPLC-UV and high-resolution mass spectrometry. Results revealed slower direct photolysis kinetics and quantum yields that were 10-100 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 nitro-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

Buchinger, H. Pollmann, M. Klaum, G. Goppelsroder

German Federal Institute of Hydrology, Koblenz, Germany

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 approach.

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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 and improve identification of environmental unknowns. Furthermore, it facilitates the possibility to check instrument comparability and to spend more time on unknowns.


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 for the detection of the target analytes in the ng/g range. After validation based on the ICH/2005 norm, this analytical method 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 fallout. 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

T. Schulte1, E.L. Schreiber2, M. Krauss3, S. Neumann4, C. Hug5, C. Gallamput6, J. Slobodnik7, W. Brack8

1UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany
2Eawag - Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
3Leibniz Institute for Plant Biochemistry - IBP, Halle, Germany
4Institute for Environmental Sciences, Uppsala University, Sweden
5Environmental Institute, Koi, Slovak Republic

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 in LC-MS analysis 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 databases. Existing LC-MS 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, EI-MS, EI-FT-MS and EI-FT-MS. The main objective of our work is the establishment of a community driven mass spectral database using the MassBank MS spectral database. Existing LC-MS databases claimed containing all types of environmental samples 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 and MS spectra to assist in candidate selection. Thus, contributions to a community driven mass spectral database within the MassBank MS spectral database containing all types of environmental samples 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 MS libraries. The database is available at http://massbank.ufz.de/MassBank and at the NORMAN website. References: [1] M. V. Salvia, J. Singer, J. Hellendil, Anal Bioanal Chem 397 (2010) 943; [2] A.C. Hogenboom, J.A. van Leerdam, P. de Vos, J Chrom A 1219 (2009) 510; [3] H. Horai, M. et al., J Mass Spec 45 (2010) 703.

MOPC4-1

Disaccharide content and cross-contamination assessment of biotechnology by-products and/or transformation products in complex environmental samples

S. Jones1, P. Lesage1, B. Buxmann1, C.M. Baylis1

1International Aluminium Institute, London, United Kingdom
2CIRIAE, École Polytechnique de Montréal, Montréal, Canada

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 these 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. Consequently, a project that would be a) well accepted by the LCA community and b) could be integrated into current LCAs to improve the quality of the final results. The project was initiated in 2009 with the aim of improving the quality of the LCI data by increasing the use of the most up-to-date data on aluminium production. The aim of the project was to develop a so-called ecoinvent database for aluminium using the ecoinvent database version 3 as a launch pad. However, the IAI realized that the ecoinvent database was not suitable for their specific needs. The ecoinvent database was designed for gate-to-gate assessment and not for the specific needs of the IAI. Therefore, the project was redesigned and named BioEnergieDat: Life Cycle Inventories and IT infrastructure for Bioenergy in Germany.

BioEnergieDat: Life Cycle Inventories and IT infrastructure for Bioenergy in Germany


1Karlsruhe Institute for Technology (KIT), Eggenstein-leopoldshafen, Germany
2GreenDelta TC GmbH, Berlin, Germany
3Universität Stuttgart, ERE, Stuttgart, Germany
4Deutsches Zentrum für Luft- und Raumfahrt e.V., Stuttgart, Germany
5Wuppertal Institut für Klima, Umwelt, Energie GmbH, Wuppertal, Germany
6Ruhr-Universität Bochum, Lehrstuhl Energie systeme und Energiewirtschaft, Bochum, Germany
7Hochschule Bremen Technische Universität, Bremen, Germany

LCAs 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. A further challenge for the development of such databases is the need of an adequate quality database. This is the case for LCAs on bioenergy, where the need of an adequate quality database has identified a two year's project for a database on energetic use of biomass for Germany has been launched named ‘BioEnergieDat’. Due to its modular approach and the related development of IT, BioEnergieDat implies a broader outreach: it shall support methodological development as to specific 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 methodology 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 that were currently 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 as to other including formats through or related using data transmission data. The combination of training on the ecoinvent 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. -bre type='nul'
MOPC4-3
Improving regional specific life cycle assessment of energy crops in Germany

Johann Heinrich von Thünen-Institut (vTi), Braunschweig, Germany

University of Bonn, Weizsäcker-Triepels, Freising, Germany

University of Rostock, Rostock, Germany

Leibniz-Centre for Agricultural Landscape Research (ZALF), Müncheberg, Germany

Christian-Albrechts-University of Kiel, Kiel, Germany

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 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, and the database of pesticides has been expanded to 90 active ingredients.

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 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.

References

MOPC4-4
Validation of PestLCI 2.0, an updated and expanded model to estimate pesticide emissions for use in LCI
T.J. Dijkman, M. Birkved, M.Z. Hauschild

Technical University of Denmark, Kgs. lynchby, Denmark

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 within 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 soil and different fate modules are used to model the fate of the pesticides after deposition on the crop surface. Determining the compartment specific fate of the pesticide is a new feature of PestLCI 2.0. The model 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, and the effect of tillage on macropore flow has been added. The algorithm sets of other modules have been updated. The applicability of the model is no longer limited to PestLCI 2.0.

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 well-suited to estimate pesticide emissions for use in LCI.

MOPC4-5
SwageLCI 1.0, an inventory model to estimate chemical specific emissions via sewage system
M. Birkved

Technical University of Denmark, Kgs. lynchby, Denmark

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 emission factor. It is 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 quantifying 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 eflluents are nation specific. In order to reflect the national specificity of SsS configurations, statistical data on the ST configurations in all EU countries were compiled from European Union (EU) governments, and the ST configurations and emission factors used in the STPWIN model were extracted from 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 to soil. Emissions from SSs are calculated by 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 receiving the sewage 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 connection.

MOPC4-6
Interpretation phase in life cycle assessment software programs: a case study for Brazilian biodiesel
R.R. Sallaberry, K.R.A. Nunes, L. Sereck

TU Darmstadt, Darmstadt, Germany

Institute for Technology Assessment and Systems Analysis, Karlsruhe, Germany

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 phase have been developed by ISO 14040 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 checks were applied in each program integrated in 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 were 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 information is used as information extraction from the software check, for example, to judge 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 the case 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 programs 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)
L. Maseroni, S. Minestrini, M. Pirillo

ISPRA - The Italian Institute for Environmental Protection and Research, Roma, Italy

Stager - ISPRA, Roma, Italy

ISPRa is involved in research activities to build and to implement a national, on-line and public database for LCA information (ILCA), 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
ISPA 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, ISPA is now planning out a research design aimed at developing 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 I-LCA 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 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 conformity with the reference standard, while other modules should be implemented in order to achieve the same degree of conformity.

The creation of a public database LCA, with quality and consistent data, methods and assessments, 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, ISPA intends to plan out a research design aimed at developing experimental application to a specific transport sector based on primary data already available.

**MOPC5-8**

**Setting up collaborative research on LCA and related topics**


*Solihim, Paris, France
*UREC, Villeurbanne, France
*EDF, Moret sur loing, France
*Bleu Safran, Lyon, France
*EDF, EDF FV, NW, Renault, Saint-Gobain, Total and Veolia, with the support of RECORD and of ADEME, are the co-founders of a new non-profit Corporation, SCORE LCA, created 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 organization (or group of organizations) 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:**

1. **Assessment methods is widely debated, especially for policy making support purposes. In this poster presentation, we intend to discuss several methodological approaches 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 provided.**

2. **Footprint is an approach to convert the environmental impacts of human activities into a physical quantity (area, volume, weight, etc.). In this, the “Footprint Family” would rephrase as 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.**

3. **Footprint is an approach to convert the environmental impacts of human activities into a physical quantity (area, volume, weight, etc.). In this, the “Footprint Family” would rephrase as 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.**

4. **Footprint for I-LCA v.3.0 according the format developed by the European Platform on LCA.**

**Comparison of life cycle-based methods to assess the consumption of Luxembourg**


*EDF, Moret sur loing, France
*Solinnen, Paris, France
*P. C. F. Osset

**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 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.**

**MOPC5-9**

Improving the relevance of carbon footprint calculations: temporal issues and global warming indicators

P. L. Favorito, IRD, Toulouse, France

**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 utilization) 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, RE, ReCiPe and Solar Energy Demand-SED) along with different environmentally extended input-output tables (EEITs) 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 EEITs. Indeed, there is a high uncertainty related to the type of environmental extensions (i.e. physical, economic, and direct and indirect contribution analyses by sector) that 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.**
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 calculations. 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.

MOPC3-5 Economic footprint of the Port of Vigo (NW Spain)
T. Antelo1, C.A.R.L. Lopez2, Á. Franco-Uría1, A. Alonso1
1IIM - CSIC, Vigo, Spain
2Instituto de Investigaciones Marinas - CSIC, Vigo, Spain

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 for nature and according 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 region (NW Spain) is the second largest along the coast of Spain in term of maritime activities. 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 obtained results 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.

MOPC3-6 Ore grade decrease as an indicator to address mineral resource scarcity in life cycle assessment
M.D.M. Vieira1, M. Goedkoop2, P. Storm3, M. Huijbregts2
1MOPC5-2, Berlin, Germany
2MOPC5-3, Netherlands
3Raw Materials Group, Solna, Sweden

Resource depletion is an important topic of 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 required. Ore grade is an inherent property of mineral resources and yet it also expresses the scarcity of the mineral. Life Cycle Assessment (LCA) has not been used as an indicator directly to assess scarcity of ore 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 decrease of ore grade (response of ore grade to the additional amount extracted of this mineral). We calculated for the following ten elements: cobalt (Co), copper (Cu), diamond (D), 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.

MOPC7-6 Development of biodiversity and resource indicators: assessing potential impacts throughout the life cycle of products, processes and services
L. Hamon1, A. de Schryver2, B. Chapper1, E. Assouti1, J.B. Bayart3, M. Bugel4, A. Flesch5, J. Serre6, C. Guignard7, A. Bouret4, S. Humbert4
1Quants, Paris, France
2Volia Environnement, Paris, France
3Veolia Environment - Research and Development, Rueil-malmaison, France
4Veolia Environnement, Research and Innovation, Rueil-malmaison, France

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 sectors 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 paper, a methodological framework has been established to link biodiversity to 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.

Recent studies have been conducted with data extracted within Volia 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.
A Setac pellston workshop on the risks from Pesticides to pollinators: feedback and basis for the future
A.O. Alice, P.D. Delorme1, E. Steeger1, T. Morarvay1, D. Fischer1, M. Goulston1, J. Frazier1, C. Lee Steere1, F. Streissl1, J. Pestic2, J. Pflugfelder3, M. Vaughan4, J. Wul5
1 Dow AgroSciences, Abingdon, United Kingdom
2 US Environmental Protection Agency, Washington, United States of America
3 BayerCropScience, Washington, United States of America
4 Syngenta Ltd, Bracknell, United Kingdom
5 Pennsylvania University, Pennsylvania, United States of America
6 Australian Environment Agency, Barton, Australia
7 ESA, Paris, Italy
8 USDA, Baltimore, United States of America
9 US Environmental Protection Agency, Washington, United States of America
10 Swiss Bee Research Center, Switzerland
11 Xerces Society for Invertebrate Conservation, Portland, United States of America
12 BSAE, Us, United States of America

Dow AgroSciences, Abingdon, United Kingdom

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. 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 the impact of pesticides on pollinators, theoretical approaches (i.e. effectiveness in reducing crop pest populations), regulatory approaches (i.e. minimal residues) or field studies (i.e. monitoring of populations) have been used in order to assess the impact of pesticides on pollinating species.

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The decline in pollinators was reported from several different regions of the world. Pollination is a very important ecosystem service for food production and maintainance 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 analyses 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.

Activities of the OECD expert group on pesticide effects on insect pollinators in the context of the risk assessment for pesticides
A.O. Alice1, A.P. Pease1, P.D. Delorme1, C.H. Hart2, S.F. Pore1
1 Dow AgroSciences, Abingdon, United Kingdom
2 Environmental Protection Agency, Washington, United States of America

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. Lunt2, C.S. Stenies3, D. Autenri
1 EFSA, Parma, Italy
2 RIVM, Bilthoven, Netherlands

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Activities of ICPBR in the context of the risk assessment for pesticides to bees
A.O. Alice1, D. Forrester1, G. Lesw1, E. Steeger1, G. Smaghe1, R. Wallner2, J. Wassenberg3
1 Dow AgroSciences, Abingdon, United Kingdom
2 The Food and Environment Research Agency, York, United Kingdom
3 Julius Kuhn Institute, Braunschweig, Germany
4 Ghent University, Ghent, Belgium
5 University of Hohenheim, Stuttgart, Germany
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Assessing the comparative risk of plant protection products to honey bees, non-target arthropods and non-apis bees

J. Miles, A. Alix
Dow AgroSciences, Abingdon, United Kingdom

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 ectotoxicological risk assessment sentinel species are employed aiming at ensuring a high level of protection. Tier 1 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 toxicological trigger. In other regions e.g. North America the acute toxicity trigger of 11 µg active substance/beee 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 of the bee as a test organism and other non-Apis bees a safety factor of 10 (for interspecies differences) was suggested. On the basis of the results of the OECD test data on the NTA and the data from NTA and NTAs, 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 identifies candidate substances to move forward for further evaluation. The suggest non-Api HQ trigger to 5 gave a similar pass / fail rate to the NTA scheme but was slightly confounded by a lack of determined end points. However, even this taking 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 compare the empirical HQ approach with methods using more specific species exposure estimates.

ET03P - Animal adaptations: methods, endpoints, and testing strategies

TH 001
Carotilage and bone malformations in the head of 6 day old zebrafish embryos after exposure to hydrazides and hydrazinases

R. Streek, N. Demir, T. Braumbeck
University of Heidelberg, Heidelberg, Germany

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 guideline 203). Using the ZFET, not only acute mortalities can be investigated, but also specific sublethal alterations can be easily observed. Hydrazides and hydrazinases 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, benzylhydrazide and phenylhydrazide 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 µg/L, whereas those of benzhydrazide, benzylhydrazide and phenylhydrazide 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 toxicity concentrations, exposure to all test chemicals resulted in notochord breaks, whereas the amount of breaks diminished with the concentration. In addition, cartilage condensations were found. Therefore, the zebrafish embryo test appears to be a sensitive and reliable acute test for the detection of hydrazides and hydrazinases and a powerful tool for high- throughput screening of changes in the gene expression induced by estrogenic chemicals. Using the zebrafish cyp19a1b-GFP in-vivo assay proved to be an excellent screening method with high sensitivity and reproducibility for 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 in-vivo assay proved to be an excellent screening method with high sensitivity and reproducibility for the 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

TH 002
Use of "moribund" stage in the OECD fish acute toxicity test and its effects on suffering and LC50-values

H. Flett
Ecotoxolutions, Basel, Switzerland

In the acute fish toxicity test according to OECD (Organisation for Economic Cooperation and Development) Guidance 203, LC50 is assessed in terms of the concentration of a test substance to 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 exposure). This criterion has been introduced to ectotoxicological test methods 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 new 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 2, maximal by a factor of about 16.

TH 003
Screening of the estrogenic activity of environmental samples using transgenic cypr9a1b-GFP zebrafish embryos

E. Fetter, 1, S. Scholz, 1, M. Krauss, 1, W. Brack, 1, K. Petersen, 1, K.E. Tollefsten, 2, F. Brion, 2, O. Kah, 2
1UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany
2Norwegian Institute for Water Research, Oslo, Norway

InERIS, Verneuil-en-halatte, France

"University of Rennes, Rennes, France

Due to the increasing environmental hazards on reproduction and population development in the aquatic ecosystem by estrogenic substances, their hazard assessment play a paramount role for regulations 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 cypr9a1b-GFP transgenic embryo, which expresses GFP under the control of the zebrafish cypr9a1b 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 cypr9a1b-GFP zebrafish embryo assay. The results were also validated by artificial mixtures of compounds, which were also 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-vivo method. The transgenic cypr9a1b-GFP in-vivo assay proved to be an excellent screening method with high sensitivity and reproducibility for environmental samples.

TH 004
Micronucleus and alkane comet assays in Japanese Medaka larvae. Integrative methods for sensitive detection of genotoxicants in aquatic ecosystems

F. Le Bihan1, A. Pichon1, B. Morin1, S. Berrada2, J. Cachot2
1EPOC, Talence, France
2Atoxigen, Pessac, France

We investigated 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 alkane comet assays are two genotoxicity assays allowing DNA damage analysis at different steps of mutagenesis. Comet assay detects repairable DNA damage as singloundouble DNA strand breaks and alkalilabile sites. Micronucleus assays (MN) detects non-reparable 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 alkane 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, calcium and cadmium chloride. Twelve 2dpf-larvae per replicate were used to prepare cell suspension using enzymatic and mechanical 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 acidine orange. Compared to comet assay, the Micronucleus assay is more sensitive, this may be due to the higher sensitivity of the micronucleus assay. 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 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

M. Gonzalez-Donde1, J.E. Garcia-Mauricio1, L. San-Segundo1, S. Sastre2, C. Fernandez2

1INIA, Madrid, Spain, 2School of Medicine, Complutense University, Madrid, Spain

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 are 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 elutheroumbryos is promising alternatives to current ecotoxicity tests without reducing the validity of the risk assessment. These stages also provide the application in of in vivo methods for the analysis and quantification of biomarkers of estrogenicity/xenoestrogenicity. We propose a battery of non-invasive morphometrical tools to examine physiological and anatomical features in embryos and elutheroumbryos of medaka (Oryzias latipes) so the effects of waterborne exposure to PB (40-400 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 assess whether these effects may result in long term toxicity. This work is being made possible thanks to Spanish Government Grants RTA2010-00004-C02 and CTM2010-17797-C02.

**TH 006**

Evaluation of genotoxicity of sediment samples using zebrafish embryo and comet assay

S. Gartts1, M. Garcia-Kaeuter2, C. Halter1, S.H. Keiter1, H. Hollert1

1Hydronox GmbH, Freiburg i.b., Germany, 2RWTH Aachen University, Aachen, Germany

Genotoxic hazard estimation of sediment-bound anthropogenic pollutants is still a challenge since many genotoxicants tend to adsorb onto particulate matter. Testing of extractions from sediments is time-consuming 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 vivo zebrafish embryo model represents an alternative scenario 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 homogenates (Cosmosb6 2006).

We investigated the genotoxic potential of sediment samples from the Rhine River and outer Vering Canal in Hamburg. 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 exposure different environmental waters.

**TH 007**

Modeling concentration-response relationships of quantitative gene expression in zebrafish embryo and its application to predict mixture effects

P. Kemmer1, N. Kluever2, D. Zittka1, R. Altenburger2, D. Jungmann2, T.U. Berendt2, S. Scholz2

1UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany, 2Technische Universität Dresden, Dresden, Germany

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 of single compounds. Over the last decades several models (concentration addition, independent action) have been tested and predicted mixtures using apical endpoints like lethality or growth inhibition. However, the effects of mixtures at a molecular level are still poorly understood. Using zebrafish embryos, we aim to investigate how gene expression patterns in zebrafish embryos reflect the exposure to mixtures of environmental pollutants. 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 up-regulation of gene expression at concentrations that are considered to be non-toxic. This phenomenon is currently under investigation.

**TH 008**

An evaluation of the zebrafish embryo model for estrogenicity testing of wastewater treatment plant effluents

A. Blaas1, A. Vitecková Wünschová2, I. Sovadinová2, B. Jarošová2, L. Bláha3

1UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany, 2Research Centre for Toxic Compounds in the Environment (RECETOX), Brno, Czech Republic, 3Aquatic Toxicology Laboratory, Institute of Hydrobiology, Slovak Academy of Sciences, Bratislava, Slovakia

Aquatic toxicity testing of chemical effluents is a central component of any wastewater treatment plant monitoring program. In order to properly assess the potential hazard, the process to test the effluents for estrogenic activity needs to be efficient and effective. The Zebrafish Embryo Toxicity (ZFET) assay, an in vitro test with the zebrafish (Danio rerio) embryo, was introduced as an alternative approach to the aquatic toxicity testing of chemical effluents. However, this test does not provide an alternative for the in vivo tests. For this purpose, the test has been coupled with specific estrogen responsive gene expression studies in combination with androgen responsive gene expression studies. These approaches have demonstrated the potential of the test for hazard identification and for prioritization of wastewater treatment plants for further investigations.

