Abstract Book

SETAC North America 34th Annual Meeting

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This book comprises the abstracts of the presentations for the platform and poster sessions of the 34th Annual Meeting in North America of the Society of Environmental Toxicology and Chemistry (SETAC), conducted at the Gaylord Opryland Hotel and Convention Center in Nashville, Tennessee, 17–21 November 2013. The abstracts are reproduced as accepted by the Scientific Program Committee and appear in numerical order. In each abstract, the presenting author's name is underlined.

The author index cross-references the corresponding abstract numbers. Affiliation, session, and keyword indices are also included.

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SETAC

A Professional Society for Environmental Scientists and Engineers and Related Disciplines Concerned with Environmental Quality

The Society of Environmental Toxicology and Chemistry (SETAC), with offices in North America and Europe, is a nonprofit, professional society established to provide a forum for individuals and institutions engaged in the study of environmental problems, management and regulation of natural resources, education, research and development, and manufacturing and distribution.

Specific goals of the society are:

- Promote research, education, and training in the environmental sciences
- Promote the systematic application of all relevant scientific disciplines to the evaluation of chemical hazards
- Participate in the scientific interpretation of issues concerned with hazard assessment and risk analysis
- Support the development of ecologically acceptable practices and principles
- Provide a forum (meetings and publications) for communication among professionals in government, business, academia, and other segments of society involved in the use, protection, and management of our environment

These goals are pursued through the conduct of numerous activities, which include:

- Conduct meetings with study and workshop sessions, platform and poster presentations, and achievement and merit awards
- Publish scientific journals, a newsletter, and special technical publications
- Provide funds for education and training through the SETAC Scholarship/Fellowship Program
- Organize and sponsor chapters and branches to provide a forum for the presentation of scientific data and for the interchange and study of information about local and regional concerns
- Provide advice and counsel to technical and nontechnical persons through a number of standing and ad hoc committees

SETAC membership currently comprises nearly 6000 individuals from government, academia, business, and nongovernmental organizations with backgrounds in chemistry, toxicology, biology, ecology, atmospheric sciences, health sciences, earth sciences, environmental engineering, hazard and risk assessment, and life cycle assessment.

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229 South Baylen Street, 2nd Floor
Pensacola, Florida, 32502 USA
T **+1 850 469 1500**
F **+1 850 469 9778**
E **setac@setac.org**

Avenue de la Toison d'Or 67
B-1060 Brussels, Belgium
T **+32 2 772 72 81**
F **+32 2 770 53 86**
E **setaceu@setac.org**

www.setac.org

Environmental Quality Through Science®

Implementing Ecological Risk Assessment Weight-of-Evidence Approaches that Contribute to Decision-Making

1 Weight-of-Evidence for Management Decision-Making: Past, Present, and Possible Futures

P.M. Chapman, Golder Associates Ltd.

The origin and development of weight-of-evidence (WOE) approaches for proactive and reactive management decision-making will be reviewed, in terms of both assessment prior to management actions and further assessment/monitoring following management actions. Appropriate and inappropriate means of combining different lines of evidence (LOE) will be discussed. Available and possible future LOE for both terrestrial and aquatic environments will related to different aspects of risk assessment (i.e., problem formulation to screening level, to detailed level; effects and exposure), including determination of causation. Key future improvements for WOE will be outlined including: limiting and bounding the use of best professional judgment; balancing generic guidance with necessary site-specific flexibility; using WOE when dealing with the 'moving targets' resulting from climate and associated changes; dealing with uncertainty (e.g., the Precautionary Principle); interpreting contradictory information supplied by different LOE; and limits to certainty and how to appropriately convey these to management decision-makers.

2 A Weight-of-Evidence Strategy for Predictive Assessments

B.K. Hope, CH2M Hill; J.R. Clarkson, ENVIRON International Corp.

Weight-of-evidence (WOE) is the process of considering strengths and weaknesses of various pieces of information in order to inform a decision among competing alternatives. A predictive assessment uses existing information relating cause and effect to estimate the probability that today's decision X will lead to tomorrow's outcome Y. There appears to be no practical guidance for use of WOE in such assessments. We propose a strategy for using WOE, within the context of the ecological risk assessment (ERA) framework, to weigh and integrate outcomes from various lines of evidence to estimate the probability of a detrimental outcome in an assessment endpoint. The ERA framework is necessary to connect the results of an assessment to the management goals of concern to decision-makers and stakeholders. Within that framework, a WOE approach provides a consistent and transparent means of interpreting the myriad types of data and information gathered during a complex ecological assessment. Impediments to application of WOE will be discussed, including limited regulatory guidance and prior regulatory use, along with persistent reliance on threshold-based decision-making.