In this work 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 009**

The normal development of the fathead minnow

S. Böhler1, R. Strecker1, B. Kais1, T. Braunbeck1

1Hydronox GmbH, Freiburg i. b., Germany

The fathead minnow (Pimephales promelas) 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 toxicity testing. Prior to the use of the fathead minnow as 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 stage was performed in petri dishes containing test waters prepared according to OECD TG 203 Annex 2 at 25 ± 1 ºC. Pictures were taken every 5 minutes between 0 - 1 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 from 3 to 12 hpf. 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

M. Gonzalez-Doncel1, C.U. Polleichtner1, N. Schumacher2, R. Schmidt2, H. Hollert2

1Federal Environmental Agency, Berlin, Germany, 2German Federal Environmental Agency, Berlin, Germany

Sediment contamination in German lakes and rivers is an ongoing problem. A 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.

Aquatic 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 28°C. At the start of exposure and also 1, 6, 24, 30, and 48 hours later the fish were weighed for lethal and sublethal toxicological endpoints. Furthermore, samples of the aqueous medium were taken. The extracts were compounded 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 01.01

Exploring animal alternatives: seeking a replacement for whole effluent toxicity testing in fish

N.H. Gonoa1, M.K. Jeffries1, J.T. Oris2

Miami University, Oxford, United States of America

In the US, the facility discharges effluents directly to surface waters. In order to comply with the effluent guidelines set by the USEPA regulations, the fish embryo toxicity test (FET) is the only method that allows for the testing of effluents with lethal and sublethal toxicological endpoints. Furthermore, samples of the aqueous medium were taken. The extracts were compounded 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 01.02

Final results of the OECD validation study on the transferability, intra- and inter-laboratory reproducibility of the Zebrafish Embryo Toxicity Test

M. Halder1, S. Belanger2, T. Braunbeck3, F. Busquet4, G. Carr5, A. Lillipp6, J. Rawlings7, R. Strecker8, S. Walter-Rohde9, A. Gourmelon1

1European Commission, Ispra, Italy
2Procter & Gamble, Cincinnati, United States of America
3University of Heidelberg, Heidelberg, Germany
4European Commission, Joint Research Centre, Ispra, Italy
5NIVA, Norway
6UBA, Oldenburg, Germany
7OECD, Paris, France

The OECD Acute Fish Toxicity Test Guideline (TG 203) is an integral component in the environmental safety assessment of industrial chemicals, agrochemicals, pharmaceuticals, and other hydrophobic substances. One of the most promising 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 TDG on Fish Embryo Toxicity Test in 2006. The validation study (TG 203) will set the stage for interlaboratory reproducibility of the Zebrafish FET (ZFET).

TH 01.03

Genotoxic effects of sediment extracts and native samples from Hamburg harbor and the Rhine River in Danio rerio embryos

B. Peddinghaus1, S. Steffen2, L. Lohel3, J. Bräuning4, H. Hollert5, S.H. Keiter1

1Institute for environmental research, Aachen, Germany
2Institute for environmental research-RWTH Aachen, Aachen, Germany
3The European Water Framework Directive (EFWD) aims to achieve a good ecological and chemical status in the surface water of European rivers. However, since particular matters are relevant for the ecological status and are not relevant for environmental samples, applied sediment toxicology plays an important role in the successful implementation of the goals of the EWFD. 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, the biological significance of the comet assay results in not yet clear yet further correlations with biotests with definite endpoints such as the micronucleus assay are recommended. Within this study both biomarkers will be compared, in order to gain a reliable genotoxic assessment of environmental samples.

TH 01.04

The threshold approach for acute fish toxicity testing of formulated plant protection products: a proposed framework

6C. Port1, J.R. White2

1NC3Rs, London, United Kingdom
2Syngenta, Bracknell, United Kingdom
3The 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, mortality is observed at full concentration-response test is triggered. To assess applicability to plant protection formulated products, Syngenta conducted 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 from other acute exposure endpoints, 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 01.05

Different test conditions for hydrophobic and fugitive substances in the Fish Embryo Toxicity Test for a reliable risk assessment

A.C. Port, S. Peddinhaus, H. Hollert, S.H. Keiter

Institute for Environmental Research, RWTH Aachen, Aachen, Germany

Background: There is an ongoing discussion for the European FLHM-LGS (FET) test conditions for lethal and sublethal toxicological endpoints. Furthermore, samples of the aqueous medium were taken. The extracts were compounded 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.
The transgenic line Tgfl-1:EGFP/y1 expresses enhanced GFP in the entire vasculature under the control of the flil promoter, and thus enables the visualisation of vascular defects in live zebrafish. We assess the detection capability of the Zebrafish embryo test (zFET). The assay is designed as a means to replace fish embryos with Zebrafish larvae and adult fish in high-throughput studies for aquatic risk assessment. The sensitivity of embryos, larvae and adult fish to toxicants was compared and discussed in detail during this study.

Conclusion: Only some significant differences, but mostly just tendencies between the different test conditions were recorded. Thus, no precise recommendation can be given for the selection of different test vessels for selected volatile and adsorptive heterocycles. However, the present study highlights the benefits of using fluorescent marker applications on zebrafish for studying chemical toxicity on vascular, myotomal and neuronal development, thereby improving the zFET.

The embryotoxicity of three selected substances (benzofuran, 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 showed that exposure to benzofuran in plastic vessels had 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 static than under semi-static conditions, which was not expected. Quinoline showed in glass vessels a slightly higher toxicity under static than under semi-static conditions in tests conducted in plastic vessels. In contrast to benzofuran, quinoline showed minor differences in toxicity with increasing exposure time. Differences in toxicity between quinoline and benzofuran are probably based on different vapour pressure as well as Henry’s law constant. Chlorpyrifos showed only a low mortality (< 30 %) under all test conditions.

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 in aquacultures. Accordingly, this work aimed to assess the sensitivities of different life stages. Moreover, differences between males and females responses should be taken into account, since different physiological mechanisms of response may be observed in aquatic organisms affected by MT effluents. Finally, the apparent higher effectiveness of MT should be carefully assessed so that proper legislation can be established for MT use in aquacultures.
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 (Aphanopus 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 same family as the zebrafish. In terms of sensitivity 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 sensitivities to 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 ecotoxicology 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 vitro toxicity experiments using an equilibrium partitioning model
J.M. Armitage, J.A. Arnot, F. Wania
University of Toronto at Scarborough, Toronto, Canada
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 common for the in vitro data will play a prominent role in the prediction of the in vivo toxicity. 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 human beings is quite rare as it requires that cellular cultures 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 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 multwell plate-based assays: requirements, possibilities and challenges
K. Tanneberger1, L. Blaha1, E.J.M. Hermens2, S. Schier3, J. L. M. Hermens3, K. Schirmer3, E. Kawag
Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
Institute for Risk Assessment Sciences, University of Utrecht, Utrecht, Nederland
UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany
Eawag - Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland
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, enabling 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, favors loss of test chemicals due to sorption and/or evaporation, leading to a reduced bioavailability of test chemicals. In the near future, critical concentrations in culture wells will rarely be done. Although, e.g. the OECD draft guideline for the fish embryo toxicity test requires that check stable chemical concentrations are maintained during the whole exposure duration. Therefore, we set out to design protocols for sample preparation, sample processing and chemical analysis applicable to the small volumes present in multi-well plates for cell line- and embryo-based toxicity testing. The main challenges were how to prepare the aqueous stock solutions 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 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 023
Characterizing the bioavailability, toxicity and metabolites of benzo[a]pyrene using animal alternatives bioassay
M.T. Hulman1, K. Schirmer1, J. Allan1, B.O. Rosseland2, K.E. Tøllefsen2
NIVA, Oslo, Norway
EAWAG, Dübendorf, Switzerland
UMR, Ås, Norway
In vitro assays is targeted to provide alternatives to animal testing by implementing the 3Rs (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 as test systems and are based on the bioaccumulation properties of chemicals due to retaining innate cellular properties such as 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. Thus, it is extremely important to consider the impact of cell culture conditions on the reporter gene expression and the exposure media requirements, in order to assess accurately the toxicity of test chemicals. In the beginning and at the end of exposure time, 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 assay results versus acute fish toxicity.

TH 024
Cytoxic, mutagenic and genotoxic effects of sediments from Czech river basin
M. Benešek1, K. Hilscherová1, L. Blaha2, H. Holletter1
Research Centre for Toxic Compounds in the Environment (RECETOX), Brno, Czech Republic
Institute for Environmental Research (Biology V), RWTH Aachen, Aachen, Germany
This study summarizes various effects of 14 sediments collected from rivers in Southern Bohemia (Czech Republic). After Soxhlet extraction, chemical analyses of sediment extracts were performed. Sediments were spiked with 14C-labelled 14C-labelled BaP was used to determine the partition coefficients and metabolic activation (bioactivation) which today represent challenges to

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because the currently used culture medium is based on mammalian serum which might inhibit piscine cell differentiation.

Exposure of ZF-L cells to 0.5 µM 5'-Azacitidine lead to hypomethylation of genes with both low and high basal methylation, indicating similarity to mechanism in mammals.

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.

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.

As BERc assay are appropriate methods to measure DNA repair in fish cell lines, the BERc assay is 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.

The present study investigates CYP1A response in Poeciliopsis lucipinosa liver 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 from two sites (A, B) close to an industrial effluent. Samplese were extracted and purified. 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 treated extracts (1.10 mg/ml for P and 0.5, 3 mg/ml for NP); EROD activities were measured in cells exposed for 24 h to two dilution of treated extracts (1.10 mg/ml for P and 0.5, 3 mg/ml for NP); TCDD (10-11 mg/ml) 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 (maximum of 15-folds respect to control at 50pM for sample A and B 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 trend of EROD induction has been observed for 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) with EC50 value of 120 pM. Overall results confirm that 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.

Use of AhR superinducers for potential improvement in the micro-EROAD assay

Evaluation of epigenetic DNA methylation in a zebrafish (Danio rerio) cell line model

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 in mammalian toxicity studies, to date it has not been considered for environmental applications in model aquatic species. Currently, whole organisms (fish) are the 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, monoculture 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 a suitable system in vitro screening tool for use in environmental toxicological monitoring.

In vitro kinetics of chloropromazine in cytotoxicity assays with Caco-2, Balb/c 3T3 and HepG2 cells

In 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 in mammalian toxicity studies, to date it has not been considered for environmental applications in model aquatic species. Currently, whole organisms (fish) are the 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, monoculture 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 a suitable system in vitro screening tool for use in environmental toxicological monitoring.

In vitro kinetics of chloropromazine in cytotoxicity assays with Caco-2, Balb/c 3T3 and HepG2 cells

In 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
Luminescence bacteria bioassay is a rapid, inexpensive (in contrast of the test on living animals), reliable method to identify toxic. Toxicity analysis of the pectin samples were 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 (2) F. Nuria, et al. 2005. Sep Sci Technol. 40:1013

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.

The on-line water toxicity monitoring system based on the luminescent bacteria 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 water toxicity monitoring 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 sample. 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 protocol was verified to be performed 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 (DWT) 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 DWT, and the discharge of treated wastewater to receiving water when water is 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.

Estimation of pectins to be used as sorbents towards bacterial biosensor


Since the early days of the development of this technique, 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.

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-robin has been the study of the interoperability and 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-robin 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.

Estimation of pectins to be used as sorbents towards bacterial biosensor


Experimental protocol was verified to be performed 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 (DWT) and also the effluent from a wastewater treatment plant.

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 exchange reactions between the solution and the algal cell surface. Pesticides and their metabolites can influence the metal speciation in solution as well. The speciation of metal ions in solution can vary with pH, redox potential and the presence of competitive metal complexes.

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In this work we present the results on the performance and reliability of an on-line, continuous monitoring 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 water toxicity monitoring 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 sample. 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 protocol was verified to be performed 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 (DWT) 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 DWT, and the discharge of treated wastewater to receiving water when water is 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.

Experimental protocol was verified to be performed 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 (DWT) and also the effluent from a wastewater treatment plant.

Acknowledgements. The authors thank the ISTC KR-1880 and FTP MES RF-GK 02.740.11.0693 for financial support.


A resin buffered nutrient solution for controlling metal speciation in the algal bottle assay

E. Sakut’, A. Vyshevhen’I’, R. Mezck

The metal speciation in solution is uncontrolled 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 this issue. The 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 exchangeable chloride (1:1 chloride:Cd+2) over the synthetic ligand solution (1:2 chloride:Cd+2) and another of dissolved Cd. The synthetic ligand treatments were prepared in the presence of Chelex®-100 (Bio-rad, small form, 0.62 mmol (g wet weight)-1) to obtain equal free Cd+2 activity (pCd=8.2) in solution. The nutrient solutions and their 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 72 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 CdZ 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 in the NaNO3 treatment (p<0.001) of the complexes in the traditional algal assay in 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
L.M. Monteiro1, M.B. Brenek2, G.A.P. Dos Santos1, W. Traunspurger3, T.M. Moens4
1Bielefeld & Gent University, Gent, Belgium
2University of Bielefeld, Department of Animal Ecology, Bielefeld, Germany
3Federal University of Viçosa, Centre of Geomicrobiology, Viçosa, Brazil
4University of Bielefeld, Dept of Animal Ecology, Bielefeld, Germany

Growth, reproduction and behavior of the free-living nematode Caenorhabditis elegans were investigated using the traditional algal, liquid and soil nematode assay. In the traditional assay, nematodes were allowed to grow and reproduce on the test material and development was assessed using the integrated fertility index (IFI) at 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 highest test results on NaCl followed by Pb, Zn and Cu. 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 homogenously 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 attractants 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
M. Ribó1, J.A. Donnemeth2, S. Latres3, M.C.A.R.M. Riva4
1Taxonomic Group, CSIC, Valencia, Spain
2Waste Agency of Catalonia, Barcelona, Spain
3Technological University of Catalonia (UPC), Terrasa, Spain
4University of Bielefeld, Dept of Animal Ecology, Bielefeld, Germany

Soil screening using springtail avoidance tests has been proposed as an alternative way to understand the biological effects of toxicants. The aim of this study is: (a) to assess sub lethal (0.01-1mg/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. Currently, 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 soil quality. 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 contaminated soils 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 sample.

TH 038
Behaviour response of the nematode, Caenorhabditis elegans after the treatments Benzene
J. Choi1, S.J. Lee1, H. Kim1, T.S. Choi2, H. Jeong1
1University of Seoul, Seoul, South-Korea (Rep)
2Pusan National University, Busan, South-Korea (Rep)

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 toxic effects parameters such as reproduction. However due to difficulty catalonia (UPC), it has not been widely used. In this study, response behaviour of Caenorhabditis elegans was observed after benzene treated at 0.5 ppm under air exposure conditions in wild-type and trioress 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 contaminated in 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 soil quality. 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. In this communication we present the results of the cobblemols avoidance test: the test organisms after exposure of soil environmental quality in areas such as contaminated sites, remediation processes, application of waste to agricultural soils, sediments, monitoring studies.

TH 039
Differences in the number of micronucleated red blood cells and other nuclear abnormalities in Ciconiiformes: potential bioindicators of genotoxicity in birds
C.S.A. Santos1, M.S. Monteiro2, A.M.V.M. Soares2, S. Loureiro2
1Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal
2University of Aveiro & CESAM, Aveiro, Portugal

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) assess the usefulness of the micronucleus test in Ciconiiformes and (ii) monitor the occurrence of genotoxic effects in Portuguese populations of Ciconiiformes, the frequency of micronuclei (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 that the levels of MN and other nuclear abnormalities, and the sum of frequencies of all other abnormalities were generally higher on the samples from 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 waste to agricultural soils, sediments, monitoring studies.

TH 040
Impact of thiol reactivity on the excess toxicity of β-Unsaturated Ketones, Esters, and Aldehydes
A. Bichne1, D. Thoens2, A. Paschke3, G. Schüürmann4
1Technische Universität München, Centre of Environmental Sciences, Munich, Germany
2University of Bielefeld, Dept of Animal Ecology, Bielefeld, Germany
3University of Bielefeld, Department of Animal Ecology, Bielefeld, Germany
4Gent University, Department of Biology, Marine Biology Section, Gent, Belgium

Toxicity tests, using single species biosassays, are a promising way to understand the biological effects of toxicants. The aims of this study are: (a) to assess sublethal (0.01-1mg/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 highest test results on NaCl followed by Pb, Zn and Cu. 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 homogenously 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 attractants 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 041
Excess toxicity of organic electrophiles in the Tetrahymena pyriformis biosay informs about reactive modes of action
A. Laqua1, A. Paschke3, G. Schüürmann4
1Universität Heidelberg, Centre for Environmental Research, Heidelberg, Germany

In aquatic toxicology, the minimum (narcosis-level) toxicity of an organic compound can be well estimated applying hydrophobicity-based quantitative-structure-activity relationships (QSAR). Using this approach, electrophiles in water are known to show a membrane affinity. By contrast, electro-positive compounds may undergo membrane 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 factor T) as compared to toxic-hydrophobic narcotics. For β-unaturated carbonyls as one class of electrophiles, Michael-type addition forms a prominent route to cause excess toxicity toward aquatic organisms. Here, the kinetic glutathione (GSH) complexation allows one to quantify chemical toxicity in terms of the second-order rate constants kN5 and kN6 and this 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 chemosensory is employed for assessing the electrophilic reactivity in terms of kC5 of β-unaturated ketones, esters, and aldehydes. Comparison with toxicity data in terms of kN5 leads to excess toxicity inhibition of Tetrahymena pyriformis. The results suggest that the predictive identification of excess-toxic compounds. Moreover, regression models for predicting both log EC50 and log T were derived, using log kN5 and log kN6 as descriptors. Financial support was provided by the European Commission through the project OSPRIS (Optimized Strategies for Risk Assessment of Industrial Chemicals through Integration of Non-Test and Test Information, EU Contract No. GOCE-CT-2007-057017), which is gratefully acknowledged.
In aquatic toxicity, the well-accepted narcosis paradigm states that every chemical substance exerts a minimum toxicity proportional to its hydrophobicity. Accordingly, 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 for assessing the response time, technical difficulty, cost, reproducibility and number of citations in the literature were defined. The total score was calculated by the sum of 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 chemical’s ability to activate so-called ‘toxicity pathways.’ In evaluating acceptability of OSRI, EPA rejected ToxCast results claiming that the reliability, responsiveness and 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 developed, exploiting different types of non-animal data such as in vitro, in chemico and in silico information.

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. 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 establishment of data requirements under the EU regulation 1107/2009, reptiles come into question regarding risk assessments of plant protection products (PPP).
TH 051 Relevant lizard species for the risk assessment of plant protection products in Southern Europe

N. Lutzmann, C. Dietzen, J. Gerlach, O. Körner, G. Vogel
RIFCON GmbH, Hirschberg, Germany

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 7 July 2010) some toxicity data are available in the open literature. These data are intended to 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/689/2009 rev 1) was conducted. The study focused on the habitat use of 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?

N. Lutzmann1, G. Vogel1, W. Böhme2
1 RIFCON GmbH, Hirschberg, Germany
2 Zoological Research Museum J. Köngig, Bonn, Germany

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). No specific background for such an addressing are reliable data on the exposure of PPP. For reptiles genetic 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 developed areas and are candidates as focal species for risk assessments for PPP in fruit orchards and olive groves 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 Perezi 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 Toxicity of eucalypt and pine leaf leachates in zebrafish (Danio rerio) and iberian frog (Pelophylax perezi) early-life stages

N. Lutzmann, C. Dietzen, J. Gerlach, O. Körner, G. Vogel
RIFCON GmbH, Hirschberg, Germany

Toxicity of eucalypt and pine leaf leachates that may be particularly damaging in intermittent streams, was evaluated using embryo toxicity testing based on the ASTM guideline on Frog Embryo Teratogenesis Assay-Xenopus (FETAX). The endpoints monitored included mortality and sub-lethal parameters in 24 h. In general, the different leachates presented similar toxicity (LC50-96 h = 15,97 and 16,66 g L-1 of eucalypt and pine leaves, respectively) . The 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 12 g L-1 of Eucalypt and 1.6 to 32 g L-1 of Pine in both species. Simultaneously, the water physico-chemical parameters and pH, were measured. 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 054 Plant protection products and juvenile amphibians: no matter of concern or unexpected risk?

T.W. Schmidt1, A. Alsheer1, S. Hoeger1, S. Pieper1, C. Bruehl2
1 Harlan Laboratories Ltd., Ittigen, Switzerland
2 Federal Environment Agency, Dessau, Germany

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 frogs 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

K.A. Siddiqua1, S.P. Wilson1, R. Alquezar2
1 CUUniversity, Gladstone, Australia
2 Queensland University of Technology, Brisbane, Australia

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 amongst 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 developmental alterations registered in both species will be presented and discussed considering the type of leaf leachate tested and their respective water physico-chemical parameters.

TH 056 Toxicity of eucalypt and pine leaf leachates in zebrafish (Danio rerio) and iberian frog (Pelophylax perezi) early-life stages

J. Medeiros1, K. Monaghan2, A.M.V.M. Soares3, M.S. Monteiro3
1 CESAM, Universidade Aveiro, Aveiro, Portugal
2 CESAM, Dep Biologia, Universidade de Aveiro, Aveiro, Portugal
3 CESAM, Dep Biologia, Universidade de Aveiro, Aveiro, Portugal

Toxicity of eucalypt and pine leaf leachates in zebrafish (Danio rerio) and iberian frog (Pelophylax perezi) early-life stages

J. Medeiros, K. Monaghan, A.M.V.M. Soares, M.S. Monteiro
1 CESAM, University of Aveiro, Aveiro, Portugal
2 CESAM, Department of Biology, university of Aveiro, Aveiro, Portugal
3 CESAM, Department of Biology, university of Aveiro, Aveiro, Portugal

Abstract

Eucalypt and pine leaf leachates are used in agricultural practices due to their environmental benefits. However, the toxicity of these leachates to aquatic organisms is not well understood. In this study, the toxicity of eucalypt and pine leaf leachates was assessed using zebrafish (Danio rerio) and iberian frog (Pelophylax perezi) early-life stages. The lethal concentrations (LC50) for eucalypt and pine leaf leachates were obtained from 96-hour tests. The LC50 values for eucalypt and pine leaf leachates were 3.32 and 12.25 g L-1 of eucalypt and pine leaf leachates, respectively. These findings are important for assessing the potential ecological impacts of eucalypt and pine leaf leachates on aquatic ecosystems.
This work has been funded by CSD2006-00044 -CONSOLIDER- and RTA2010-00004-C02-01 projects. 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.

Peckford, B., Sarrozo, S.

Brunel University, University, United Kingdom.

Assessment of potential toxicity of wastewater treatment plants (WWTPs) that release effluents with classical endpoints, such as mortality, malformation rates and growth, as well as evaluating the expression levels of several genes as 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, 1:25 and 1:50) for 96 hours. At the end of the assay, mortality and malformation rates were recorded and larval length was measured. Embryos for generic analyses 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 semi-quantitative RT-PCR.

To value the sensitivity of this gene expression approach, the transcriptomic results were analyzed and compared with morphological abnormalities rate and length embryonic data.

This work has been funded by CID2006-00044 - CONSOLIDER- and RTA2010-00004-C02-01 projects. LSS was supported by an FPI-INIA fellowship.

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 effects of metals on enzymatic activities were observed in all populations. 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.

TH 059

Effects of larval estrogen exposure on adult reproductive potential in the amphibian model, Silurana tropicalis.

B. Pickford, S. Larrozo

University of Porto: CIIMAR, Porto, Portugal

Since amphibian age is 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 endogenous 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 abnormalities can be correlated with apical endpoints, such as altered hormone levels. Using 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 ethynyl estradiol (EE2; 10, 50, 100ng, 300, 3000 ng l-1), on metamorphic and adult reproductive endpoints. Exposure of larvae to EE2 from stage 51/2 until stage 66 (completion of metamorphosis) decreased the sex ratio toward females in the exposures (2pgs, though at completion of metamorphosis there were a significant number of under differentiated 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 observed in female frogs that had been exposed to EE2 at concentrations > 30 ng l-1. Regressed testicular oocytes from adult males from all treatment groups (including control) and incidence appeared to be not EE2 related. 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 activity suggests that bullfrog tadpoles can develop adaptations to the pro-oxidant challenges provoked by environmental contaminants signaling increased H2O2 and LPO levels.

TH 060

Exposure to cadmium: evaluation of the effects on the larval epidermis of Lithobates catesbeianus.


Universidade Federal de Säo Carlos, Sorocaba, Brazil

Since amphibian epidermis directly interacts with the environmental constituents (water, air and soil), we investigated the acute toxicity of the cadmium (Cd, lppb-48h) on the skin of bullfrog tadpoles (25 Gosner stage, Gosner, 1960). Lithobates catesbeianus. Enzyme activities (CAT, catalase and GSH, 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, lppb-48h (n=8). After exposure, tadpoles were sacrificed and the skin was removed to assays. LPO (7.3±1.2 ng/mlmg), MT (100,130 μmol/Hmg protein) and GSH (0,08 mM) concentrations and CAT (26.4±0.27 U/mg protein) and GST (244.5±19.22 U/mg protein) to evaluate the stress response to cadmium exposure to metal that directly or indirectly, generates reactive oxygen species (ROS). In contrast, an antioxidant system deficiency will induce a stress response, making biological species more susceptible to toxic agents. GST and MT, which represents the bulk of non-protein thiols, are synthesized in the liver and then exported to other tissues functioning in the detoxification of heavy metals. 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 levels.

TH 061

Intersexed assessment of the effects posed by historical exposure to chemical contamination in natural populations of the Perez's frog, Pelophylix perezi (Seoane, 1885) in the Pontevedra (Galicia) estuary,


Departamento de Biologia, Universidade de Vigo, Pontevedra, Spain

Evaluation of reproductive fitness in the adult phase remain unclear. Assessing impacts of gonadal abnormalities, such as testicular oocytes, requires models in which incidence of these abnormalities can be correlated with apical endpoints, such as altered hormone levels. Using 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 ethynyl estradiol (EE2; 10, 50, 100ng, 300, 3000 ng l-1), on metamorphic and adult reproductive endpoints. Exposure of larvae to EE2 from stage 51/2 until stage 66 (completion of metamorphosis) decreased the sex ratio toward females in the exposures (2pgs, though at completion of metamorphosis there were a significant number of under differentiated 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 observed in female frogs that had been exposed to EE2 at concentrations > 30 ng l-1. Regressed testicular oocytes from adult males from all treatment groups (including control) and incidence appeared to be not EE2 related. 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 levels.

TH 062

Development of new non-destructive biomarkers in the helleborine serpent: first evidence of induction of CYP1A in skin biopsy slices treated with PAHs, OCs and PBDEs.

M. Giannetti, S. Casini, L. Marsili, S. Maltese, T. Campani, L. Carletti, M.C. Fossi

University of Siena, Siena, Italy

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,
TH 063
Effects of lead shot pellets contamination on oxidative stress biomarkers and plasma biochemistry in turtles
M. Martinez-Haro1, A. J. Green2, R. Mateo3
1IMAR, Compostela Port, Pontevedra, Spain
2Department of Weather Ecology, Instituto Biologico de Doña (CSIC), Sevilla, Spain
3Instituto de Investigación en Recursos Cientificos, IREC (CSIC-UCLM-JCCM), Ciudad real, Spain
Lead shot pellets are a major threat to many wildlife species, and C. caretta is an invasive species 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 developed and tested in standard skin (biopsies) and sediments from C. caretta. The 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 is the first of its kind in the literature, demonstrated the presence and inducibility of CYP1A in skin biopsy slices of C. caretta, and validated this non-destructive biomarker for application in field studies. Future results from small scale experiments (laboratory experiments) may be validated by conducting studies at large spatial and temporal scales with a higher ecological relevance.

TH 064
Transition metals contamination on marine turtle eggshell from Thailand
1, Areekijseree, 2, Chuen-Iim, 3, Sribuddhachart, 4, Thepsithar
1Silpakorn University, Nakorn pathom, Thailand
2Department of Biology Faculty of Science, Silpakorn University, Nakorn Pathom 730, Nakorn pathom, Thailand
3Department of Biology Faculty of Science, Silpakorn University, Nakorn Pathom, Thailand
4Department of Biology Faculty of Science, Silpakorn University, Nakorn Pathom, Thailand
Elemental analysis of eggshells in 3 marine turtle samples (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). 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 eggshell was present. Trituration metals and other metals; including lead (Pb), palladium (Pd), and molybdenum (Mo), were detected in all 3 marine turtle types. Moreover, lead and molybdenum were positively associated with PbB (F1,53=1.538, p<0.05). Our results evidenced that lead in turtle eggshell contaminated wetlands showed sublethal effects. Nonetheless, further research is needed to elucidate Pb effects on individual and population health and dynamics.

TH 065
Analytical microstructure on eggshells of green turtle (Chelonia mydas), hawksbill turtle (Eretmochelys imbricata), and leatherback turtle (Dermochelys coriacea)

Areekijseree, Chuen-Iim, Sribuddhachart, Thepsithar
Silpakorn University, Nakorn pathom, Thailand
Department of Biology Faculty of Science, Silpakorn University, Nakorn Pathom 730, Nakorn pathom, Thailand
Department of Biology Faculty of Science, Silpakorn University, Nakorn Pathom, Thailand
Department of Biology Faculty of Science, Silpakorn University, Nakorn Pathom, Thailand

Analytical microstructure on eggshells of 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). 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 eggshell was present. Trituration metals and other metals; including lead (Pb), palladium (Pd), and molybdenum (Mo), were detected in all 3 marine turtle types. Moreover, lead and molybdenum were positively associated with PbB (F1,53=1.538, p<0.05). Our results evidenced that lead in turtle eggshell contaminated wetlands showed sublethal effects. Nonetheless, further research is needed to elucidate Pb effects on individual and population health and dynamics.

TH 066
Will global warming have an influence on the adverse effects caused by pesticides? A first approach using amphibians as a model
E.G.M. Garcia-Muñoz1, L. Lopez2, M.A.C. Carretero3, A.M.V.M. Soares1
1CESAM, Aveiro, Portugal
2CIBIO, Vairao, Portugal
3Universidade de Aveiro & CESAM, Aveiro, Portugal

Over the last years the change in climate 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 indirectly and directly responsible for amphibian's decline. Although amphibians are considered less sensitive to shifts than other groups, small changes can trigger cascade effects when thermal disturbance is combined with pollutants. Among these indirect consequences, temperature changes can alter the acid雨 (pH) in systems. 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 (biomolecular interactions), individual level (survival, growth, development, behaviour) or population level (reproduction).

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? Will global warming have an influence on the adverse effects caused by pesticides on amphibians? A first approach using amphibians as a model

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 (biomolecular interactions), individual level (survival, growth, development, behaviour) or population level (reproduction).
As a further step, the studies as one proposed here, exploring the interactions between climate change and pesticides contamination at different hierarchical levels, will provide a better understanding of the responses of species to novel situations, such as global warming and, hence, the basis for prevention, management and mitigation measures.

**TH 073**

Occurrence and seasonality of cyclic volatile methyl siloxanes in Arctic air
S. Newton, T.F. Builman, M. Tysklind, K. Wiberg

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 they are persistent organic pollutants 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 predicted to result in increased ranges for sea-ice cover, primary productivity, and changes in the transport and environment of Arctic contaminants.

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 predicted to result in increased ranges for sea-ice cover, primary productivity, and changes in the transport and environment of Arctic contaminants.

The potential influence of global climate change on the Arctic results in improved ecosystem functions and higher overall productivity. The increased productivity is due to changes in the cryosphere that are predicted to result in increased ranges for sea-ice cover, primary productivity, and changes in the transport and environment of Arctic contaminants.

**TH 074**

Emerging persistent organic pollutants in arctic air, surface water and snow in Ny-Alesund, Svalbard
M. H. Hermanson

Emerging persistent organic pollutants (ePOP) may reach ecologically sensitive Arctic environment via atmospheric and/or oceanic long range transport. They are subject to seasonal variations in the amount of sunlight and cold temperatures in the winter season slows down chemical reactions. 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 little is known about these processes. In this study we present results from an active air sampling method using solid phase extraction cartridges, previously validated and applied for decamethylcyclopentasiloxane (D5) in Sweden, and adapted for Arctic conditions and further evaluated for hexamethyldisiloxane (D3), octamethyldisiloxane (D4) and dodecamethylocyclohexasiloxane (D6) in addition to D5. The method was applied to collect air samples at the Zeppelin observatory in Ny Alesund (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 075**

Brominated flame retardants in ice cores from Svalbard: an east-west comparison
M.H. Hermanson, E. Isaksson, C. Teixeira, O. Brandt, S. Forstrom, T. Aasen, D.C.G. Mørk

University Center on Svalbard, Longyearbyen, Norway

Brominated flame retardants (BFR) are a group of compounds that have been widely used in electronic and electrical products, plastics, and a variety of other consumer goods due to their ability to increase fire resistance and help reduce the spread of fire. However, their use is often associated with environmental and health concerns due to their persistence, bioaccumulation, and potential for bioconcentration.

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 predicted to result in increased ranges for sea-ice cover, primary productivity, and changes in the transport and environment of Arctic contaminants.

In this study, we present data on the occurrence and distribution of BFRs in ice cores from Svalbard, Norway (78°N), collected at different sites across the archipelago. The sites sampled include the eastern coast of Spitsbergen and the western coast of Nordaustlandet. The results show that BFRs are widespread in the Arctic environment, with high concentrations observed in the southeastern part of Svalbard. The presence of BFRs in ice cores provides valuable insight into their long-range atmospheric transport and potential for bioaccumulation in Arctic ecosystems.

**TH 076**

Atmospheric deposition of legacy and emerging persistent organic pollutants in Northern Sweden
S.R. Newton, D.W. Builman, M. Tysklind, K. Wiberg

University of Stockholm, Stockholm, Sweden

University of Umeå University, Umeå, Sweden

Swedish University of Agricultural Sciences, Uppsala, Sweden

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 predicted to result in increased ranges for sea-ice cover, primary productivity, and changes in the transport and environment of Arctic contaminants.

In this study, we present data on the occurrence and distribution of legacy and emerging persistent organic pollutants (POPs) in Northern Sweden. The sites sampled include the eastern coast of Spitsbergen and the western coast of Nordaustlandet. The results show that legacy and emerging POPs are widespread in the Arctic environment, with high concentrations observed in the southeastern part of Svalbard. The presence of legacy and emerging POPs in ice cores provides valuable insight into their long-range atmospheric transport and potential for bioaccumulation in Arctic ecosystems.
Increasing environmental concentrations of POPs in the Arctic in a future warmer climate

Aarhus University, Roskilde, Denmark

The Danish Eulirian Hemispheric Model (DEHM) was applied to study the impact of climate change on contaminant levels in the Arctic. DEHM is a 3-D atmospheric chemical transport model developed for studying the long-range transport of the Northern Hemisphere, which contains the Northern Hemisphere, which has been developed further to include marine primary, and Persistent Organic Pollutants (POPs).

The model pays attention to a broadly defined region of the Arctic, including all of Europe, North America, and the Southern Hemisphere. The results of the model suggest that the levels of POPs in the Arctic are expected to increase significantly by the end of the 21st century. This is due to the projected increase in atmospheric temperatures and the subsequent increase in the rate of chemical transport.

The implications of these results are significant for policymakers and environmentalists. They highlight the need for further research into the impacts of climate change on POPs and the need for urgent action to reduce emissions of these pollutants.
synergy and integration. The TF HTAP collaboration enhanced 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 appreciation 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 evaluation of results. Collaborations are voluntary, and key areas, like global POPs emission estimates, remain bottlenecks for further progress. Collaborations 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 collapsed together, and sometimes emissions are given without uncertainties. Continued monitoring will be essential to discern the effects of climate change and prospective reforming emissions. The five TF HTAP volumes provide an extensive synthesis of the state-of-science and a guide to future research and policy needs. https://www.htap.org/activities/2010_Final_Report.htm

TH 083

Brominated flame retardants and polychlorinated biphenyls in the Antarctic fish food web
H. Wolschke1, A. Moller2
1Helmholtz-Zentrum Geesthacht, Institute of Coastal Research, Geesthacht, Germany
2Helmholtz-Zentrum Geesthacht, Geesthacht, Germany

Antarctica is a pristine desert continent on which industrial agriculture and agriculture activities never existed. Therefore, contaminants primarily reach the continent by long-range atmospheric transport (LRT). Since semi-volatile persistent organic pollutants have a documented LRT potential these substances are of special importance for Antarctic research. The potential for lipidophilic and persistent POPs to accumulate 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 Dichlorane 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).

TH 084

POPs affect the plasma steroid levels in polar bears, Ursus maritimus
B. Styrischave1, M. Hansen2, J. Byringsvik3, T. Ciesielski4, I.T. Hansen5, L. Gustavson6, J. Aars7, E. Lie8, B.M. Jensen9

1Section of Toxicology, Copenhagen, Denmark
2Section of Toxicology, University of Copenhagen, Copenhagen, Denmark
3Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway
4Norwegian Polar Institute, Prolarmiljøsenester, Tromsø, Norway
5Norwegian Polar Institute, Prolarmiljøsenester, Tromsø, Norway
6Department of Chemistry, University of Oulu, Oulu, Finland

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 indicated a possible link between POP exposure 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(o)-estradiol, and 17(l)-estradiol) in plasma from 35 adult and subadult polar bears [3-23 years] collected in April 2008, Svalbard, Norway. 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 085

Temporal trends of legacy and new persistent organic pollutants in landlocked char in High Arctic Lakes in Canada
C.G. Muir1, G. Kock2, X. Wang1, D. Jakupcik1, M. Williamson1, G. Lescord2, K. Kidd3, E. Barresse4, E. Sverko5

1Environment Canada, Burlington, Canada
2University of New Brunswick, St. John, Canada
3Florida International University, Miami, Austria
4Resolute Bay, Resolute bay, Canada
5University of New Brunswick, St. John, Canada

Landlocked char are important sentinel species in Arctic lakes. As the top only 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 (Amitsuk, 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 (>500 mg) samples have been analysed for PCBs, chlortrichlorobenzene, DDTs, dichlorophenols, dieldrin, and toxaphene, as well as new POPs (endosulfan, HBHC, HCHs, hexachlorobutadiene, PBDEs, PFOs) to assess trends over time. PCB concentrations declined significantly in Char Lake (1993-2010), Amitsuk (1992-2010) and Hazen (1990-2010), but not in Resolute (1997-2010). HCH and 2,4,6-HCH also declined significantly and at a more rapid rate than other POPs. Toxicological concentrations have also declined although the trend is signs changing. The data indicates biomagnification throughout the food web from herbivores to carnivores and char and Resolute hazen are now and have been declining.