3 Transforming the weight of evidence estimation to a Bayesian network calculation

W.G. Landis, K.K. Ayre, Western Washington University / Institute of Environmental Toxicology; E.E. Hines, Western Washington University / Institute of Environmental Toxicology, Huxley College of the Environment

Bayesian networks will be demonstrated as effective means of incorporating disparate type of evidence for estimating risk and assigning causality in order to establish management programs. Lines of evidence are expressed as combinations of casual pathways from source to stressor to habitat to effect and impact. The interactions between a stressor, the environment and the receptor are expressed in conditional probability tables (CPT). Uncertainty due to a lack of site-specific data, extrapolation between species or other factors can be documented and expressed in the distributions represented in the CPT and the distributions of the parent nodes. A specific advantage of this approach is that the mathematics of Bayesian networks is clearly established, in contrast to current formulations to combine various lines of evidence. Information for mechanistic data, population modeling and expert elicitation can be integrated using the framework. Risk can be calculated. Inversely, current conditions can be put into a parameterized Bayesian network and the likely causes estimated with a concurrent sensitivity and uncertainty analysis. Examples will be presented from our current research sites as well as demonstrating how the weight-of-evidence formulation for the decline of the Pacific herring as presented by Landis and Bryant.

4 Assessment of the Environmental Situation of Tecolutla, Veracruz, México

P. Ramirez Romero, X. Guzman-Garcia, U.A.M. Iztapalapa / Hidrobiologia; G. Barrera-Escorcia, Universidad Autonoma Metropolitana / Hidrobiologia; M. Miranda Arce, U.A.M. Iztapalapa / Hidrobiologia

Tecolutla, Veracruz is a municipality of the Gulf of Mexico with valuable natural resources that are being deteriorated by unrestricted and unplanned development. Unlawful land use changes are shrinking the vegetation cover of Mangroves and therefore the habitat of commercially important species. Overfishing and uneducated tourists exert a pressure over the resources that can only be remediated with effective enforcement and long term commitment of the municipal authorities. Fishermen and other habitants have organized themselves to try to find solutions to their problems through knowledge and communication with the authorities. The Ecotoxicology Group of the Universidad Autonoma Metropolitana is developing an environmental assessment of the situation through the use of multiple indicators like water chemistry, water and sediment toxicity evaluated with Microtox and histopatological studies of fish and bivalves among others. From the results obtained to date, it is clear that in spite of the amounts of pollutants entering the river, the wetlands retain efficiently the nutrient inputs, however bacterial water pollution is important in those sites where the untreated waste waters enter the environment directly. Pollutants accumulated in sediments are not high enough to cause acute toxicity on *Vibrio fischeri* but are present in sufficient quantities to cause sublethal effects on fish and clams. Once the environmental assessment is completed a series of recommendation will be presented to the local authorites, NGOs and residents so they can start planning the best way to implement them.

5 Employing weight-of-evidence approaches: a journey from Massachusetts to British Colombia

C.A. Menzie, Exponent; A. Fairbrother, Exponent, Inc. / EcoSciences

Over the past two decades we have had the opportunity to apply weight-of-evidence approaches for assessing ecological risks and using those results to inform environmental decisions. An early effort in the mid-1990s to develop a formal structured approach involved working with the Massachusetts Weight-of-Evidence Workgroup to develop guidance for use in the Massachusetts hazardous waste site program and for Superfund sites within USEPA Region 1. That guidance was published and subsequently picked up by other states and some Canadian provinces. More recently, we had the opportunity to update and refine the approach reflected in the Massachusetts guidance and to develop new guidance on weight-of-evidence applications for use in British Colombia. While many of the basic concepts remained the same, experience with regulatory applications over the past fifteen years has shown us that there are a number of useful ways to improve upon how to make the weight-of-evidence approach more useful for decision-making. These ideas and lessons learned are reflected in the British Colombia guidance. A key aspect of the new guidance is that it provides a step-wise approach that can guide work plan development. The most critical step is planning the approach as part of Problem Formulation. This is when the Lines of Evidence (LOE) are selected along with how these will be used in the assessment. The guidance provides criteria for selecting these LOE. The guidance outlines three additional steps for applying the weight-of-evidence method: adjustments of the weights assigned to LOEs after data collection to reflect quality of information, determining the magnitude and nature of responses in LOEs, and integrating the information from the various LOEs into an overall weight-of-evidence related to particular conclusions. The guidance provides examples and work tables to support the assessments. The usefulness of a weight-of-evidence approach for informing environmental decisions depends on effective communication of the results as well as the technical reliability of the analysis. The guidance provides insights into how to communicate results of a technical analysis to decision makers and others who may not have the same level of technical knowledge. In addition to providing an overview of this particular journey from Massachusetts to British Colombia, we will provide examples of where the method has been effectively used.