TH 086

Modelling and monitoring reveals time trends of POPs in Arctic biota
E. de Laender1, J. Hammer2, A. J. Hendriks1, K. Soetaert3, C.R. Janssen1

1Ghent University, Gengt, Belgium
2University of Amsterdam, Amsterdam, Netherlands
3Radboud University, Nijmegen, Netherlands
4NIOO CEME, Yerseke, Netherlands

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 of a broad range of POPs in Arctic biota. Our model approach for the Barents/Norwegian Sea ecosystem and 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 suggest the summation of POP burden remains in line with 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 (Euphausia superba)

IADEA CSC, Barcelona, Spain

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 has for a reduced number of samples and covering a limited spatial extension. Hexachlorocyclohexanes (HCHs), Polybrominated diphenyl ethers (PBDEs), and Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs and PCDFs), dibenzo dioxins and furans (PCDDs and PCDFs) were detected in the Antarctic krill. The results indicate biomagnification throughout the food web from the herbivores to the carnivores and krill and Resolute hazen are now and have been declining.

TH 088

Effects of a warmer climate on temporal trends of persistent organic pollutants in Greenland biota
K. Vorkamp, R. Dietz, F.E. Riget

Aarhus University, Roskilde, Denmark

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 hexachlorobenzododecanee.
Impact of dense shelf water cascading in the transfer of organohalogenated contaminants offshore Svalbard, Arctic Ocean

C.J. Stock, M. Balkanz, N. Bertrand, M. J. Ojeda, J. Dachs

Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain

In Greenland, systematic monitoring of POPs has covered the last 16 and 28 years for organochlorines and polybrominated diphenyl ethers (PBDEs), respectively. In general, concentrations of POPs in surface seawater have decreased over time, with the exception of PBDEs in more recent years. 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.

Temporal trends of cadmium in landlocked Arctic char (Salvelinus alpinus) in the Canadian High Arctic: the possible influence of climate change


University of New Brunswick, St. John, Canada

TH 090

Biochemical and physical controls on legacy persistent organic pollutants in the Antarctic atmosphere, seawater and plankton

H.S. Sanei, P. Outridge, G. Stern, J. Carrie, F. Wang

Department of Fisheries and Oceans, Winnipeg, Canada

TH 091

Relationships between mercury, organic matter, and climate change

H.S. Sanei, P. Outridge, G. Stern, J. Carrie, F. Wang

University of Victoria, Victoria, Canada

TH 092

Temporal trends of mercury in landlocked char in High Arctic Lakes in Canada: Possible influence of climate warming?

H.S. Sanei, P. Outridge, G. Stern, J. Carrie, F. Wang

Resolute Bay, Resolute bay, Canada

TH 093
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 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, atmosphere, and the snowpacks in the Arctic. Atmospheric pathways, source attribution and trends of mercury concentrations and deposition were analyzed. The model simulates transport and transformations of mercury in the atmosphere and its exchange with Earth’s surfaces on a global scale. 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 on mercury. At present, Arctic temperatures are rising at a rate 3 times faster than the global average. A significant portion of the deposited mercury to the snowpacks is observed to be volatilized 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 other aquatic or air ecosystems. 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.

**ECO3P - In situ remediation of metal(loids) 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
  - B. Montalban, M.C. Lobos, A.E. Pradas del Real, A. Plaza, A. Pérez-Sanz
  - IMIDRA, Madrid, alcalá de henares, Spain
  - Helianthus tuberosus L. (Compositae) offers wide technological interest as a bio-ethanol due to its high potential for biomass production. Recently studied shows 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 heavy metals that can no longer be used. 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 solution. The plants were harvested after two weeks, separated into leaves, stems, roots and tubers, dried in an oven at 70°C, and weighted. The concentration of metals in plant samples were determined by FAAS and ICP-MS. The results show that Helianthus tuberosus is a phytoremediation plant material, since it can assimilate all the studied metals at different rates. The plant growth was not significantly affected at different stages of development, showing a high tolerance to the studied heavy metals. The interaction between metals was not evident, indicating that Helianthus tuberosus is a good phytoremediation plant material for heavy metals.

- **TH 098**
  - Numerical modeling of a Phyto-extraction process
  - F. Lugli
  - COPPE / Federal University of Rio de Janeiro, Rio de janeiro, Brazil
  - The study focused on developing an integrated bioaccumulation model involving plants that extract contaminants and accumulate them in plant tissues. Despite its low cost and low impact present 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 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
  - I. Twardawa, E. Miszczak, S. Stefanian
  - Polish Academy of Sciences, Zabrze, Poland
  - Vacuum irradiation of soils contaminated with metal(l)oids, should fulfill 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 5.53); of hydraulic conductivity 10^-10 m/s, specific surface with respect to polar adsorbate (H2O) 216 m2/g, CEC=283cmol/kg, with predominance of Ca and Mg as exchangeable ions, OM 51% and inorganic 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
In situ arsenic and lead stabilization and remediation in agricultural soils using green chemistry and nano iron welding fumes

M. Miller, K.G.S. Kirk Gi, T.L. Todd, A.Z. Zimmeth

Institute of Problem of Chemical Technology, Byskek, Kyrgyzstan

Influence of humic-based sorbents on bioavailability of lead in soil

M.A. Pukhalchik, V.A. Terekhova, A.A. Iosimovs, O.S. Takimenkos, K.A. Kydryvaleva

Institute of Problem of Chemical Technology, Byskek, Kyrgyzstan

In situ arsenic and lead stabilization and remediation in agricultural soils using green chemistry and nano iron welding fumes

M. Miller, K.G.S. Kirk Gi, T.L. Todd, A.Z. Zimmeth

Institute of Problem of Chemical Technology, Byskek, Kyrgyzstan

Arsenic (As) and lead (Pb) are highly toxic naturally occurring elements found in soils worldwide and is recognized as a carcinogenic responsible for neurologic, gastrointestinal, cardiovascular, and reproductive illnesses. Toxic concentrations of As and Pb pose a public health threat particularly to children because of their tendency to consume soil via hand-to-mouth contact. Arsenic (As) and lead (Pb) are highly toxic naturally occurring elements found in soils worldwide and is recognized as a carcinogenic responsible for neurologic, gastrointestinal, cardiovascular, and reproductive illnesses. Toxic concentrations of As and Pb pose a public health threat particularly to children because of their tendency to consume soil via hand-to-mouth contact. Arsenic (As) and lead (Pb) are highly toxic naturally occurring elements found in soils worldwide and is recognized as a carcinogenic responsible for neurologic, gastrointestinal, cardiovascular, and reproductive illnesses. Toxic concentrations of As and Pb pose a public health threat particularly to children because of their tendency to consume soil via hand-to-mouth contact.
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 on the soluble fraction of U in these organs. Thus, subcellular distribution of uranium was performed using ultracentrifugation followed by uranium-specific ICP-MS. Following this step, uranium distribution within cytosolic proteins was assessed by 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 be studied by 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 cystosols shows similar patterns for control samples and contaminated samples while intensity of 3 fractions at 4, 7 and 10 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 the element.

**TH 107**
**Determination of Se species in fish tissues by HPLC-ICPMS**
R.I. Jagtap¹, F. Krikowa¹, W.A. Maher¹, S. Foster¹, E. Ellwood³
¹University of Canberra, Canberra, Australia
²Australian National University, Canberra, Australia
³Se primary 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 from in general. Many enzymes and other proteins require Se for their activity and the forms required by animals are the selenoamino acids or Se containing proteins. Since, selenoamino acids 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 physicochemical 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 derivatization of selenoamino acids. 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 Se-containing bonds. Further, the sample preparation step with diithothretol 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 carboxamidomethyl 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**
R.I. Jagtap¹, F. Krikowa¹, W.A. Maher¹, S. Foster¹, E. Ellwood³
¹University of Canberra, Canberra, Australia
²Australian National University, Canberra, Australia
³Methyl 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 the 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 concentrations in the sediment reference material CRM C: 380 and biological certified reference materials, NRCC DORM-2 Dogfish muscle, NRCC Dole-3 Dogfish liver, NIST RM Albacore tuna and IRMM IEMP-20 tuna fish were in agreement with the certified values.

**TH 109**
**EDTA: a marker of anthropogenic pollution in the Suquia River basin**
1, M.E. Valdéz², M.V. Monterrón³, M.A. Bistoni³, D.A. Wunderlin¹
¹Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Córdoba, Argentina
²ISIDSA-ICTTAC, UNC-CONICET, Córdoba, Argentina
³Diversidad Animal II.Pas. Cs. Exactas,Fis. y Naturales, Univ. Nat. de Córdoba, Córdoba, Argentina

Anthropogenic inputs, such as uraninite, at very high levels are transported using the atmosphere over long distances and in the earth's crust and oceans, resulting up the sedimentary record. EDTA has 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 environmental ecosystems. 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 (Cu, B, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Ba, Mo, Ag, Cd, Ba, Ti, 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 at least 3 locations. Amounts of EDTA varied along stations. At higher 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 (Córdoba). Some metals, as Cd, Cu, Fe, Zn, Ag, Zn and Pb, 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 quality parameters.

**TH 110**
**Speciation and bioavailability of arsenic and heavy metals in contaminated stream sediments**
P.J.C. Stavas
University of Trás-os-Montes e Alto Douro, Vila real, Portugal

The application of the Selective Chemical Extraction Technique on the sediments of the stream that drains the “Vale da Gata”/Minas area, allowed us to notice that: 1) the most part of the contents of Cu, Zn, 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 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 interesting 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 stations where the 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 to AMD and heavy metal tailing, exchangeable fractions of Cu, Zn, Cd and Pb are the most important.

EDTA was found in at least 3 locations. Amounts of EDTA varied along stations. At higher 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 (Córdoba). Some metals, as Cd, Cu, Fe, Zn, Ag, Zn and Pb, 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 quality parameters.
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 phytochelatins (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 metallothiol compounds 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), concentrations ranging from 0-200mg/L.

The quantification and determination were performed by HPLC-ICPMS and HPLC-ESI-MS. The same chromatographic conditions were used for both instruments. Quantification by ICMS does not ensure unequivocal identification of molecules but allows the determination at low detection limits and is not significantly affected by the matrix. The use of ESMS allows both low concentration quantification and unequivocal identification 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**

J. Kuta1, J. Machát2, M. Vítová2

Masaryk University, Brno, Czech Republic

The authors also are grateful for financial support of MŠMT (MSM0021622412).

Se 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 selenium in the environment. In this study attention is focused on the identification of selenium species in different parts of algae. Particular emphasis will be made on the use of smaller internal standard for quantification and its effect on the results. The results show that As-GSH complexes are formed in Chlorella vulgaris cells after exposure to arsenic and contribute to elucidate the mechanisms of arsenic detoxification used by microalgae.

**TH 113**

**Speciation of arsenic and selenium in surface water**

M.C. Kotte, R. Breidenbach, I. Bakker, H. van den Heuvel, O.J. Epema

WRS Centre for Watermanagement, Lelystad, Netherlands

Watershed studies of various international River basin programs are aimed at the identification of non-toxic selenium and arsenic with the help of different sample types.

**TH 114**

**Ion chromatography with XSERIES 2 for metal speciation applications**

D.K. Kutsch, J. Willis

Thermo Fisher Scientific, Bremen, Germany

Ion chromatography with XSERIES 2 is described. This combination provides a single powerful, flexible solution for both ion chromatography and ion exchange chromatography applications as well as traditional HPLC methods. The combined system is ideally suited for trace metal speciation as it provides a way to overcome the drawbacks in mobile phase reduction risk and improving robustness of methods 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.

**TH 115**

**Speciation of Bromine in water using sample introduction techniques**

R.C.H. Chemnitzer

Bruker Daltonics GmbH, Berlin, Germany

Foods and agricultural products contain various concentrations of nutrients, which can range from trace amounts to percentage levels. However, whilst most of their nutritional value is needed 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 minor, minor trace and elemental analysis. In combination with separation techniques, ICP-MS allows the determination of the chemical form of elements 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 is a 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 chromatographic technique to an ICP-MS provides a powerful and very sensitive tool to separate bromine and bromate.

The presentation will compare 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)**

R. González Gago1, D. Profrick2, A. Prange1, J.J. García Alonso1, D. Swart3, D. Schief1, R. Philipp4

1 Helmholtz-Zentrum Geesthacht/Institute for Coastal Research, Geesthacht, Germany
2 University of Oviedo/Department of Physical and Analytical Chemistry, Oviedo, Spain
3 Physikalisch-Technische Bundesanstalt (PTB)/Department 3.1, Metrology in Chemistry, Braunschweig, Germany
4 Bundesanstalt für Materialforschung und -prüfung (BAM)/Department 1, Analytical, Berlin, Germany

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 in the list of priority substances that need to be measured in all surface waters covered by the WFD. Environmental Quality Standards (EQS) are defined to the maximum allowable concentrations, have been defined at very low levels for PBDEs (2PBDEs ≤ 0.5 ng/L).

Analytical methods intended for their monitoring must meet certain minimum performance criteria in terms of uncertainty (≤ 50% at EQS, 95% confidence) and limits of quantification (LOQ) ≤ 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 in combination 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 work, a gas chromatography-mass spectrometry (GC-MS) or electron capture negative ionization (ECNI) mass spectrometry provide interesting possibilities as it offers high sensitivity for the detection of heterocyclics 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.
Improved extraction, preservation and analysis of hexavalent Chromium from soil extracts and water

R.F. Jack1, C.G. Giuriati1, L.B. Basumalik1, F.A. Abballe1, R.J. Rohrer1

Thermo Fisher Scientific, Sunnyvale, United States of America

Caterina Giuriati, Ferrara, Italy

Chromates are oxoanions (e.g. CrO42-, Cr2O72-) of chromium in soil extraction methods. 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, the USEPA sampling method is based on ion exchange chromatography on an IonPac AS17 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 of 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 accuracy 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 with a sample detection 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 and Drinking Water to create a new EPA method based on these final modifications, EPA 218.7

Class 1 and 2 integrons, sul resistance genes and antibiotic resistance in Escherichia coli isolated from the East River, South China

G.C. Ying

Guangzhou Institute of Geochemistry, Guangzhou, China

Extensive use of antibiotics by humans and animals has 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.

In the East River, 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 1,403 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 including 22-gene cassette aadA1, adaA2, and adaA3 were identified for the first-time in the East River samples. Multiple antibiotic resistances (MAR) index was significantly correlated with multiple 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 showed 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.

Antibiotic contamination and promotion of antibiotic resistance in microbial communities of three reservoirs

B. Huerta1, E.M. Martín1, M.G. Gros1, S.R.M. Rodriguez-Mozaz1, J.L.B. Balcazar1, D. Barcelona1, R.M. Marce1

Catalan Institute for Water Research, Girona, Spain

IDAEA-CSIC, Girona, Spain

Pharmaceuticals are continuously released into surface waters from discharge 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 very low concentrations. Pharmaceutical 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 on 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 that impair their natural fitness 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. The water 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 qrn and sul gene copies (index of antibiotic resistance for the quinolone and sulfamide family, respectively) found in the reservoirs. Resistance genes were found at 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.

Persistence of heavy metal and antibiotic resistance downstream a point source pollution of a river ecosystem

C. Seiter1, W Von Tümpling2, A. Lupo1, T.U. Berendonk1

1Dresden University of Technology, Dresden, Germany

2UFZ - Helmholtz Centre for Environmental Research, Magdeburg, Germany

The impact of heavy metals and antibiotic resistances in the aquatic environment 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 act as selective agents for antibiotic resistance genes. 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 river characterized 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 harboring sul and heavy metal resistance genes were selected for 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. Therefore, 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 resistance genes, while Cr is not linked to the concentrations of the antibiotics resistance genes. These findings show that the combination of heavy metal and antibiotic resistances might contribute to the persistence of the corresponding antibiotics resistance genes.

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 in the Western Bug River.
the mobilisation of blaCTX-M genes, was found ahead of blaCTX-M-14 gene. RAPD analysis indicated a relatedness between 4 E. coli harbouring blaCTX-M-14 of which 2 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 Netherlands. Further, it is an important case study demonstrating how organic pollution of rivers may represent a critical 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

V.E.I.J. O Isanad, V. Voolaid

University of Tartu, Tartu, Estonia

In addition to medical setting, antibiotic resistance is wide spread in environment. 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 horizontal transfer of the aquatic bacteria. Total bacterial DNA isolated from several samples was isolated from a lake and its run off river. The bacteria were isolated on antibiotic (including ampicillin) containing selective media. The isolation 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 nearby. Total bacterial DNA was isolated from the run off river. The lake was also isolated by E. 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

I. Muziasari1, A.N.T.T. Jarkkan1, O. Mitsuki2, S. Suzuki2, M.A.R.K.O Virra1

1University of Helsinki, Helsinki, Finland

2Agricultural Research Centre, Tartu, Estonia

Aquaculture environment has been suggested to be a reservoir of antibiotic resistance genes due to prophyactic and therapeutic use of antibiotics. It has been observed that different resistance genes are present 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.

**TH 127**

Diversity and antibiotic resistance of Acinetobacter spp. in water from the source to the tap

C.A. Narciso Da Rocha1, A. Ronaldo1, O. Barraud2, I. Vaz Moreira1, R. L. Svendsen3, Moore4, C.M. Manafa5

1School of Biosciences, University of Tartu, Tartu, Estonia

2Universidade do Porto, Porto, Portugal

3Aix-Marseille University, Marseilles, France

4Department of Microbiology and Immunology New York Medical College, Valhalla, ny 10595, United States of America

5Universite de Limoges, Limoges, France

Diversity and antibiotic resistance of Acinetobacter spp. in water from the source to the tap. Acinetobacter spp. (A. lwoffii, A. johnsonii) were isolated only from tap water. Most of the isolates (80 %) were susceptible to all of the 12 antibiotics used, except for the strains of A. johnsonii and A. lwoffii tested, which were resistant to both imipenem and meropenem.

**TH 128**

Resistance to broad-spectrum antibiotics in aquatic systems: do anthropogenic activities affect the dissemination?

M. M. C. Cabello1, F. C. Cabello1, A. Correia1

1University of Aveiro, Aveiro, Portugal

Significant amounts of antibiotics and antibiotic-resistant bacteria are released daily into the environment. The prevalence and molecular diversity of resistance genes in water may be an indicator of water quality, useful to assess the risk of environmental antibiotic resistance to public health. Production of extended-spectrum beta-lactamases (ESLs) is one of the mechanisms of resistance. In fact, Gram-negative bacteria have developed strategies to cope with antimicrobial pressure. Several schemes have been developed to assess the horizontal transfer of resistance genes. In conclusion, results showed clear differences between P and UP lotic ecosystems, concerning prevalence, phylogenetic diversity and antimicrobial susceptibility profiles of CTX-M, beta-lactam and production of clinically relevant ESBL genes. This validates the hypothesis that anthropogenic activities modify the environmental resistance gene pool and are promoting antibiotic resistance dissemination. Also, it suggests that CTX-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

T.S. Stalder1, O. Barraud2, M.C. Casellas1, C.D. Dagot1, M.C.P. Poy1

1University of Limoges, Limoges cedex, France

2Universitat Autònoma de Barcelona, Bellaterra, Spain

GRESE, Limoges, France

The spread of antibiotic-resistant bacteria is a growing problem and a public health issue. Wastewater 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 and 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 were equivalent sampled. After a 24 h flow proportionate sampling were sampled, effluents were concentrated and total genomic DNA was extracted using the PowerWaterTM 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 resistance genes. CTX-M harboring class 1 MI were harbored by A. baumannii and A. lwoffii, suggesting that the horizontal transfer of CTX-M gene cassette occurred in hospital environment. The most common gene cassette was CTXM-14, followed by CTX-M-1, CTX-M-5 and CTX-M-10. These results suggest that the density of CTX-M-14 gene cassette is higher in hospital effluents than in urban effluents. To our knowledge, this is the first study which assessed the impact of hospital effluents on the WWTP. Even if the prevalence of MI was higher in hospital effluents, it was not significant different from urban effluents. However, antibiotic resistance genes are more prevalent in wastewater from hospital area, which suggest that hospital effluents are a potential source of antibiotic resistance dissemination in the receiving WWTP.

**TH 130**

Occurrence of antimicrobial resistance among environmental bacterial and fish of farm ponds

Q.A. Shah1, T.M.L. 1Abie-Lund1, E.C. Cabello2, D.J. Colquhoun1, H.S. Sørum2

1Norwegian School of Veterinary Science, Oslo, Norway

2Departments of microbiology and Immunology New York Medical College, Valhalla, ny 10595, United States of America

Department of Microbiology and Immunology Department of Microbiology and Immunology New York Medical College, Valhalla, ny 10595, United States of America

Occurrence of antimicrobial resistance among environmental bacterial and fish of farm ponds. Q.A. Shah1, T.M.L. 1Abie-Lund1, E.C. Cabello2, D.J. Colquhoun1, H.S. Sørum2

1Deparment of microbiology and Immunology New York Medical College, Valhalla, ny 10595, United States of America

2Department of microbiology and Immunology New York Medical College, Valhalla, ny 10595, United States of America

In future studies, resistant bacteria from sediment, fish and fish guts will be isolated by using independent and 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.
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 investigated for the presence of antibiotic resistance genes (343 samples of marine bacteria from the Norwegian fjords and 135 isolates of freshwater bacteria from lakes and rivers in Poland). 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 Poland and Romania implies a large number of antibiotic treatments, however, higher in fresh water isolates than in marine isolates. The prominent resistance genes found in the bacterial isolates studied were TetA, TetG and TetE operons (7.6 % of isolates), sul1, sul2 (23.5 % of isolates), qnrA, qnrB, qnrS (10.7 %), catF (21.2 %) and mefA (13.5 %), causing resistance against tetracycline, sulphonamides, trimethoprim, ciprofloxacin and erythromycin, respectively. The tet genes were abundant on all four sites in association with R-plasmids. Carbapenem resistant fresh water environmental isolates were screened for the presence of the blaNDM-1 (New Delhi metallo-beta-lactamase) gene, but blaNDM-1 was not found. All isolates were also negative for plasmid mediated qnrA, qnrB and qnrS genes.

Extensive use of antibiotics in aquaculture and in livestock farming during the 1980s and early 1990s appears 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 resistant bacteria in the aquatic 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**

N. Czekalski, R. Sigdel, H. Bürgmann

Eawag, Kastanienbaum, Switzerland

Antibiotic resistance in aquatic 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 treatment 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. In order to determine the potential impact 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 this process, antibiotics 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.

As antibiotic resistance is commonly found in freshwater bacteria, its presence seems to be obvious. The factors determining the persistence of antibiotic resistance are largely unknown. Antibiotic resistance is advantageous for resistant bacterial strains as it allows it to compete in its niche. 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 is studied. This multi-resistant P. aeruginosa was enriched by clinical as well as municipal WW effluent. Antibiotic resistance and antibiotic use are closely linked with the reduction of antibiotic resistance. Pseudomonas aeruginosa is capable of degrading a broad diversity of xenobiotics. P. aeruginosa is a well-known bioremediator of soil and water contamination. The persistence of antibiotic resistance is a relevant concern that is not well understood. Antibiotic resistance was assessed through successive sampling campaigns of raw hospital effluent and treated wastewater of the municipal wastewater treatment plants in the watershed, etc.

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, enteric bacteria and aeromonads on different culture supplemented or not with amoxicillin or ciprofloxacin (1). 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 most frequently mentioned is the necessity to determine more accurate indicators.

**TH 132**

**Differential gene expression and biofilm formation of multiple antibiotic-resistant Pseudomonas aeruginosa in municipal wastewater**

T. Van, S. L. Ferreira, H. L. Brandt, A. Bähr, A. H. Coelho, M. Chaves

Karlsruhe Institute of Technology / Institute of Functional Interfaces, Eggenstein-leopoldshafen, Germany

Occurrence of antibiotic resistance genes in wastewater used for irrigation in the Mézquital Valley

M. B. Brozarov, J. Huber, E. Grohmann

University Medical Center Freiburg, Freiburg, Germany

Wastewater (WW) reuse for irrigation is widely practiced 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 is the world’s largest irrigation area in Mexico. WW from Mexico City is reused for crop irrigation, which poses risks for farmers and the consumers of the agricultural produce. 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; sulfonamides and ciprofloxacin, and five antibiotic resistance genes (sul1, sul2, qnrA, qnrB, qnrS) that mediate resistance against these antibiotics. Furthermore, the blaSHV genes that are frequently found in bacterial isolates from patients in Mexican hospitals was selected. This gene encodes for Extended Spectrum Beta-Lactamases (ESBL). Furthermore, we determined the concentration of the faecal indicator Enterococcus spp. and the total bacterial concentration. WW was collected at the WW treatment plant in Mexico City. For the second sampling campaign the influents of the WW effluent treated in Mexico City and the WW effluent before water treatment were sampled from the WW treatment plants in the watershed, etc.

For the different samples, the total heterotrophs, total enteric bacteria and total aeromonads were determined. Total DNA was extracted from all samples and was used for quantitative PCR specific for each resistance gene, total bacteria (16S rRNA gene) and the most abundant antibiotic resistance genes (aadA1, aadA2, dfrA12, dfrA13, dfrA14, dfrA15, dfrA7, dfrA1 and dfrA13) 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 gene copies/100 ml water to 10^1 gene copies/100 ml water and qnrB decreased by 4 logs from 10^4 gene copies/100 ml water to 10^0 gene copies/100 ml water. The concentration of total bacteria decreased by 3 logs from 10^6 gene copies/100 ml water to 10^3 gene copies/100 ml water and the concentration of Enterococcus spp. by 4 logs from 10^2 gene copies/100 ml water to 10^0 gene copies/100 ml water. The recovered water still contained a high concentration of total bacteria and faecal indicators. Therefore, without further treatment it is not suited for crop irrigation.

**TH 133**

**Occurrence of antibiotic resistance genes in wastewater used for irrigation in the Mézquital Valley**

M. B. Brozarov, J. Huber, E. Grohmann

University Medical Center Freiburg, Freiburg, Germany

**TH 134**

**Development of treatment methods for the degradation of antimicrobial compounds present in wastewater**

A.C. Reis1, C.M. Manaia2, O.C. Nunes3

1Faculdade de Engenharia, Universidade do Porto, Porto, Portugal

2Escola Superior de Biotecnologia, Universidade Católica Portuguesa, Porto, Portugal

3Laboratório de Estudos de Microrganismos e desinfeção, Instituto de Microbiologia, Universidade do Porto, Porto, Portugal

The increase of antibiotic resistance to any other compound leading to the necessity of antibiotic detoxication and/or contamination with these microorganisms or with their toxic degradation products. The widespread distribution of these contaminants may have harmful effects, such as the increase 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 most frequently mentioned is the necessity to determine more accurate indicators. The implementation of degradation methods of MRX is urgently needed. The objective of this study aims at developing low cost effluent 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 amoxicillin to non-active degradation products were obtained. The members of these cultures were identified and their antibiotic resistance profile 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**

A.R. Varela1, C.M. Manaia2, O.C. Nunes3

1Escola Superior de Biotecnologia - Universidade Católica Portuguesa, Porto, Portugal

2LPEAF-Dep. Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Porto, Portugal

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, studies on the fate of AR bacteria from the hospital effluents to the receiving sewer 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).
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.

Horizontal transfer of certain antibiotics, such as vancomycin, carbapenems and quinolones.

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 which the last resort antibiotics, such as vancomycin, carbapenems and quinolones.

Horizontal transfer in the effluents. The culturable antibiotic resistance rates were documented on Novo & Manaia (2010) study. Some of the most relevant DGGE bands were selected for further identification.

Methods: 

Background: Bacterial communities were compared using DGGE analysis. Antibiotics and heavy metals were determined in the inflow and in the treated effluent. The culturable antibiotic resistance rates were documented on Novo & Manaia, 2010 study. Some of the most relevant DGGE bands were selected for further identification.

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 concentrations, percentages of antibiotic resistance among culturable bacteria, and climate or plant operation conditions).

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.

Quantification of resistance genes and resistance of bacterial communities for last resort antibiotics from wastewater treatment plants

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.

The variation in bacterial communities in the hospital effluent and in the WWTP raw and treated effluent is analyzed.

Vancomycin and ciprofloxacin resistance in enterococci from a Hospital effluent and in the receiving Municipal Wastewater Treatment Plant

Vancomycin and ciprofloxacin resistance in enterococci from a Hospital effluent and in the receiving Municipal Wastewater Treatment Plant

Occurrence of beta-lactamases, namely GES-5 carbapenemase, among Gram-negative isolates from wastewater samples in Northern Portugal

The results of this study were to evaluate some AR mechanisms in Gram-negative bacteria isolates from surface and raw and treated wastewater 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 Knyvaya spp., 2 Enterobacter spp., 1 Hafnia alvei, 1 Pantoea agglomerans, 1 Pseudomonas luteola, 1 Rodella ornithobacteriosa, 1 Serratia spp.) was performed by disk diffusion method. The search for antibiotic resistant genes was based on the detection of antibiotic resistance genes (ARGs) and the occurrence of ARGs in the effluent of the hospital effluent and the treated wastewater.

Results: Overall, 13 isolates were multidrug resistant, suggesting a great diversity of ARG mechanisms. Two isolates showed non-susceptibility to carbapenems, one of the last resort antibiotics. Their phylogenetic and molecular characterization revealed the expression of a chromosomal beta-lactamase in P. lacta 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 beta-lactam酶, with
distinct promoters, 4 blaTEM (2 blaSHV, and 2 blaTEM), and also different chromosomal AmpC beta-lactamases, namely CMY-65. Class 1 integrons were detected among 60 of TEM-1-producing isolates. Together, these beta-lactamase levels exhibited 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 as it represents a liable reservoir of potential pathogenic resistant bacteria.


TH 141
Pseudononas spp. in drinking waters - diversity and antibiotic resistance
I. Vaz-Moreira1, O.C. Nunes2, C.M. Manaia2
1Escola Superior de Biotecnologia, Porto, Portugal
2Laboratório de Biologia Molecular, Unv. Porto, Porto, Portugal

Pseudomonas are common inhabitants of aquatic environments, including drinking waters. 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 explore the presence and extent of antibiotic resistance 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 rDNA, rpoB, rpsL, recA, rpoD and 16S rRNA). Biochemical and effusion tested isolates were classified based on the STs present, and in some and in brand of mineral water. Twenty eight different sequence types (ST) were observed by the housekeeping genes analysis, with the genes rpsL and rpoD 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 batches of water. 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 cephaplatin, and no resistance phenotypes were observed to ciprofloxacin, tetracycline, pipercillin, imipenem, meropenem and colistin.

This study, based on 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
M.E. Falcone-Dias1, I. Vaz-Moreira1, C.M. Manaia2
1Ministry of Education of Brazil, Brasilia-df, Brazil
2Centro de Biociencias, Unv. Catolica Portuguesa, Porto, Portugal

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 classes of antibiotics (ATB, Bio-Rad). Resistance was assessed by turbidimetry (determination of antibiotic resistance profiles). The Actinobacteria presented high resistance levels to the antibiotics cetrimide, meropenem and ciprofloxacin according to minimum inhibitory concentrations (Etest and MLI-26).

Bottled mineral water was confirmed as a source of antibiotic resistance mechanisms, with the potential to spread the resistance genes.

TH 143
Important vectors of antibiotic resistance genes in agricultural systems
S. Rother1, B. Sinha1, C. Kopmann2, U. Zimmerling2, E. Krogeruskenfort2, H.H. Heuer1
1JCL, Braunschweig, Germany
2Spreading land management on agricultural soils was recently proposed to promote spread of transferable antibiotic resistance and residual veterinary medicines in agricultural soils. For this study, soil of treatment with soil with manure spiked with the antibiotic sulfolauzidine (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 plasmid which were captured into E. coli recipients from soil samples of mesocosm, mesocosm and field samples by probing and antibiotic resistances carriers. The plasmids captured were mainly assigned to LowGC and IncP-1PE 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-1PE, the ability to efficiently transfer under soil conditions and the wide host range of IncP-1PE plasmids strongly suggest that these plasmids are important vectors for spreading antibiotic resistances in the agro-ecosystem. LowGC plasmids have in contrast to IncP-1PE 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 hosters are emerging pathogens of the genus Acinetobacter.

TH 144
Spread of antibiotic resistance determinants in wastewater irrigated soils in the Mézquital Valley, Mexico
M.M. Broszat1, B. Prado2, C. Siebe3, J. Siemens3, J. Huebner1, E. Grohmann1
1University Medical Centre Freiburg, Freiburg, Germany
2Universidad Nacional Autonoma de Mexico, Institute of Geology, Ciudad Universita, Mexico city, Mexico
3Universite Bonn, Institute of Crop Science and Resource Conservation, Bonn, Germany

We investigated the spread of antibiotic resistance determinants by horizontal gene transfer (HGT) in wastewater (WW)-irrigated soils from the Mézquital Valley (60 km south of Mexico City). WW from Mexico City is reused for irrigation. WW contains a huge amount of potentially harmful characters such as resistance determinants, pathogens and antibiotic. 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 non-mobilizable plasmid labelled with the Red Fluorescent Protein (RFP) were added to land at 1 year and 100 years-irrigated soil, each in soil columns of 20 cm height and 13 cm diameter. At each irrigation 1013 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 from top soil and at 1 cm from the soil of the experiment from different heights (2 cm to 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
J. Modrzynski, K.K. Brands, J. Berg, O. Nybroe

University of Copenhagen, Faculty of Life Sciences, Frederiksberg, Denmark

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 [14C]leucine incorporation assay for measuring growth rates of bacteria extracted from soil. The [14C]leucine incorporation rates in 5-ml soil bacterial sub-samples were subsequently subjected to growth rate analysis 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 [14C]leucine incorporation incubations based on a single 5-ml soil sample.

The optimized micro-scale [14C]leucine incorporation assay was subsequently used to measure pollution-induced community tolerance to Cu and co-tolerance to selected antibiotics in Cu-contaminated soil samples. Cu-loaded bacterial diversity was analyzed by a downscaled version of an existing whole-cell bacterial bioassay analysis (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-ml 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
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 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.

Aspects of antibiotics resistance in Salmonella spp. isolates
S. Parkovická, K. Koudalová, J. Pátilarová, K. Demnerová
Institute of Chemical Technology Prague, Prague 6, Czech Republic
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 and also to 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 the livestock and medical practice or in wastewater treatment plants, supported by biofilm formation. The aim of this work was to study the different aspects of antibiotic resistance in 50 strains of Salmonella spp. isolated from different sources in Czech Republic (domestic wastewater, 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, ciprofloxacin, nalidixic acid, trimethoprim, tetracycline, chloramphenicol, nitrofurantoin, kanamycin, sulfonamide) (tetA, tetB, tetC, tetG) was detected by PCR method together with the cluster SGI-1 (Salmonella Genomic Island-1). The strains resistent minimally to one antibiotics (tetracycline, ampicillin, streptomycine, nalidixic acid, ciprofloxacin or chloramphenicol) were examined for the ability to 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.

Adoption of bacteria to ameliorate the metabolic burden of carrying antibiotic resistance genes (a systems biology modeling analysis)
Northwestern University, Evanston, Illinois, United States of America
Bacterial resistance to antibiotics is an important problem, and it is unclear what leads to the relatively high abundance of resistant bacteria in the 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, because these behaviors can be predicted from basic molecular biology (e.g., metabolic models). Tetracycline and tetracycline are 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, 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 bacterial biomass is considered 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 biology". The model reproduces observations from the literature, including growth rate vs. substrate concentration, competition between resistant and sensitive strains in the absence of selective pressure, 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 of the carrier is lethal for the resistant strain but not the susceptible strain. 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.
Uptake and distribution of multiwalled carbon nanotubes in zebrafish Danio rerio

E. de Souza, E. Durante, B. Steffen, H. Deimann, F. Hueschen, W. Baumgarner, H.T. Ratte, A. Schäffer

BWT Hachen University, Aachen, Germany

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 on the toxicology of this nanomaterial, which need to be examined 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. Using 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 (Lumbricus variegatus). Measurements were performed 24, 48, 96 h after water exposure in absence of DOC and 48, 72, 96 h after dietary exposure. Essential component of the elimination experiment was a set point was 168 h. Elimination was measured after an exposure time of 168 h, at 72, 96 h and 120 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 solubilisation. After exposure via water, DOC was added. TEM imaging, CNT detection, and morphological changes were observed by STEM.

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.

Salinity and pH-dependent silver nanotoxicity in post-hatch embryos of Japanese medaka


Toyo University, Itakura oura, Japan

Our previous investigation of nano-sized silver toxicity using Japanese medaka embryos and elucidated that silver nanocollodius cause severe inhibition of embryogenesis and morphological changes such as blood clots, percardio vascular edema, tubular hearts, small eyes, shortend 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 nanocollodius (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 fish 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 0.5 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 size 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) would influence and potentiate on environmental fate of nanomaterials. Tested SNC changed zeta-potential smaller in pH9 (-29.3 mV) than in 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 pH-dependent toxicity of SNC using post-hatch embryos as well.

Development of a test chamber to enable homogeneous aqueous phase dispersions of nanoparticles for testing nanoparticle toxicity in fish

H. Boran1, D. Boyle2, R.D. Handy3, I. Atkinson4, T.B. Henry4

Kaiseri Technical University, Trabzon, Turkey

1University of Plymouth, Plymouth, United Kingdom

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 is difficult due to the heterogeneous properties and due 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 standardised 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 codigned to (mainly) CAs, MgO, KI, NaI . NSPs 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 larvae zebrafish to establish 96-h LC50 values for TiO2 NPs, AgNPs, CuNPs, and CdNPs; and compared to metal ions 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), which determined that homogenous dispersions were maintained during the exposure (total loss of NPs from water column <10% over 96h). 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 AgNPs, CuNPs, CdNPs, and ZnO-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 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 chamber 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.
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 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 content of glutathione and superoxide dismutase, and the activation of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT).

In this work, we evaluated the effects of CdSe/ZnS QDs on the marine diatom Phaeodactylum tricornutum, which is a sensitive and widely used bioindicator of coastal environmental contamination. The results of this study are relevant to understanding the toxic mechanism of SNC and will help us to understand the toxic phenomenon of SNC in aquatic ecosystems.

Silver nanoparticles (AgNPs) have gained much popularity on account of their antimicrobial properties. However, the environmental impact and the mechanism of toxicity still have not been fully elucidated. Phytolipid represents the first link of the food chain in natural waters, thus it represents the mechanisms of uptake, accumulation and toxicity of nanoparticles in these unicellular organisms. The results of this study are presented here with the aim of understanding the role of AgNPs in marine ecosystems.
Effects of silver nanoparticles in the freshwater snail Physa acuta
S. Gonçalves1, M.D. Pavlaki1, S. Loureiro1
1Universidade de Aveiro & CESAM, Aveiro, Portugal

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 used in different products, such as cosmetics, 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 Mysisidopsis Juniae
C. Vaz1, T.M.N. Oliveira1, R.F.S. Bojin1, R.A. Gonçalves1, E.C. Spintrzer1, T. Kleine1, S.P. Melegari2, C.H. Costa1, F. Perreault1, R. Popovic1, W.G. Matisa2
1UNIVILLE, Joinville, Brazil
2IFIC, Florianópolis, Brazil

The copper oxide nanoparticles are components of anti fouling paints that are used for painting the hulls of boats. Their decomposition can provide both the soluble ionic form of copper which is highly toxic to many marine organisms and the formation of copper oxide nanoparticles. 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. In this study was aimed at assessing the chronic toxicity of copper oxide nanoparticles on the marine microcrustacean Mysisidopsis 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 maintenance and test protocols 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 Dunnet 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 TiO2 on Mytilus immune parameters
C. Bregant1, T.M.A. Corda1, G. Gallo1, A. Marcomini1, G. Pojana1, L. Canesi1
1University of Genoa, Genoa, Italy
2University of Urbino, Urbino, Italy
3University Ca’ Foscari Venice, Venice, Italy

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 ENPs in aquatic ecosystems.