6 The application of Activity and Fugacity to increase Weight of Evidence in Environmental Risk Assessments

F.A. Gobas, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment); V. Otton, Simon Fraser University / Resource & Environmental Management; M. Crawford, School of Resource and Environmental Management (Faculty of Environment)

One of the challenges in environmental risk assessments of chemical contaminants is that while there is often a considerable amount of information available on a variety of characteristics of the chemical stressor, only limited information is used in the risk assessment. In addition, information of various nature can lead to apparent inconsistencies and incoherencies which sheds doubt on the outcome of the risk assessment. Also, "selective use" of data and information can provoke criticism and may be judged as bias. This paper discusses several examples where the application of activity and fugacity theory has helped to enhance the weight of evidence, by providing a method to include information of different nature in a risk assessment, and to improve the internal consistency in the risk assessment. We will briefly explain the basic requirements and assumptions for the application of activity and fugacity to environmental risk assessment and then illustrate the application of the activity and fugacity approach to risk assessments of decamethylpentacyclosiloxane (D5), di-ethylhexylphthalate esters (DEHP), and polyaromatic hydrocarbons (PAHs). Finally, we will discuss the lessons learned from these experiences and evaluate the activity and fugacity approach for their merits and limitations and suggest possible risk assessment applications where such an approach may be useful.

7 A quantitative approach to weight of evidence in ecotoxicological risk assessment

G.J. Van Der Kraak, University of Guelph / Department of Integrative Biology; M.L. Hanson, University of Manitoba / Department of Environment and Geography; A.J. Hosmer, Syngenta Crop Protection, Inc.; W. Kloas, Liebniz Inst. of Freshwater Ecology & Inland Fish.; K.R. Solomon, University of Guelph / School of Environmental Sciences

A quantitative weight-of-evidence (WoE) approach was developed to evaluate studies in the open literature that report the effects of the herbicide atrazine on fish, reptiles, and amphibians. The methodology involves a detailed assessment of 1) effects related to outcomes that are of relevance to whole animal performance and 2) the strength of the experimental methods employed. Each study end-point examined was considered in relation to the statistical significance and consistency of the response, the relevance of the response to apical endpoints (e.g., survival, growth, reproduction, and development; SGRD) and the underlying biological plausibility of the mechanisms leading to the observed effect. We characterized the strength of the experimental methods by scoring the hypothesis being tested and the experimental design, the use of QA/QC measures such as GLP, numbers of concentrations used, and the testing of environmentally realistic concentrations of atrazine. The means of the scores of these two scores were then used to summarize and weigh the evidence for atrazine contributing to ecologically significant changes to the organisms of interest. This WoE differs from many of the past assessments in that the scoring was inclusive of all studies reported in the literature except for older acute/chronic toxicity studies. The quantitative analysis conducted considered over 200 articles from the primary literature, and assessments were conducted on over 30 distinct endpoints including aspects of sexual development and differentiation, reproduction, endocrine physiology and immune function. Overall, the WoE showed that atrazine might affect biomarker type responses, such as gene expression, hormone levels, and biochemical processes (e.g., induce detoxification responses) at concentrations sometimes found in the environment. However, these effects were not translated to adverse outcomes in terms of apical endpoints including SGRD. The WoE approach developed provides a transparent, reproducible and robust framework that can be used to assist the decision-making process when assessing environmental chemicals and helps to identify areas where future studies can be best directed.

8 An Empirical Activity and Fugacity-Based Approach to Risk Assessment of Petroleum Hydrocarbons

M. Crawford, School of Resource and Environmental Management (Faculty of Environment); F.A. Gobas, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment)

Petroleum hydrocarbons (PHCs) are a group of chemicals present in the environment from petrogenic, pyrogenic, and biogenic sources. An organism's exposure to PHCs is evaluated by measuring the chemical concentration in many different environmental compartments, including in sediment, soil, water, air, and tissue. Typically PHCs are present as mixtures of multiple individual PHCs each with different physical-chemical properties that determine their distribution between the different environmental compartments (e.g., water and sediment). The potential risk to aquatic and terrestrial organisms can be evaluated by comparing their environmental exposure to the concentrations associated with various effects (e.g., LC50). However, describing this risk from exposure to PHC mixtures can be a challenge because a lot of toxicity data are measured for individual species, based on individual PHCs in a single medium (especially water). Non-polar narcosis is a mode of toxic action thought to be shared by all PHCs, implying that the internal concentration of chemicals eliciting this response will be equal regardless of chemical, media, or species. This research proposes the use of fugacity and activity, two thermodynamic properties, to describe bioavailable quantities of PHCs in organisms. Fugacity (expressed in units of pressure; e.g., pascals) and activity (a unitless ratio describing how close chemical concentrations are to saturation in a particular medium) are two complementary ways of normalizing exposure concentration and toxicity data, and expressing all data in the same units so that they may be compared directly. This research converts existing PHC toxicity endpoints and exposure concentrations into fugacity and activity to look for similarities between a variety of different species, endpoints, individual PHCs, and media types. Toxicity data of PHC mixtures expressed as fugacity and activity will also be compared to individual PHC data to test the hypothesis that fugacity and activity are additive. Ultimately, this approach is evaluated as a potential method to enhance ecological risk assessments, as well as criteria development, by allowing an enhanced weight of evidence approach that integrates toxicity and exposure data from multiple media, multiple species, a110nd multiple PHC chemicals.