We have previously demonstrated that in the marine bivalve Mytilus galloprovincialis, short term in vivo exposure to TiO2, 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 vivo toxicity of different types of ENPs, including TiO2. Biomarkers of immunotoxicity may reveal changes in the immune function induced by ENP exposure, possibly leading to changes in organisms’ health.

In the this work, in the in vivo effects of TiO2 on immune function were investigated in mussels exposed to different nominal concentrations of TiO2 (1, 10 and 100 mg/L) for 4 days. Hemolymph immune parameters were evaluated: hemocyte lysosome membrane stability (LMS), ROS and NO production, phagocytic activity and serum lysozyme activity. Expression of selected genes was evaluated in hemocytes by RT-PCR.

The potential ecotoxological risks of nanoparticles (NPs) have received increasing research interest 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 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 fullerene, 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). The results showed that exposure to nTiO2 induced significant changes in different functional parameters and molecular immune biomarkers in mussel hemocytes. Moreover, decreases in lysosomal biomarkers (lysosomal membrane stability) and oxidative stress biomarkers (catalase, GPx, GPx-Se and GST activities) were observed in the digestive gland of the test organisms under stress conditions. These data indicate that exposure to TiO2, 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 cells 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
C.C. Waiss-Leinonen1, E.J. Petersen1, K. Fakarinen1, J. Akkanen1, M.T. Leppinen1, J.V.K. Kukkonen1
1University of Eastern Finland, Joensuu, Finland
2Biochemical Science Division, National Institute of Standards and Technology, Gaithersburg, United States of America

The possible ecotoxicological risks of nanoparticles (NPs) have received increasing research interest 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 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 fullerene, 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). The results showed that exposure to nTiO2 induced significant changes in different functional parameters and molecular immune biomarkers in mussel hemocytes. Moreover, decreases in lysosomal biomarkers (lysosomal membrane stability) and oxidative stress biomarkers (catalase, GPx, GPx-Se and GST activities) were observed in the digestive gland of the test organisms under stress conditions. These data indicate that exposure to TiO2, 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 cells 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 173
Multibiomarker assessment of cerium dioxide nanoparticle (nCeO2) sublethal effects on the freshwater invertebrate Dreissena polymorpha and Gammarus roeseli
J.V. Kukkonen1, C. Agar1, C. Cossa Leguil1, V. Felten1, S. Pain-Dest4, P. Rousselle1, P. Rousselle2, P. Wagner3, L. Guambéna1
1Laboratory LIEBE - CNRS UMR 7146 - Université de Lorraine (UdL), Metz, France
2Laboratoire CNRS - UMR 7146 - Université de Lorraine (UdL), Metz, France
3Laboratoire CNRS - UMR 7146 - Université de Lorraine (UdL), Metz, France
4Laboratoire CNRS - UMR 7146 - Université de Lorraine (UdL), Metz, France

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 appear to be caused to morphological changes, thus inhibiting the larvae growth.

TH 174
Effects of TiO2 nanoparticles on Mytilus galloprovincialis 48-h old larvae
G. Galasso1, D. Minetto1, C. Lusso1, L. Manodori1, E. Sallaboni1, A. Volpi Ghirardin1
1University Ca’ Foscari Venice, Venice, Italy

In this study, 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 rosei, at concentrations close to potential 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 anti-oxidative defences (catalase, GPx, Gpx-S and GST activities, metallothionein concentration and lipid peroxidation); 2) locomotion and ventilation (Na+/K+-ATPase activity and Na⁺, Cl⁻ and Ca²⁺ haemolymph concentrations); 4) lysosomal 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.
applications and as an additive in paints, papers, inks 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 effects to the aquatic ecosystem.

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 nanoparticle to aquatic organisms may be either due to the effects of dissolved silver ions or to specific effects of nanoparticles.

The presence of Ag 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 Ag NPs are bioavailable for mussels, and are accumulated in the mussels 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.

Acknowledgements: TEM and X-ray microanalysis were carried out at the Centre for Ultrastructural Imaging, King's College London. Work funded by the European Community's 7th FP (FP7/2007-2013) under grant agreement n° CP-FP 21478-2 and by the Basque Government (GIC07/67-IT-393-07). A.J-R is recipient of a pre-doctoral fellowship from the University of the Basque Country.

TH 176

Metal-particle size affects metal bioaccumulation from sediment and depuration in a deposit-feeding snail
C. Panizza, A. Belhaoui, S.K. Misra, E. Valsami-Jones, V.E. Forbes

Roskilde University, Roskilde, Denmark

Department of Environmental, Social and Spatial Change, Roskilde University, Roskilde, Denmark

Mineralscience, Natural History Museum, Cromwell Road, London SW7 2BD, UK, London, United Kingdom

School of Geography, Earth and Environmental Sciences, University of Birmingham, Birmingham, United Kingdom

University of Nebraska-Lincoln, Lincoln, United States of America

With the increasing use of Cu 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, i.e., CuO nanoparticles, Cu-spiked sediment and Cu-spiked 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
S.A. O'Rourke, V. Stone, T.F. Fernandes

Heriot-Watt University, Edinburgh, United Kingdom

Since the 1990s 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 NPs to be released into the environment. The novel properties of these particles (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 processes etc. As a food additive, it is used as a food additive. Although soil and freshwater ecosystems are as important to terrestrial 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, Lumbricillus 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 Lumbricillus 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 Sodium Sulfate (Na2SO4). There were no significant differences in the toxicological effects between the various concentrations. Concentrations were significantly different from the controls. No significant differences in the 1.25mg/L concentration were observed between the different treatments.

TH 178

Silver nanoparticle dissolution and Ag speciation as key parameters for toxicity of AgNP to algae
L. Sigf, F. Piccapreta, U. Lindauer, N. Oezdek, R. Sofield, R. Behra

Eawag, Dübendorf, Switzerland

Western Washington University, Bellingham wa 98225, United States of America

Because of their multiple applications in consumer products silver nanoparticles may reach the aquatic environment and affect aquatic organisms. Toxic effects of silver nanoparticles are expected to occur at sites of high bioaccumulation or where particles may be dragged out of AgNP to aquatic organisms, namely agglomeration of nanoparticles, dissolution and speciation of dissolved AgI. Dissolution of AgNP is of fundamental importance, as dissolved Ag+ ions appear to be the species directly causing toxic effects. The present study aimed to determine the extent of dissolution of AgNP under a range of conditions. 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 AgI. Dissolution of AgNP is of fundamental importance, as dissolved Ag+ ions appear to be the species directly causing toxic effects. The present study aimed to determine the extent of dissolution of AgNP under a range of conditions.

Silver nanoparticles to aquatic organisms may be either due to the effects of dissolved silver ions or to specific effects of nanoparticles.
The toxic potential of nanoparticles in sewage plant processes 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 use in metal decontamination.

The ecotoxicity and behaviour of nanoparticles in the environment is yet almost entirely unknown. Manufacturing processes, handling, use and disposal increase their appearance in soil, water and living matter. Nanoparticles can reach the soil through the application of sewage sludge. This agricultural practice has potential for use in metal decontamination.

This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2011-0015985).
The functionality of the treatment process in terms of DOC-elimination and nitrification was not affected. The overall TiO₂ after exposure to the highest concentration of Cd²⁺ in the absence of polyhydroxyl fullerene. Membrane disruption and ROS accumulation decreased with increasing concentration of polyhydroxyl fullerene and with the increase of 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


University of Soroa/ Brazil, Brazil

Embrapa, São Carlos, Brazil

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 nanomaterials 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 cellulose nanofibers (white cotton, brown cotton, rubber cotton and gray 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 effects than other cottons than others. Furthermore, the results showed that the nanofibers are used 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

A. Laurent†, M.Z. Hauschild†, S.I. Olsen†, O. Jøller‡

Technical University of Denmark (DTU), DTU, Denmark

University of Michigan, Ann arbor, United States of America

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 current NO-modelling capabilities for quantifying the exposure, exposure effects of NO currently present 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 NO/polymerisation to provide a tool to model the health effects from inhalation of NO, with specific focus on TiO₂ and nanosilver. We apply a physiologically-based pharmacokinetic (PBPK) model to obtain the concentrations of NO 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 metric, 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 situations where the 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 vivo assays results to chronic in vivo effects for which we discuss opportunities for 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

A. Mozeta, D.R. Hristovski, A. Critto, A. Marcoumi

Univesità di Firenze, Venice, Italy

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. 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) has been developed and evolved 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 (environmental) exposure model is still required to support proper risk and impact assessment from nanomaterials.

TH 189
Mass balance of nanoscale titanium dioxide in laboratory wastewater treatment plants according to OECD 303

S. Gartner†, C. Nickel†, M. Stein†, S. Damme†, L. Erdinger‡, T.A.J. Kühbush‡

Hydrovex GmbH & Co. Kg, Germany

Institute of Energy and Environmental Technology e.V., Duisburg, Germany

Technische Universität Dresden, Dresden, Germany

IPEK, Heidelberg University Hospital, Heidelberg, Germany

Heidelberg University, Heidelberg, Germany

Titanium dioxide is used in huge amounts as white pigment in consumer products and industrial applications. The use of nanoscale TiO₂ is predicted to increase in the near future due to the efficiency of microparticle TiO₂ (> 0.7 µm) 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 TiO₂ in STP are available so far. In the present study the fate and behaviour of TiO₂ nanomaterials (P25 with an average particle size < 250 nm) in the laboratory sewage treatment plants simulation test according to OECD 303 has been performed. The nanomaterial were prepared with sodium metaphosphate (SMP) using an ultrasonic homogenizer and added under continuously stirring in a ten-fold concentrated suspension via a flexible-tube pump to reach a final concentration of 1, 5, and 10 mg L⁻¹ TiO₂ and 1, 5, and 10 µg L⁻¹ SMP. The nanosilver 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 were performed. The biodegradation of TiO₂ in the outflow and in the surplus sludge was measured via IC/ICP after HCl, HNO₃, HF digestion. The functionality of the treatment process in terms of DOC-elimination and nitritation was not affected. The overall TiO₂ balance indicated that 4% (trial 1) and 3% (trial 2) of the total TiO₂ added is discharged with the outflow of the STP and that more than 95% of the TiO₂ is adsorbed to the activated surplus sludge. Glass fiber filtering of samples indicated that the main part of TiO₂ in the outflow has to be attributed to particles below 0.6 µm. The TiO₂ balance showed a gap of 18% (trial 1) and 37% (trial 2) probably due to the recovery error of the OECD Guideline 303A. Significant time 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. The study was sponsored by the German Federal Environment Agency (UBA) (FKZ 3709 63 417) Supporting.

Bioaccumulation and effects of sediment-associated silver in different forms for the deposit-feeders, Capitella teleta and Macoma balthica

M. M. Gladkova, V. A. Terekhova

Roskilde University, Roskilde, Denmark

European Commission, Joint Research Centre, Ispra, Italy

University of Nebraska Lincoln, Lincoln, United States of America

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 metallic 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 factor) at the organism level, although Ag nanoparticles of different metals, Ag nano-particles were 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.

Silver nanoparticle toxicity in Caenorhabditis elegans

J.N. Meyer

Duke University, Durham, United States of America

We studied the short-term toxicity of silver nanoparticles (NPs) to a nematode 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 factors (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-acetylcysteine 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.

Importantly, the toxicity of the tested AgNPs was almost never greater than what 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.

Impact of zero-valent iron (ZVI) nanoparticles on soil microbial structure and functionality: a molecular approach


Universidad Complutense de Madrid, Madrid, Spain

Camps de Excelencia Moncloa, Madrid, Spain

Instituto de Investigacion y Desarrollo Rural Agrario y Alimentario, Madrid, Spain

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 for the removal of cost-effective toxic metals from soils and groundwater. 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, the use of binding agents containing nanoscale materials has led to a new generation of environmental remediation technologies for the removal of cost-effective toxic metals from soils and groundwater. Thus, 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.

Molecular toxicocology of nanosilver guided using in vitro prognosis

Y. Hayashi, L.H. Heckmann, P. Engmann, H. Antrup, D.S. Sutherland, J.J. Scott-Fordsmand

Aarhus University, Aarhus c, Denmark

University of Pécs, Pécs, Hungary

To study the molecular and cellular basis of silver nanoparticle (AgNP) toxicity, we used a recently established in vitro model of earthworm coelomocytes in comparison to the conventional in vivo molecular ecotoxicology approach. Compared to the latter, the in vitro test organism was exposed to NPs of defined size but held in an environmental matrix, in vitro models benefit from the ease of controlling exposure conditions in a defined set of biochemical milieu 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 protein addition. Physical characterisation of AgNPs, the size of silver ions 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 organ level (measured as total accumulated silver). The results indicate that the uptake of AgNPs to selected silver exposure to AgNPs result in 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 study. 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.

Zero-valent iron nanoparticles for soil remediation

M. Roda, N. Arroyo, A. Pérez Sanz, J. Alonso, M. Martin, M.C. Lobo

1, IMIDRA, Alcalá de henares (madrid), Spain

2, Universidad Complutense, Madrid, Spain.

 Soil contamination by the presence of pollutants and the need to manage needs 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, agricultural production, manufacturing, and land utilization of urban residues, have resulted in the contamination of our ecosystem, in particular, the soils. Among other pollution sources, mining activity contributes to the environment and human health as a result of their mobility and solubility. So, remediation strategies must be focused 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.

The bioavailability of these metals in soils was determined after a sequential extraction procedure, analyzing soluble, exchangeable, linked to carbonates and residual fractions. In back-extracted 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. The 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.

Acknowledgements. Projects CTM 2010-20617-C02-02 and S2009/AMB-1478.

Phytotoxicity of nano-TiO2 and effect of humus preparation

M.M. Gladkova, V.A. Terekhova

SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting
The objective of this study was to determine the bioactivity of nano-TiO$_2$ and to assess their toxicity in presence of humic substances (HS) which are natural components of soil necessary for higher plants growth.

In this study, we have chosen the well-known nanomaterials - nanodioxide titanium (nano-TiO$_2$), which are characterized <75 nm in size of particles in 10 weights % of water suspension, manufactured in the company "Sigma-Aldrich", USA. Nano-TiO$_2$ represents oxide the titanium (IV), the mix of two known crystal modifications of dioxide titanium - anatase and rutile.

According to the studies we have chosen "Pow-Humus" (le-PkK) (K-humate, originated from leonardite), manufactured by German firm "Humintech".

Concentration of nano-TiO$_2$ was varied in range 5-500 mg/l. Humate of le-PK 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-TiO$_2$ 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) but the most effect was shown at the higher concentration: probe with nano-TiO$_2$ 500 mg/l was toxic because the deviation of average roots length was more than 25 % from control.

Inhibitory effect of nano-TiO$_2$ was reduced in presence of humate Le-PK 5 mg/l. Samples with nano-TiO$_2$ 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-TiO$_2$ presence in growing medium 2); the degree toxicity of nano-TiO$_2$ depends on the concentration of nanoparticles; 3) humus substances eliminate the toxic effect of nano-TiO$_2$ 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

S.L. Gomes 1, A.M.V.M. Soares 2, J.J. Scott-Fordon 1, M.J.B. Amorim 1

1Department of Biology & CESAM, University of Aveiro, Aveiro, Portugal

2University of Aveiro & CESAM, Aveiro, Portugal

Aarhus University, Silkeborg, Denmark

University of Aveiro, Dept Biology & CESAM, Aveiro, Portugal

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 is important to provide toxicity data for these organisms, and if possible to understand more in regard the mode of action of AgNPs within organism. To study this we performed toxicology experiments using the OECD standard test, testing AgNPs and AgNO$_3$, and relating this to Ag$^+$ activity of the soil 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 (Ison selective electrode) in the soil 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


University of Seoul, Seoul, South-Korea (Rep)

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 toxicity data 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. The present study is 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 p38 MAPK pmk-1 in oxidative stress, DNA damage & apoptosis by using p38 siRNA knock down in mammalian in vitro system and Ag pmk-1 mutant C.elegans strain followed by AgNPs exposure and determine the toxic endpoint end points. This study highlights the importance of comparative toxicity approach in extrapolation of in vivo nanotoxicity data for human and ecological health evaluation.

Acknowledgements: This work was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology (2011-003214)

TH 198

Toxicity of ZnO nanoparticles on soil receptors compared with bulk ZnO and dissolved free ionic zinc

C.G. Garcia-Gomez,1 M. Babint,1 A.O. Obrador,2 J.M. Alvarez,2 C.R. del Rio,1 J.L.P. Pareja,1 G.D. Fernandez1

1INIA, Madrid, Spain

2ETH Agronomos (UPM), Madrid, Spain

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 environmental. To compare to their effects on soil microorganisms in oxidative stress, DNA damage & apoptosis by using p38 siRNA knock down in mammalian in vitro system and pmk-1 mutant C.elegans strain followed by AgNPs exposure and determine the toxic end point end points. This study highlights the importance of comparative toxicity approach in extrapolation of in vivo nanotoxicity data for human and ecological health evaluation.

Acknowledgements: This work was supported by Basic Science Research Program through the National Research Foundation of Korea(NRF) funded by the Ministry of Education, Science and Technology (2011-003214)

TH 199

Mixture toxicity of nano-TiO$_2$ and ingredients of personal care products

A.J. Schneider1, A. Meister-Werner2, R. Petro1, H.M. Mäs3, H. Hollert1, A. Schaaf3

1FWH Aachen University, Aachen, Germany

2Institute for Biological Analysis and Consulting (IBACON), Rossdorf, Germany

3In the last years many studies have focused on the ecotoxicology of nano-TiO$_2$. However only a few studies have investigated the mixture toxicity of nano-TiO$_2$ and organic contaminants. Mixture toxicity has to be considered during risk assessment of nano-TiO$_2$ because both substance groups often have the same entry pathway into the environment. Nano-TiO$_2$ and biocides are both ingredients of personal care products. Consequently, they have the potential to be released into water treatment plants and subsequently through biosolid amendment into soils.

In this study the mixture toxicity of nano-TiO$_2$ and TCC to terrestrial organisms is examined. The acute and chronic toxicity of nano-TiO$_2$ (10-1000 mg/kg dw) and TCC (42-675 mg/kg dw) together with either 400 or 1000 mg/kg dw of nano-TiO$_2$ to Eisenia fetida is tested in accordance to OECD 207 (1994) 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, LC$_50$ 40 mg/kg). Nano-TiO$_2$ 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 paper presentation.

The investigation of mixture toxicity of nanoparticles 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 Porcellio scaber

S. Tourinho1, C.A.M. Van Gestel2, C. Svendsen3, A.M.V.M. Soares4, S. Loureiro1

1University of Aveiro, Aveiro, Portugal

2Vrije Universiteit, Amsterdam, Nederland

The influence of soil pH on the toxicity of ZnO nanoparticles to the isopod Porcellio scaber


TH 200

The influence of soil pH on the toxicity of ZnO nanoparticles to the isopod Porcellio scaber

S. Tourinho1, C.A.M. Van Gestel2, C. Svendsen3, A.M.V.M. Soares4, S. Loureiro1

1University of Aveiro, Aveiro, Portugal

2Vrije Universiteit, Amsterdam, Nederland
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 stability of NPs and may alter their toxic effects. In the present study, NP stability was investigated in four different TiO$_2$ systems. 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. In this study, we compared the effect of four different TiO$_2$-NPs, as NPs and non-nano sized particles, and ZnCl$_2$ 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/o %), in order to achieve an extra soil pH of 7.3. Soil was then spiked with ZnO NPs (30nm), non-nano ZnO (200nm) or ZnCl$_2$. The isopod Porcellionides pruinosus was used at concentrations of 200 and 4000 mg Zn/kg dry soil. Juvenile 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$_{50}$ of the soil was measured at the beginning and at the end of the 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 an increase soil pHe of 0.3, whereas 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 ZnCl$_2$, 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 ZnCl$_2$, respectively. For both pH, results were observed at Zn concentrations at pH 4.5, but at pH 7.3, a 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 ZnCl$_2$, 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 ZnCl$_2$. 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 pH, and in a different way from the corresponding ionic Zn exposure based on ZnCl$_2$ salt.

**TH 201**

Effect of different TiO$_2$ nanoparticles on growth of Rhizobium trifolii in liquid culture

J. Moll, A. Gogos, M.G.A. van de Heijden, T.D. Bucheli, F. Widmer

Agroscope Reckenholz-Tänikon Research Station ART, Zurich, Switzerland

Initial results with two TiO$_2$ NPs - one with and one without coating, with concentrations between 200 mg/l and 1 mg/l - indicate that the different particles show similar effects and growth rates of rice cultures. Applied at concentrations between 10 mg/l and 1 mg/l, similar to those possibly used in practice, the two TiO$_2$ NP did not show any effects on R. trifolii growth as compared to controls without NP. The objective of further experiments will be to test 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

J.M. Hansch, C. Emmerling

University of Trier, Trier, Germany

The harmful effects of silver nanoparticles to microorganisms are commonly accepted. The application of contaminated sewage sludge to soil is the main potential pathway into the environment. Thus, 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 into the cell. For this purpose, we conducted two TiO$_2$ systems with different diameters and performed a sublethal study 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 203**

Do nanoparticles influence cell physiology and/or function?

A. Simon$^1$, H.M. Mues$^2$, H. Holller$^1$, A. Schaffer$^1$

$^1$RWTH Aachen-University, Aachen, Germany

$^2$RWTH Aachen-University, Institute for Environmental Research, Aachen, Germany

Silver nanoparticles (NPs) are the fastest growing NP class. Silver NPs are a new dimension of pollutants in the environment. A novel, non-invasive electrophysiological 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 silver nanoparticles ecotoxicological assessment: Novel electrophysiology biomarker and the potential role of P-glycoprotein in modulating toxicity in earthworms and aquatic organisms


TH 204

Uptake routes of silver nanoparticles in earthworms

M. D. S. Spurgeon$^1$, A. Simon$^2$, C. A. M. Van Gestel$^1$

$^1$Centre for Ecological and Hydrological, Wallingford, United Kingdom

$^2$Vrije Universiteit, Amsterdam, Netherlands

Thanes and effects of silver nanoparticles on earthworms are rare. In this study, the contribution of dermal and oral uptake of silver nanoparticles (AgNPs) 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 study the mechanisms by which silver nanoparticles enter into dermal and oral pathways into the body of the organisms exposed. Earthworms are representatives of 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 this purpose, experiments will be performed with the earthworm Lumbricus rubellus, applying silver nanoparticles (AgNPs) to soil of different pH values. Gilded and ungilded earthworms will be exposed to Luma 2.2 soil spiked with 100 and 500 mg kg$^{-1}$ dry soil AgNPs for 7 days. An exposure of gilded and unglided earthworms to soil spiked with 20 and 100 mg kg$^{-1}$ AgNPs will also be included to address the interaction of silver with organic matter. By measuring total silver body content, a comparison of both with 1/24, 5 for any of the Zn forms has been made. In 48 h following exposure to AgNPs and AgNO$_3$
concentrations of LC0.001, LC0.01, LC0.1, 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 treatments were renewed 2 times a week, taking 7 h to be used in the experiments. For AgNO3, 2 experiments were carried out, at both 7 and 8 h. In the first 3 h, the AgNO3 treatments resulted in a CV decrease of 20% and 50% for L. variegatus and A. caliginosa, respectively. These results were then confirmed using MGF cells with the toxicant alone. These results indicate that AgNP is more toxic than AgNO3 for both species in terms of behavioral changes.

The authors thank the EU FP7-funded ENPRA project, the MEI center, Osaka University, Osaka, Japan, and the MEI center, Osaka Research Consortium, Osaka, Japan, for providing financial support and technical assistance. The MEI center, Osaka University, Osaka, Japan, is acknowledged for providing the necessary facilities and expertise.

**References**


**Acknowledgement**

The authors are grateful to the EU FP7-funded ENPRA project for providing financial support and technical assistance. The authors are also grateful to the MEI center, Osaka Research Consortium, Osaka, Japan, for providing financial support and technical assistance. The MEI center, Osaka University, Osaka, Japan, is acknowledged for providing the necessary facilities and expertise.

**Conclusions**

The results of this study indicate that AgNP is more toxic than AgNO3 for both species in terms of behavioral changes. This is consistent with previous studies that have shown that AgNP is more toxic than AgNO3 for various species, including insects and fish. Therefore, it is important to consider the potential toxicity of AgNP in future research and regulatory assessments.

**Keywords**

AgNP, AgNO3, toxicity, behavioral changes, L. variegatus, A. caliginosa.

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**TH 206**

A weight of evidence approach for occupational risk assessment of engineered nanomaterials

D.R. Hristovski, S. Gottardo, A. Zabori, A. Critto, A. Marcocini

University Ca Foscari Venice, Venice, Italy

Venece Research Consortium, Venice-marghera, Italy

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 their decision-making. In this context, gaps have been gradually filled by new emerging tools like the FPA or the MixTox approaches. It is preliminary to use these tools to provide regulatory guidance, but they have been widely used in the literature for the last 5 years. The MixTox approach is based on the combination of single-agent toxicokinetic and pharmacokinetic data with that of the mixture, using FPA to define the toxicological effect of the mixture. MixTox has been demonstrated to be an effective tool to assess the synergistic or antagonistic effects of mixtures of agents, which is important when assessing the risk of ENMs.

The authors thank the EU FP7-funded ENPRA project for providing financial support and technical assistance. The authors are also grateful to the MEI center, Osaka Research Consortium, Osaka, Japan, for providing financial support and technical assistance.

**References**

[1] Hiroyuki Kurosawa

**Acknowledgement**

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**Keywords**

AgNP, AgNO3, toxicity, behavioral changes, L. variegatus, A. caliginosa.

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**TH 207**

Toxicity assessment of nanoclay Clay2, on human hepatic cell line HepG2

M. Patra, M. Pedrazzoli, M. Jorí, J.M. Bermúdez, S. Aucejo, A. Joss

University of Seville, Seville, Spain

Wageningen University and Research Centre, Wageningen, Nederland

Area of Packaging Materials and Systems, ITENE, Valencia, Spain

Nanoparticles (NPs) attract a great deal of attention due to their unique properties. Their high surface to volume ratio, specific chemical composition, surface structure and solubility, shape and aggregation effects in matrices that are qualitatively different from their bulk counterparts. These properties make them suitable for numerous applications. The NPs are also emerging technology. The most promising uses of NPs in the food industry are in food packaging. Dispersions 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, 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 a result, several NPs will be transferred from the packaging to the food products created also lead to Hansen contaminates in the NPs. At larger concentrations of NPs, the Hansen contaminants in the NPs will be transferred to the food products. To study the NPs effects on HepG2 cells, the nanoclay Clay2 has been selected due to its toxicological potential and unknown toxicity of these particles on biological systems. Since NPs can differ in their physico-chemical properties, their behaviour characteristics in solutions, and their effects on cells, it is important to study the toxicity of NPs.

The authors thank the EU FP7-funded ENPRA project for providing financial support and technical assistance. The authors are also grateful to the MEI center, Osaka Research Consortium, Osaka, Japan, for providing financial support and technical assistance.

**References**

[1] Hiroyuki Kurosawa

**Acknowledgement**

The authors thank the EU FP7-funded ENPRA project for providing financial support and technical assistance. The authors are also grateful to the MEI center, Osaka Research Consortium, Osaka, Japan, for providing financial support and technical assistance.

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**TH 208**

Characterization of aqueous metal nanoparticles and assessment of exposure on immune cell viability and effector function

A. Ortega, C. Riopelle, J. Stafford, G. Goss

University of Alberta, Edmonton, Canada

The authors thank the EU FP7-funded ENPRA project for providing financial support and technical assistance. The authors are also grateful to the MEI center, Osaka Research Consortium, Osaka, Japan, for providing financial support and technical assistance.

**Conclusions**

The results of this study indicate that AgNP is more toxic than AgNO3 for both species in terms of behavioral changes. This is consistent with previous studies that have shown that AgNP is more toxic than AgNO3 for various species, including insects and fish. Therefore, it is important to consider the potential toxicity of AgNP in future research and regulatory assessments.

**Keywords**

AgNP, AgNO3, toxicity, behavioral changes, L. variegatus, A. caliginosa.

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**TH 209**

Cellular and genomic effects of nanoparticles on human cells

A.Y. Yamashita, M. Katsuyama, N. Negri, B.B. Burlando

University of Eastern Piedmont “A. Avogadro”, Alessandria, Italy

The authors thank the EU FP7-funded ENPRA project for providing financial support and technical assistance. The authors are also grateful to the MEI center, Osaka Research Consortium, Osaka, Japan, for providing financial support and technical assistance.

**Conclusions**

The results of this study indicate that AgNP is more toxic than AgNO3 for both species in terms of behavioral changes. This is consistent with previous studies that have shown that AgNP is more toxic than AgNO3 for various species, including insects and fish. Therefore, it is important to consider the potential toxicity of AgNP in future research and regulatory assessments.

**Keywords**

AgNP, AgNO3, toxicity, behavioral changes, L. variegatus, A. caliginosa.

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**TH 210**

The basic safety assessment of fullerene C60 after oral administration


Osaka University, Osaka, Japan

MIET center, Osaka University, Osaka, Japan

National Institute of Biomedical Innovation (NIBIO), Osaka, Japan

Vitamin C60 BioResearch Corporation, Tokyo, Japan

Summary: More recently, the development of nanomaterials with particle sizes below 100 nm is promoted extensively. These nanomaterials have already been 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 developed 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.

Acknowledgement: This study was supported in part by Health Labour Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan.

TH 211
In vitro effects of amorphous nanosilica particles on the bone metabolism
H. Nabesh1, T. Yoshikawa1, T. Akase1, T. Yoshida1, T. Hirai1, M. Uji1, H. Takahashi1, K. Misato1, K. Ichihashi1, A. Udaka1, T. Morii1, Y. Yoshokoya1, Y. Tsusumi1
1Graduate school of Pharmaceutical Sciences, Osaka University, Suita, Japan

Summary: Recently, applications of amorphous nanomaterials 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 concentrations of nSPs are often used in oral care products, it is important to investigate the effects of nSPs on human oral health. In this study, we evaluated the potential adverse effects of amorphous nanosilica particles (nSPs) on osteoclastogenesis of RAW264.7 cell line, a murine macrophage cell line. The results suggested that the osteoclastogenesis inhibitory effect of nSPs was observed at a low concentration of 10 µg/mL.

TH 212
Surface interaction of coagulation factor and amorphous nanosilica particles plays critical role for acute toxicity
T.Y. Yoshida1, T. Yoshikawa1, H. Nabesh1, K. Matsuyma1, Y. Nakazato1, S. Tomig1, T. Hirai1, M. Uji1, K. Ichihashi1, T. Akase1, Y. Yoshokoya1, Y. Tsusumi1
1Graduate School of Pharmaceutical Sciences, Osaka University, Osaka, Japan

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 and foods. Thus, the exposure against NMs is unavoidable for us in our living environment. Under this circumstance, there is increasing concern about 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/vitro distribution analysis by transmission electron microscopy. In previous study, we examined in vivo distribution of oral 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 intrinsic coagulation factors could be suppressed, the safety of nSP70 may be ensured. As a result, the calculated quantiative and exposure level of nSP70 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
C. Steenken1, A. Coolsen1, M. Hazelslo1, S. Loureiro3, C.A.M. van Gestel1, F. Dondero1, A. Johnson1, L.A. Walker1, D. Spurgeon1
1Centre for Ecology and Hydrology, Wallingford, United Kingdom
2University of Piemonte Orientale, Alessandria, Italy
3University of Aveiro & CESAM, Aveiro, Portugal

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 hindering sound assessment of environmental risks posed by engineered nanomaterials (ENPs). Our vision is to assess environmental and human health risks of nanomaterials with particle size below 100 nm, because ENPs 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 intrinsic coagulation factors could be suppressed, the safety of nSP70 may be ensured. As a result, the calculated quantiative and exposure level of nSP70 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 242

Does probabilistic approach relevant to derive Health occupational exposure levels limits?


Institut national de la recherche agronomique, Paris, France

In order to derive Occupational exposure levels professional (OELs) for hazardous substances, the scientific community needs to find a robust tool for risk assessment to ensure the appropriate and safe working conditions of workers. We compare a deterministic, a two-stage, and a probabilistic approach for the derivation of OELs. We present a probabilistic approach for the derivation of OELs. We compare the deterministic, a two-stage, and a probabilistic approach for the derivation of OELs. We present a probabilistic approach for the derivation of OELs.

Current methods for risk assessment are mostly determinist. 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 toxicities.

Current methods for risk assessment try to avoid 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 what values within the range are most likely. The result of a probabilistic risk assessment can also be shown as a distribution, showing which impacts within the 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 levels professional (OEL). OELs for hazardous substances are 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

M. Swarooski, E. Streisik, S. K. Bopp

European Food Safety Authority, Parma, Italy

EFSA, Parma, Italy

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 ecosystem function) a reference tier should be identified, based on the most sophisticated experimental or modeling 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 of the lower tier risk assessment using the new SPGs. To do so we used published data from Draft assessment reports that have recently been summarized by EFSA in a database. Results will be presented at the conference.

References:


TH 244

How much of a difference in assessing ecological risk between effects of organism-level and population-level?

B. Lin

RISS/AIST, Tsukuba, Japan

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 (requiring more ecotoxicity data than organism-level), assessing the ecological risk of toxic chemicals 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 (PRERAs) for risk calculation from approaches of organism-level and population-level. To derive the reference value defined as Clamped-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

F. Gabris, M. Hammers-Wirtz, A. Schaffer, T. G. Preuss

Institute for Environmental Research - RWTH Aachen University, Aachen, Germany

Research Institute for Ecosystem Analysis and Assessment (giax), Aachen, Germany

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 both chronic and acute data. The model was validated using GUTS 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 model predicted the 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 risk. 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 were considered separately for risk rating, 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

F. Nicotte, C. Colombo, R. Wening, M. Sorensen, S. Deacon

ENVIROS International Corporation, Atlanta, United States of America

ENVIRON Italy, Milan, Italy

ENVIRON, Box, United Kingdom

Remote 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) of the proposed actions. Incorporating 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.
service value) associated with the implementation of various remedial alternatives based upon prescribed protection goals. In this regard, a formal quantification of the effect of remedial actions 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 that can demonstrate that remediation can improve or protect 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 NICER project (National Institute for Public Health and the Environment) have been developed to provide a comprehensive framework for improving exposure scenarios within REACH system, for monitoring environmental health, and for increasing degree of circularity of resource and substance flows.

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 case study 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? E. Bonneris1, R. Charge2, G. Weyman3
1, J. Spijker1
1, R. Dittrich2
2, G. Weyman
3
SETAC 6th World Congress/SETAC Europe 22nd Annual Meeting

Bird community monitoring in chlorpyrifos-treated citrus. Results of years 1&2 of 3 year program in Valencia region
M. Hahn, A. Schotthöfer, M. Geisthardt, J. Schmitz, P. Lenhardt, C. A. Brühl

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TH 249
Caterpillars and protection goals: the role of field margins as habitats and the effects of pesticide applications
M. Hahn, A. Schotthöfer, M. Geisthardt, J. Schmitz, P. Lenhardt, C. A. Brühl

TH 250
Bird community monitoring in chlorpyrifos-treated citrus. Results of years 1&2 of 3 year program in Valencia region
M. Hahn, A. Schotthöfer, M. Geisthardt, J. Schmitz, P. Lenhardt, C. A. Brühl

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
F. C. Colt, E. Bonneris, S. Barkevitch, M. Ebelling, R. Juliard

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2. European Commission Environment Directorate General, Oct. 2007, REACH in brief
Screening metal impacts from tissue residues for a calibrated biomonitor using DYM BAM and monitoring data in 6 Luxembourgish rivers

T. Gallé, S. Massarin, R. Carafa

CRP Henri Tudor, Ech-sur-Alzette, Luxembourg

Metals remain a pollution source for most rivers although they have been pushed from the limelit 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 DYM BAM and the calibrated biomonitor for hydropore 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 hydropore assuming that it feeds on suspended matter (hydropore 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 well as to suspended matter concentration) and elimination to run an uncertainty analysis in STELLA with the DYM BAM equation for 6 differently polluted sites in Luxembourg. Results showed the dynamic 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-threshold suspended matter has a strong influence on the results. As for the DYM BAM parameters: uptake from the water phase had a negligible part in tissue residues while ingestion rate and in particular elimination rates where governing the metal concentration. The results showed a need 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.