Ecological Risk and Impacts Relevant to Tennessee and the Region

9 Environmental Impact of Photovoltaics in the Southeast Region

A. Anctil, K. Tisza, Clemson University / Environmental Engineering and Earth Sciences

The development of solar photovoltaic (PV) has emerged first in the Southwest of the United States due to the high solar potential and availability of already cleared land. In the Southeast, the PV market has shown a much slower growth, mainly due to the low cost of current energy production, but also due to other factor such as rough surface terrain and forested area which increases the cost and environmental impact of the PV system associated with land transformation. Current rapid population growth in the region combined to new renewable energy portfolio policies could create an opportunity for photovoltaic to provide some of the additional required energy. In this work, the potential and environmental impact of PV development in Tennessee, North Carolina, South Carolina and Georgia is presented. These states were chosen as the experienced a population growth significantly higher than the US average over 2000-2012 and are currently not main solar energy producer states. Using a life-cycle approach (LCA), including system manufacturing, land preparation (deforestation and flattening), construction and operation, the potential for air pollutant reduction and carbon dioxide from increasing PV energy production as well as other relevant environmental impact will be presented for various types of PV installations. The potential for PV installation and selection of case-study site was performed using a multi-criteria approach combining specific GIS data on the solar insolation, topographic limitations, environmental and land-use constraints for the particular states. Two types of installations, rooftop on residential and commercial structures in urban area and utility scale in rural area were considered.

10 Risks and Opportunities Related to Energy Use and Development in Tennessee

V. Dale, Oak Ridge National Laboratory

Largely as a result of use of fossil fuels, Tennessee is projected to warm between 5 and 9 °F by the end of the 21st century. Temperatures are projected to increase in all months and in all ecological provinces of Tennessee. Expected precipitation differences from the long-term average are more complex but less striking. Climate change will have a strong effect on Tennessee forests by changing the typical species composition of forests across the State and by causing initial decline in total forest biomass. As tree species that are better adapted to the warmer climate take over, biomass will rise again. Changes in tree diversity and species composition occur in all ecological provinces of Tennessee with the greatest changes in the Southern Mixed Forest province. The high diversity of trees in Tennessee forests conveys resilience to disturbance such as climate change. Tennessee has traditionally been a leader in hydropower generation and has recently become a national leader in developing "green" renewable energy sources such as wind, solar and bioenergy. The potential of biofuels production in the State of Tennessee largely rests on the relative abundance of woody resources and the potential to develop and expand the production of switchgrass (a perennial warm-season native grass) as well as other bioenergy resources such as agricultural and forest residues. Through recent State investments, East Tennessee currently leads the country in cellulosic biofuel production. For example, a demonstration-scale (250M gal/year) cellulosic ethanol plant in Vonore, Tennessee run by Genera Energy is the first of its kind in the nation. However the low price of fossil fuels is detracting from development of renewable fuel alternatives.

11 Elemental Analysis of Human Placenta and its Relationship with Birth Weight in Hamilton County, TN

C. Mikelson, University of Tennessee at Chattanooga / Department of Biological and Environmental Sciences; J. Troisi, Laboratorio Chimico Merceologico; S. Symes, M. Kovach, University of Tennessee at Chattanooga; D. Adair, University of Tennessee School of Medicine; K. Johnson, Z. Lin, Southern Illinois University; S. Richards, University of Tennessee at Chattanooga

Chattanooga, TN, USA was historically heavily industrialized. Contemporary investigations have shown elevated levels of metals and metalloids in area soils and remediation for soil lead contamination in the southern portions of the city has been ongoing. Chattanooga has an above average incidence of infants born at low birth weight (LBW), defined as birth weight under 2,500 g. Metals and metalloids have been shown to affect birth outcomes. Concern over a potential correlation between concentrations of metals and metalloids in human placenta and low birth weight prompted the present study. We compared the concentration of 12 metals detected, via ICP-MS, in 415 dried human placental tissue (As, Al, Mn, Fe, Co, Ni, Cu, Zn, Se, Cd, Ba and Pb) to several maternal factors and birth outcomes (i.e., birth weight, gestational age, and a calculated birth weight centile). Birth weight was negatively correlated with Mn and Pb, but positively correlated with Co, Zn and Ba. Levels of Mn, Co, Zn and Ba were all significantly different among LBW and normal birth weight infants. Most maternal parameters correlated with significant differences in at least one metal concentration. These maternal parameters and associated metals included: marital status (Fe), maternal BMI (Co and Cd), race (Mn, Co, Ni, Cu, Se, Ba and Pb), maternal education (Cu, Ba, and Pb), maternal age (Co, Se), tobacco use (As, Se, Cd and Ba) and parity (Pb). Birth weight was significantly different according to marital status, BMI, race, and education. The present study is the first to quantify such a large array of metals/metalloids in the human placenta. Significant negative correlations of placental Mn and Pb concentrations to birth weight may be the result of a toxic effect. Elevated Co, Zn and Ba concentrations were associated with normal birth weight. This may reflect the importance of essential trace metals in maternal diet during gestation. Thus, it is important to consider trace metal and proper function of the placenta, as well as potential toxic effects when examining the human placenta for metal concentrations.

12 Analysis of state and federal agencies' actions and their effects on water pollution

R. Hoyos, Tennessee Clean Water Network

State and Federal agencies are mandated to uphold environmental laws, to promulgate regulations based on scientific knowledge and to use agency discretion when issuing permits that may harm threatened and endangered species. However, in the State of Tennessee, the Tennessee Department of Conservation (TDEC), the Office of Surface Mining (OSM) and the US Fish and Wildlife Service (the Service) have ignored their mandated responsibilities potentially causing the extirpation of threatened species and increased pollution. This presentation will focus on two cases: one in which the Service ignored its 1996 Biological Opinion in order to allow surface mining to be permitted by OSM that would discharge effluent with higher conductivity than is tolerable by Blackside Dace and the Cumberland Darter; and, the second in which TDEC refused to defend a permit that would decrease effluent from the Cookeville Sewage Treatment Plant. Recent empirical studies suggest that Blackside Dace cannot maintain healthy, sustaining populations at steam conductivities above 240 µS/cm and are visibly distressed at conductivities above 500 µS/cm. The same likely applies to the Cumberland Darter. The Zeb Mountain mine regularly discharges mining waste into Lick Fork with conductivities ranging from 538 µS/cm – 886 µS/cm. The City of Cookeville's wastewater treatment plant has discharged pollutants in excess of their permit to Pigeon Roost Creek, an urban stream that is effluent dominated. The City has discharged nitrogen and phosphorus in amounts detrimental to fish and aquatic life based on macroinvertebrate scores falling as low as 14/32 beneath the outfall of the treatment plant. TDEC has not initiated an enforcement action nor has it defended its permits from numerous appeals by the City of Cookeville going back to the 1980s. Lack of enforcement has contributed to decreases in aquatic life as well as increased pollution. This presentation will evaluate the effects of these agencies actions on water pollution and T&E species.

13 Anthropogenic Induced Decline and Restoration of Tennessee's Mussel Fauna

D. Hubbs, TWRA / Environmental Services; S. Chance, United States Fish and Wildlife Service / Ecological Services Field Office; S. Ahlstedt, United States Geological Survey (retired)

The State of Tennessee historically supported 129 of the nation's 300 native freshwater mussel species. The Tennessee and Cumberland River systems compose the Cumberlandian Region. Historically, this region supported the richest freshwater mussel (Bivalvia: Unionacea) fauna in the world with at least 107 mussel taxa and greater than one-third of this fauna is endemic to the Cumberlandian Region. Freshwater mussels are proportionally the most imperiled faunal group in Tennessee and the United States. Dozens of major impoundments have altered thermal, hydrological, and chemical regimes downstream from dams including the loss of hundreds of miles of riverine habitat. Other factors contributing to the demise of this extraordinary fauna includes episodic and chronic chemical spills, channelization, mining, agriculture, and sedimentation. The environmental impacts to this fauna have resulted in the extinction or significant decline of over 70% of nation's mussel species. Within Tennessee, the US Fish and Wildlife Service has listed 45 mussel species as endangered under provisions of the Endangered Species Act of 1973. Fourteen species known from the region, including several endemics, are now considered extinct. The numbers of imperiled mussel species continue to increase as divergent forms are recognized and environmental problems continue unabated. Focused research during the last two decades has developed conservation strategies aimed at reversing this downward trend. Some of these strategies include minimum flow releases below dams, water quality improvements (oxygen aeration), and the development of mussel population assessment, propagation, and restoration techniques. The Tennessee Wildlife Resources Agency is actively involved with the restoration of native mussel species back into state protected waters. A joint state and federal plan was developed to prioritize mussel species and rivers for restoration efforts in the Cumberlandian Region. Restoration efforts are currently ongoing and include the translocation and augmentation of adult mussels including cultured and propagated individuals raised at culture facilities.