Active monitoring programs: useful tools for monitoring effects of some plant protection products on birds and mammals - Feedback on a French approach

T. Quintaine, R. Bonneris, R. Barfknecht, P. Radui, A. Decors

1ONCF, Auffargis, France
2Bayer CropScience, Lyon, France
3Bayer CropScience AG, Monheim, Germany

Bird and mammal risk assessments for plant protection products are carried out under EU regulation 1107/2009 using the guidance document issued by EFSA. This document outlines the conservative first-tier assessment procedure for a large range of crops/uses dependent scenarios and proposes different options for refinement if needed. Field experiments were conducted in France. 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-authorisation monitoring data may be additionally useful to assist in the regulatory process. In this case, the majority of the investigations performed in France, particularly those conducted in citrus orchards, have been undertaken for the purpose of risk assessment. The results of these studies are presented in this congress. In particular, it should be noted that the Sardinian warbler (Sylvia melanocephala) has been the subject of several studies in Spain and France with the objective of assessing the potential impact of chlorpyrifos on this species through several population monitoring approaches. However, many of these studies were conducted before the publication of the EFSA Guidance Document on Risk Assessment for Birds & Mammals and the results obtained were not fully aligned with the EFSA recommendations. In this context, a case study is presented in this congress. The Sardinian warbler is the smallest predominantly insectivorous bird species inhabiting conventionally treated Spanish citrus orchards, where chlorpyrifos is regularly applied to protect the crops and fruits from pests and diseases. The population status of this species in citrus orchards has been monitored in Spain since the early 1990s. The results obtained in this study indicate that chlorpyrifos treatment does not have a significant impact on the population dynamics of the Sardinian warbler in citrus orchards. However, the impact of other plant protection products, such as neonicotinoids, on the population dynamics of the Sardinian warbler should be further investigated in the future. In conclusion, the results of this study demonstrate the usefulness of active monitoring programs for assessing the potential impact of plant protection products on birds and mammals, and highlight the need for further research to better understand the ecological effects of these products.
Gutierrez 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? J. Pistorius1, I.P. Joachimsmeier1, U. Heimbach2, D. Schenke1, W. Kirchner2 1JKL, Braunschweig, Germany 2Ruhr-Universität Bochum, Bochum, Germany

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, such as guttation flow, it will be necessary to estimate 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 75m distance to treated crop. From August to November 2011 the mortality of bees was assessed on a weekly basis and 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

G.C. O’Brien1, N.J. Smi2, V. Wepener1, N. West1, J. Vonk1 1University of Johannesburg, Aucklandpark, South Africa 2North West University, South Africa

The Umvoti River is a developed, highly utilised, stressed aquatic ecosystem that is often referred to as a ‘working river’. Potential sources of impacts include numerous urban and rural areas and associated activities, informal settlements, agricultural activities and forestry industries, activities, 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 fit of these outcomes. Various sensitivity and validity tests were applied 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 alterations. 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 source. The model also uses a distance to 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 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

G. Lomack, A. Byer, H.M. Summers 1Western Washington University, Bellingham, United States of America

The stressors and their relative contributions to risk assessment of regional risk assessment lends itself to the construction of Bayesian networks (BNs) for the calculation of the 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 incorporates 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 one of the eastern US east coast, 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 sensitivity analysis will be explored 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

L. Roche1, S.J. Marshall1, O.R. Price1, M.J. Whelan1, A.B. Gilf2 1University of Edinburgh, United Kingdom 2Cranfield University, Bedfordshire, United Kingdom

Discharge of untreated domestic wastewater into rivers can result in high concentrations of unionised ammonia (NH3), nitrite (NO2) and biochemical oxygen demand (BOD). Such chemicals are used in home and personal care products (HPCPs), where aquatic organisms can be severely impacted by conventional waste water constituents (sanitary determinants), 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 source. The model also uses a distance to 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 261

Effects of toxic and non-toxic stressors on fish community in the fish community in the Rhine

A. Fedorenko1, J. Voisk1, H.J.R.B.O. Benders1, A.N.T.O.N. Breure1, A.J.A.N. Hendriks1, R.S.E.W. Leuven1 1Radboud University Nijmegen, Nijmegen, Netherlands

Predation and fish predation 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 challenge is the identification of ecological conditions for the Rhine. The fish community in the Rhine has been intensively studied and much effort has been put into the ecological study of fish diversity and habitat. The diversity, richness and composition of the fish community in the Rhine has changed remarkably since the 1970s due to both the species-dependent sensitivity of species and the introduction of invasive species. Therefore, we hypothesize that the sensitivity of the fish community 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, water quality). The following purposes are pursued: (1) to determine the identity and tolerance in toxic and non-toxic stressors is the level of the river Rhine and (2) to 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

N.M. Pain-Devins1, S. Devins1, C. Cossu-Leguille1, A. Gefard1, L. Giambérrini1, T. Jouenne1, L. Minguet1, M. Param1, F. Rodius1, P. Rousseille1, N. Brulé2, K. Tarnowska1, C. 1Dutch National Institute for Public Health and the Environment, Bilthoven, Netherlands 2Radboud University Nijmegen, Nijmegen, Netherlands

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 challenge is the identification of ecological conditions for the Rhine. The fish community in the Rhine has been intensively studied and much effort has been put into the ecological study of fish diversity and habitat. The diversity, richness and composition of the fish community in the Rhine has changed remarkably since the 1970s due to both the species-dependent sensitivity of species and the introduction of invasive species. Therefore, we hypothesize that the sensitivity of the fish community 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, water quality). The following purposes are pursued: (1) to determine the identity and tolerance in toxic and non-toxic stressors is the level of the river Rhine and (2) to 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.
Temperature-dependent toxicity: is an assessment factor of 10 appropriate for ecological risk assessment? 

T.C. E. Lau, J. Li, J. Li, Y. G., N. E. Karraker, K. M. Y. Leung*  
*The University of Hong Kong, Hong Kong, Hongkong 
**The Institute of Marine Sciences, Hong Kong, Hongkong 

Laboratory test results 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 toxicity tests are run at a constant temperature which usually represents the temperature for the reference condition. Changes in temperature may influence toxicity 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 laboratory-dependent temperature variability adequately. Here, we compared median lethal concentration (LC50) data of several seaweed 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 (1TUref). The relative differences in the copper toxicity to the same species at different temperatures (T) expressed as a relative TU value (RTU) which is equal to LC50ref/LC50. A smaller RTU indicates a higher toxicity. For copper, we detected an average decrease of 0.139 TU/ºC increase in temperature and a decrease of 0.076 TU/ºC decrease in temperature. 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 20ºC, while 30ºC was found to be sub-lethal for simulating physical stress. Chemical stress was reproduced at the level of EC30 for each toxicant (3, 850 and 500 mg/L for Cu and Cr, respectively) with an increased level of lipid peroxidation (LPO) indicating oxidative damage and inhibition of cytochrome oxidase activity indicating alterations of chloroplastic machinery. Further, at 20ºC 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 copper and/copper and even more at high temperatures.
TH 268

Acute versus chronic temperature dependence of cadmium toxicity in zebrafish

L. Viana, A. Hagemann, I. Lust

Universiteit Antwerpen, Antwerpen, Belgium

Toxicity studies are mainly performed in standardized conditions, most often at the optimal rearing temperature of the test organism. However, environmental temperature has a large influence on cadmium toxicity. The impact of temperature on toxicity is generally receptor-mediated (bioaccumulation 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, 284 or 435 µM cadmium at 18, 26, 30 or 34°C for 5 days. 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 (6 hr at +10°C over seasonal local sea water temperature) and 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 haemocyte count, morphological differences in haemocytes, 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 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.

TH 269

The effects of thermal anomalies in mussels exposed to Cd at different seasons: cell and tissue level biomarkers

M. Muigca, U. Izagirre, I. Marigomez

UVU/UEHU, Leioa, Spain

Mussels 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 is still a matter of debate. In this study, the effects of temperature on Cd bioaccumulation and biological responsiveness were evaluated in mussel samples collected in coastal Galicia (Biscay Bay) in summer 2011 and winter 2012 and acclimated to low 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 exposed to temperature for the following 5d. At Day 21, haemolymph Cd and Cu were determined in mussel haemolymph. 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 stress. 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 stress.
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°C, 12°C, 20°C, 24°C and 28°C), using survival and biomass loss as endpoints. For this standard acute tests were performed, and adaptions 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 interactive action (IA) and synergistic or antagonistic interactions, dose-level and dose-ratio dependency were used. From the results we can changes the toxicity in 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 F.N. Cardoso1, M.P. Lima1, A.M.M. Saura1, J. Lourenço1, A. Guimarães1, S. Mendo1, S. Loureiro1
1CESAM/ Aveiro University, Aveiro, Portugal
2University of Aveiro & CESAM, Aveiro, Portugal
Terrestrial isopods 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 the study effects of carbaryl to soil-dwelling collembolan Folsomia candida at different abiotic conditions, evaluating their reproductive effort and genotoxicity. Different concentrations of carbaryl were tested in natural stressors such as 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 M.I. Mietelka, D.M. Jortve, R. Laskowski
Institute of Environmental Sciences, Jagiellonian University, Krakow, Poland
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 than copper contamination. However, the interspecific interactions could be more important than copper contamination for 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 experimental design to determine 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 275 Toxicity of copper, zinc and nickel mixtures to fathead minnows (Pimephales promelas): implications for Biotic Ligand Model for metal mixtures T.C. Hoang, N.R. Lynch
Loyola University Chicago, Chicago, United States of America
Residues and effluents in aquatic environments have been extensively monitored. However, the 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 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 was 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 exposure mixture. Similarly, mortality (97%) produced by a mixture of 200 µg/L Cu and 400 µg/L Zn was greater than the total mortality (40%) produced by individual Cu and Zn. Similarly, mortality (97%) produced by a mixture of 200 µg/L Cu and 400 µg/L Zn was greater than the total mortality (40%) produced by individual Cu and Zn. Similarly, mortality (97%) produced by a mixture of 200 µg/L Cu and 400 µg/L Zn was greater than the total mortality (40%) produced by individual Cu and Zn. Similarly, mortality (97%) produced by a mixture of 200 µg/L Cu and 400 µg/L Zn was greater than the total mortality (40%) produced by individual Cu and Zn. Similarly, mortality (97%) produced by a mixture of 200 µg/L Cu and 400 µg/L Zn was greater than the total mortality (40%) produced by individual Cu and Zn. Similarly, mortality (97%) produced by a mixture of 200 µg/L Cu and 400 µg/L Zn was greater than the total mortality (40%) produced by individual Cu and Zn. Similarly, mortality (97%) produced by a mixture of 200 µg/L Cu and 400 µg/L Zn was greater than the total mortality (40%) produced by individual Cu and Zn. Similarly, mortality (97%) produced by a mixture of 200 µg/L Cu and 400 µg/L Zn was greater than the total mortality (40%) produced by individual Cu and Zn.

TH 276 Health of and contaminants in fishes from the Slave/Athabasca River System, Canada, relative to oilsands extraction activities D. Jones, E.H. Ohnozeba1, A.H. Hil1, B.T. Tendler1, J.G. Gnesi1, P.H. Hodson2, E.K. Kelly1, J.S. Short1, G.C. Codling1
1University of Greenwich, Medway, United Kingdom
2University of Calgary, Calgary, Canada

TH 277 Influence of environmental factors on the response of Daphnia magna population to pesticide applications L. Dolciotti, K. Fost, A. Herkelrath, M. Katrinke, M. Liess
UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany
The potential risk of pesticide effects has been confirmed using standard toxicity tests on Daphnia magna cultured individually. However in the environment organisms experience abiotic and biotic interactions like inter-specific and intra-specific competition that are of key importance to obtain a response to toxicants’ activity. Moreover, input related to water qualities from agrochemicals typically occurs in pulses or in repeated exposures due to agricultural runoff and spray drift. However, the literature on the ecological and physiological responses in populations to fluctuating and repeated pollutant exposure is very limited. In our mimicry test system we investigated how inter-specific competition affects the response of Daphnia magna to pulses of pesticide. Daphnia magna were cultured to exhaustion 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 concentrations c1, c2, c3, 10, 24, 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 toxicological and physiological responses of populations to fluctuating and repeated pollutant exposure is very limited.
The cell transformation assay (CTA) is 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 Kd values to predict the solid-solution partitioning (log Kd) for selected elements in soils of the GeoEcoSurveys geochemical mapping of agricultural and grazing land soils of Europe (GEMAS). The strong spectral correlations for the partitioning of metals in soils were found to be in the range for carbonate (2500, 1810, and 1550 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 1700-1710 cm⁻¹). The PLS regression plus soil pH models were found to predict experimental log Kd values for volatile (Co, lead (Pb), manganese (Mn), nickel (Ni), and zinc (Zn)), non-volatile (V, W, Mo, Os, Se, Bi, and Pt), antimony (Sb), vanadium (V), tellurium (Te), and molybdenum (Mo) with moderate accuracy (R²=0.6 to 0.8), and copper (Cu), selenium (Se) and silver (Ag), and tin (Sn) with poor accuracy (R²<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.

Levels and trends of PBDEs and PBBs in fish (Rutilus rutilus and Cyprinus carpio) and sediment from South Kazakhstan

Evaluation of carcinogenic potential of perfluorinated compounds in vitro and in silico alternative approaches

How to cope with variability in laboratory aquatic gnotobiotic microcosms?

Evaluation of carcinogenic potential of perfluorinated compounds in vitro and in silico alternative approaches

Differences between dossier-based PNECs and EQSs based on all available literature: the case of Imidacloprid

Derivation of quality standards for protection products - the importance of open literature

How to cope with variability in laboratory aquatic gnotobiotic microcosms

Evaluation of carcinogenic potential of perfluorinated compounds in vitro and in silico alternative approaches

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 critical endpoint.
Toxicity of 9 shampoos to Daphnia magna and their detoxification by activated sludge
R.G. Dave, K.B. Boll
University of Gothenburg, Gothenburg, Sweden

Shampoos are primarily used for hair care, 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 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 glycolide, C6 alkyl glycolide, sodium caprylylmethylpyrrolidone, 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 24h EC50s ranged from 2 μg L⁻¹ for the most toxic substance (zinc pyrithione) to 2 g L⁻¹ for the least toxic compound (C8 alkyl glycolide). 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 assessed 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 observed 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.

Sediment toxicity testing for prospective risk assessment - a new framework and how to establish it
M.A. Bektov, N. Cedergreen, I.Y. Wick, M. Kattwinkel, S. Duquesne, M. Liess
UFZ - Helmholtz Centre for Environmental Research, Leipzig, Germany

The development of ecologically realistic and practical sediment toxicity testing for prospective ecological risk assessment remains a considerable challenge. There is a recognized 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 strategies to the key methodological challenges that hinder establishment of this framework. Strategies include, for example, different exposure routes, adaptations 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, relative sensitivity distribution analysis, toxicity tests that combine bacteria and arthropods, micro- and mesocosm 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.

Improved OECD 105 water solubility test design
P.C. Thomas, V. Burosse
CEHTRA, Vaulx milieux, France

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 normally formally excluded, the column elution can therefore be used in cases that are 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 difference in solubility values 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.

Assessment of Cadmium effects using standard single-species and non standard microcosm bioassays
B.P. Clement, H.E. Delahay, G.E. Triffault-Bouchet
1ENTPE, Vaux en velin, France

1University of North Texas, Denton, United States of America

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 freshwater minnow 96 h toxicity database was analyzed. Approximately 8% of the tests failed assumption 1 validation and should be considered invalid. 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. Furthermore, failure to comply with assumption 3 validation. Improvements in toxicity test design and interpretation, including explicit specification of models, routine validation of exposure times (lack of steady-state), metabolic biodegradation, and body size differences, remain embedded in LC50 estimates rendering them inaccurate 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 freshwater minnow 96 h toxicity database was analyzed. Approximately 8% of the tests failed assumption 1 validation and should be considered invalid. 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.

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1, S. Duquesne
1, M. Liess
2, L.Y. Wick
1, M. Kattwinkel
1, S. Duquesne
1, M. Liess

University of Copenhagen, Frederiksberg, Denmark

The tests produced results from standard protocols that are 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 freshwater minnow 96 h toxicity database was analyzed. Approximately 8% of the tests failed assumption 1 validation and should be considered invalid. 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.
Influence of pH, light cycle, and temperature on ecotoxicity of four sulfonyleurea herbicides towards Lemma gibba

R.T. Rosenkranz1, N. Cedergreen2, A. Baun3, K.O. Kusk3

1Technical University of Denmark, Kgs. Lyngby, Denmark
2University of Copenhagen, Department of Basic Sciences and Environment, Copenhagen, Denmark

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 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 compounds, and thereby obtain a better basis for risk assessment extrapolations.

As model compounds four sulfonyleurea herbicides (SU), 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 SU's 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 SU's. Decreasing the pH below the standard test value resulted in no change or an increase in EC50. In general, the EC50 increased between 2.2 and 10 times for the four SU's 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.

New short term toxicity test using single algal species and natural phytoplankton

R.T. Rosenkranz, D. Cupi, A. Baun, K.O. Kusk

Technical University of Denmark, Kgs. Lyngby, Denmark

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 (eNPs) 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 algal 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 pharmaceuticals, carbamates, acetylcholinesterase (AChE) 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 AChE inhibiting herbicides neither for the natural algal community nor the laboratory concentration at the test conditions.

Quality evaluation of existing critical body residue data for baseline neutral narcosis

L.S. McCarty1, J.A. Arndt2, D. Mackay3

1L.S. McCarty Scientific Research & Consulting, Newmarket on, Canada
2University of Toronto at Scarborough, Toronto, Canada
3Trent University, Peterborough, Canada

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 chemical exposure database available. A data quality evaluation of available CCB-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 effects criteria (e.g., toxic effect, mixing toxicity, and experimental conditions) that were reduced to 9 effects reported metrically. 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. The quality screening evaluation proceeded by rating data either as poor (4 rules) or rejected (11 rules). The rules were based on experimental issues such as number of replicates and quality of exposure to interpret data according to the Verhaar classification scheme. The final evaluation was compared with baseline neutral narcosis from 14 chemical subgroups, the establishment of a link between cadmium concentration and effects, and thus the comparison of simple single-species tests and microcosm assays. However, these 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.

Validity criteria from standard method: the need for assessor common understanding

C. Adékolu1, B. Jørgensen1, P. Adékolu, P.E. Thomas1

1CEHTRA, Sainte-éulalie, France
2CEHTRA Europe SPRRI, Brussels, Belgium

CEHTRA methodology, as recommended and internationally agreed test methods (e.g. OECD Test Guideline, IU Testing Methods). Test guidelines are well described providing technical information on principles 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 appropriate validation and evaluation is required. This is why common understanding of appropriate validation/inclusion is required. The framework for this work is to describe the lack of common understanding of one of the validation criteria: the guideline for the introduction of correct assessment of toxicity of Freshwater Algae and Cyanobacteria, Growth Inhibition Test. We explore the implications for reliability assessment and the possible ways to increase assessor common understanding of the appropriate test method and evaluation.

Endpoints and dose-response relationships of low-dose and standardized studies of Bisphenol A

L. Molander1, A. Beronius2, A. Hanberg3, C. Rudén3

1Royal Institute of Technology (KTH), Stockholm, Sweden
2Karolinska Institute, Stockholm, Sweden
3Karolinska Institute, Stockholm, Sweden

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
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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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T.A. Moermond, R. van Herwijnen
RIVM, Bithoven, Nederland

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=unassignable). 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 presented makes it difficult to use them as ‘publication sources’ or ‘study report’, or ‘grey literature’. Thus, public literature is included in the dossiers, 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 the dossiers, a number of scores may be applied to a record, only 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.

Recent EQS 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 Plocus cupreus

C.A. Jenkins, D. Gray
Huntingdon Life Sciences, Suffolk, United Kingdom

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 Plocus 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 introduced 24 hours after application. Control treatments were applied using the same methods. Post application one Musca domestica fly pupa 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.

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Modification of the OECD 117 Partition coefficient method using the HPLC method

V. Burse1, J. Kurtz2, P. Thomas3
1CHETRA, Vailly milieu,2France

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 is 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-slip 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 < 200, 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 demonstrated to validate 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 structures a mirror image to a given test substance. A total of 18 candidates between 6.57 and 10.89 was identified, among which 9 of analytical grade are commercially available. However, the 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 (Ecosom)

S.K.E. Damme1, H.T. Ram1, A. Schaffer2, H. Henner2, K. Rettinger2, J. Steber2, A. Coors4, T. Knacker4, H. Schulze4
1FIRMENICH SA, Geneva, Switzerland
2ECT Oekotoxikologie GmbH, Flörsheim, Germany
3German Cosmetic Toiletry, Perfumery and Detergent Association, Frankfurt am main, Germany
4RWTH Aachen, Aachen, Germany

German Cosmetic Toiletry, Perfumery and Detergent Association, Frankfurt am main, Germany

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, high lipophilicity and thus extensive partition 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 soluble approach' is one promising approach 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 (ETNCaqu) 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 ETNCaqu = 1,9 μg/l. For the screening of potential ecotoxicological substances with PBT-properties the so-called 'simplified approach' also seems to be convenient. Due to its potential relevance for assessing the environmental risks of highly lipophilic substances, the scope of the project Ecosom 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 pass 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

E.M. Mihaičič1, L.S. Ortego2, C.A. Staples3, N. Caspers4, G.M. Klecka5, S.S. Dimond6, S.G. Hentges7
1Environmental & Regulatory Resources, Durham, nc, United States of America
2Bayview Chemical Company, Triangle park, nc, United States of America
3Assessment Technologies, Keswick, va, United States of America
4Currenta, Leverkusen, Germany
5German Cosmetic Toiletry, Perfumery and Detergent Association, Frankfurt am main, Germany
6SABIC Innovative Plastics, Pittsfield, ma, United States of America
7American Chemistry Council, Washington, dc, United States of America

Apacl 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 pressure to make 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), an important high production volume compound used in the production of epoxy resins and polycarbonate plastics, 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 growth, survival 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,