14 Freshwater fish and mussel distributions related to ecological flow regimes and water chemistry in the Clinch River in Virginia and Tennessee

G.C. Johnson, US Geological Survey / WRD / Tennessee Water Science Center

The Clinch River, which flows from southwestern Virginia through eastern Tennessee, supports one of the richest assemblages of endemic and endangered freshwater mussels in the United States. Surveys of mussel community structure over the past three decades have shown catastrophic declines in mussel abundance and diversity adjacent to areas with coal mines and natural gas extraction along a 50-mile segment of Clinch River between

Carbo and Clinchport, Virginia. Mussel assemblages downstream of this section remain healthy, and some communities upstream appear to be recovering. Sections of the Clinch River with declining mussel populations experience greater specific conductance, major-ion concentrations, and metals concentrations in the water column than sections with healthy mussel populations. Although surveys along the Clinch River indicate high scores for fish community metrics, the composition of fish communities has not been compared to patterns of mussel decline, nor have host fish distributions been compared to basin physical characteristic or stream flow regime. Explanatory relationships between fish distribution and other physical, chemical and ecological influences were evaluated for sites where fish and mussel community data were available. This included specific analysis of correlations among fish community metrics, mussel distributions, ecological flow regime, and water chemistry.

15 Restoration of the Copper Basin Mining District; Polk County, TN

C. Zeller, USEPA – Region 4 / Superfund Division; L. Carr, USEPA / Superfund Division

For nearly 150 years between the late 1830s and the 1980s, the Copper Basin Mining District in southeastern Tennessee was host to extensive copper, zinc and iron mining/processing; and sulfuric acid production that resulted in environmental degradation on a massive scale. Total ore production from the nine ore bodies comprising the mining district exceeded 95 million tons. Historically, over 30 square miles of Polk County, TN and Fannin County in northern Georgia were a deforested, barren, eroded landscape caused by hard rock mining and sulfuric acid processing. Tens of thousands of acre-feet of sediment/soil were eroded from the denuded hillsides and stream banks and transported to the Ocoee River. Acid rock drainage impacted large portions of the 4,000-acre Lower North Potato Creek watershed, the 3,000-acre Davis Mill Creek watershed, and 26 miles of the Ocoee River. As a result, over 8,600 pounds of metals and greater than 19,000 pounds of acid were discharging to the Ocoee River every day. In the early 1960s through the 1970's, various government agencies and private parties accelerated efforts to re-vegetate the area. In January 2001, the story of the Copper Basin changed from one of environmental degradation to a restoration success story built upon unprecedented teamwork. The USEPA, the Tennessee Department of Environment and Conservation (TDEC) and Glenn Springs Holdings, Inc. (subsidiary of OXY USA) entered into a Memorandum of Understanding (MOU) and a series of enforceable state/federal legal agreements. The MOU applies an adaptive management approach with immediate short-term actions to protect the Ocoee River while long-term source control strategies for the basin are designed and implemented. Lime precipitation water treatment plants have been constructed on North Potato Creek and Davis Mill Creek near the confluence with the Ocoee River to remove metals and neutralize the pH prior to discharge. These systems have the capacity to store the volume of water generated by a 10 year/24 hour storm event (≈ 6 inches of rainfall). Diversion systems have also been constructed to route clean waters around the most heavily contaminated parts of the watersheds thus reducing the volume of water requiring treatment. Combined, these engineered systems have reduced the metals/acidity loading to the Ocoee River by over 98%.

Chemical Analysis for Pesticides and Their Metabolite or Degradation Products: New and Updated Methods

16 Using chiral identification of metolachlor ethane sulfonic acid as a groundwater dating tool

C.P. Rice, US Department of Agriculture / Environmental Management and Byproduct Utilization Laboratory; K. Bialek Kalinski, G. McCarty, US Department of Agriculture

We have studied the hydrologic fate of metolachlor and its two predominant metabolites, metolachlor ethane sulfonic acid (MESA) and metolachlor oxanilic acid, in groundwater and base flows of streams for several years. These two metabolites are excellent markers for groundwater processes related to agriculture and they appear to have extended retention in aquifers. Chiral separations were carried out on selected archived samples from a specific site extending from 2000 to 2005. Enantiomeric excess values for the *S* and *R* racemic forms show a clear signal for increasing levels of the *S*-enriched form of MESA over this time interval. This shift in chiral abundance agrees with the change in usage patterns for metolachlor were the *S*-enriched form was introduced in 1997, replacing the less effective racemic product used prior to that date. Hydrologic dating with this very specific agricultural marker appears to be a promising application for this information.

17 Direct Aqueous Injection-Liquid Chromatography/Tandem Mass Spectrometry for Determination of Pesticides and Pesticide Degradates in Stream Samples

M.W. Sandstrom, US Geological Survey / National Water Quality Laboratory; L.K. Kanagy, C.A. Anderson, C.J. Kanagy, US Geological Survey

The influence of the sample matrix in the determination of 238 current-use pesticides and pesticide degradates by direct aqueous injection-liquid chromatography/tandem mass spectrometry (DAI-LC/MSMS) was evaluated for stream samples. Stream water typically contains humic and fulvic acids, other biomolecules, and additional matrix components that might cause ionization enhancement or suppression in the LC/MSMS analysis. Environmental samples, as well as spike and spike replicate samples were collected two to six times from each of 47 streams sites between May and September 2012. The sites selected are part of the US Geological Survey's National Water Quality Assessment Program and reflect a range of watershed size and land-use conditions throughout the US. The analytes determined in the samples represent a broad range of pesticide classes—including acetanilide and triazine herbicides, fungicides and organophosphate insecticides—along with120 pesticide degradates. The method uses direct injection of a filtered 100-µL water sample into the LC/MSMS instrument; no other sample preparation is necessary. Two injections are used—one in electrospray ionization (ESI) positive mode and one in ESI negative mode—reflecting the optimal ionization requirements of the pesticides determined. Data acquisition is conducted with dynamic multiple reaction monitoring (MRM), using two MRM transitions for each analyte. Recoveries at the nominal concentration of 250 ng/L for most of the pesticides were within 70 to 130 percent, with standard deviations of less than 30 percent, the criteria defining acceptable performance. The analytes with low recoveries (20-67 %) included asulam, fonofos, terbufos oxon and many sulfonylureas. Several others showed high (>130 %) recoveries, including butralin, 2-hydroxy-6-ethyl-4-amino-s-triazine, didealkylatrazine, or higher variability in their recovery (>30 %) compared to reagent water, owing to matrix effects. However, the overall performance of the DAI-LC/MSMS method, in terms of acceptable bias and variability, was excellent for most of the 238 pesticide analytes, considering the broad range of stream matrices sampled.

18 Rapid sample preparation and fast GC-MS/MS for the analysis of >200 pesticides and environmental contaminants in fish

Y. Sapozhnikova, USDA / ARS

Here we present a simple and fast analytical method for simultaneous determination of >140 pesticides, and >60 environmental contaminants, including persistent organic pollutants: polychlorinated biphenyl (PCBs), polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ether (PBDE), and novel flame retardants in fish. The sample preparation is based on a QuEChERS (quick, easy, cheap, effective, rugged, safe) extraction with acetonitrile and dispersive solid-phase extraction clean-up with zirconium-based sorbent. Sample preparation for a batch of 10 homogenized samples takes approximately 1 hour per analyst. Fast, low pressure vacuum outlet gas chromatography tandem mass spectrometry (LP-GC/MS-MS) provides fast separation of multiple analytes within 9 min achieving high throughput. The developed method was evaluated at 4 spiking levels and further validated by analysis of NIST Standard Reference Materials (SRMs) 1974B and 1947. With the use of isotopically labeled internal standards, recoveries of most analytes were 70-120% with relative standard deviations less than 20% (n = 5), even at low spiking levels, making the method applicable for analysis at environmentally relevant concentrations. The measured values for both SRMs agreed with certified/reference values (72–119% accuracy) for the majority of analytes. The detection limits were 0.1–0.5 ng/g for PCBs, 0.5–10 ng/g for PBDEs, 0.5–5 ng/g for pesticides and PAHs and 1–10 ng/g for flame retardants. The calculated sample preparation cost is less than $3/sample using bulk materials, and small amounts of organic solvent used for extraction leads to reduced solvent waste and minimal environmental impact. The developed method was compared and the results agreed well with the traditional method based on pressurized fluid extraction, gel permeation chromatography and solid-phase extraction clean-up sample preparation and conventional GC-MS for the analysis of wreckfish samples. The developed method allows to analyze a large number of pesticides from one sample

combined with novel flame retardants and other legacy and emerging classes of environmental contaminants. The generated data on occurrence of these contaminants may advance the understanding of the potential risk posed by these chemicals and aid in future risk assessment and regulations.

19 Development of Methods for Analysis of a Wide Variety of Current-Use Pesticides in Sediments and Tissue

K.M. Kuivila, K.L. Smalling, M.L. Hladik, US Geological Survey

Current-use pesticides vary considerably in their physical–chemical properties, which makes it difficult to develop multi-residue methods for different environmental media. Although most monitoring studies have focused on analyzing water, studies by the US Geological Survey have shown that current-use pesticides are often bound to sediments and do accumulate in aquatic organisms. Our methods for analyzing pesticides in sediment and tissue are designed to include new or increasing use pesticides and potential degradates. Sediment samples are extracted at high temperature and pressure using pressurize liquid extraction (PLE) followed by gel permeation chromatography to remove sulfur. Co-extracted organic-rich compounds from the sediments, which can interfere with instrumental analysis, are traditionally removed using solid phase extraction (SPE). A two-step elution method to remove interferences utilized stacked graphitized carbon and alumina SPE cartridges with different solvents. The addition of azole and strobilurin fungicides to the method were more problematic and required splitting the extract for parallel cleanup steps. While one fraction went through the stacked carbon/alumina SPE, the fungicide fraction was subjected to Florisil cleanup. The current sediment method includes analysis of 121 pesticides and degradates. For tissue, the initial method used cold extraction with dichloromethane, lipid removal using GPC, and final cleanup with deactivated silica gel. Again, the addition of fungicides to the method required a change in the cleanup step. The current method includes extraction by PLE and lipid removal using GPC, followed by further cleanup using Florisil for the analysis of 95 pesticides and degradates. Sediments and tissue were analyzed by gas chromatography with an ion-trap mass spectrometer initially and then with a quadrupole mass spectrometer, both operating in electron ionization mode. When lower method detection limits are needed, analysis is done with tandem mass spectrometry to further reduce the sample background. Using these multi-residue methods, a number of current-use pesticides are being detected in both bed and suspended sediments and in tissue from a variety of organisms. Future directions include optimizing methods for biologically important metabolites and to use both gas-chromatography and liquid-chromatography platforms for analysis.

20 Routine to Difficult Pesticide and Degradation Product Analyses: Options for Improving Separation and Detection

R.J. Raina-Fulton, University of Regina / Dept. of Chemistry & Biochemistry

New advances in development of LC/MS/MS, GC/MS, and GC/MS/MS methods for the analysis of a range of pesticides in air and water samples will be presented. Multi-residue analysis methods do not always include pesticides that are difficult to analyze because of stability in solvents used in sample preparation procedures or thermal stability in injector ports, or when matrix issues are more severe. Analytical methods have been developed for specific chemical classes of pesticides or target pesticides of high concern to obtain low detection limits required for analysis of air or water environmental samples. Pesticides include organochlorines (OCs), organophosphorus pesticides (OPs), phenoxyacid herbicides, N-trihalomethyl fungicides, carbamates, phenylureas, triazoles, sulfonylureas, pyrethroids and other selected pesticides along with a range of their degradation products. New LC/MS/MS methods have been developed to simultaneously analyze pesticide active ingredients and their degradation products for chemical classes including OPs, phenoxyacid herbicides, carbamates, and triazoles. New LC columns, mobile phase composition, and post-column reagent addition to improve selectivity of separation or MS sensitivity will be discussed. GC/MS methods options to handle more difficult pesticide analysis at trace level include use of features such as large volume cold on-column injection and PTV dirty matrix injection. Comparisons of GC methods with electron impact and negative chemical ionization using selected ion monitoring and selected reaction monitoring have also been made for evaluation of method detection limits and confirmation. Selected samples taken from air and water will be presented to illustrate capabilities of the various methods.

21 Refined Methodologies for the Determination of Neonicotinoid Insecticides and their Metabolites in Foliage, Pollen, Nectar, Bees and Bee Products

A. Kamel, USEPA OCSPP

Refined analytical methodologies for the determination of the neonicotinoid insecticides imidacloprid, dinotefuran and thiamethoxam and their metabolites in pumpkin foliage, pollen and nectar as well as in honey bees and bee products are presented. The neonicotinoid insecticides and some of their metabolites have been associated with subchronic toxicity to bees. Studies of the sublethal effects of neonicotinoids on bees require an analytical method capable of detecting sub-parts-per-billion levels, in order to understand their role in pollinator decline. Modifications in the extraction solvent by adding 2% triethylamine (TEA) to acetonitrile improved the recoveries of the neonicotinoid metabolites, and detection by ultra (high)-performance liquid chromatography/ tandem mass spectrometry achieved low detection limits ranging from 0.2-1 ppb for all tested analytes.

22 Development of an on-line analytical method for the quantification of carbamate pesticides and metabolites in human matrices

P. Leonards, VU University, Institute for Environmental Studies / Chemistry & Biology; J. Koekkoek, VU University, Institute for Environmental Studies

Worldwide, serious concern has arisen about the increased incidence of learning and developmental disorders in children. From a scientific point of view, there is no doubt that exposure to neurotoxic chemicals during early brain development can adversely affect learning and development. Various recent epidemiological studies have indicated that exposure to low doses of environmental biologically active contaminants during human development can have deleterious effects on cognitive development in childhood. The European commission-funded project DENAMIC "Developmental Neurotoxicity Assessment of Mixtures in Children" investigates neurotoxic effects (e.g., learning and developmental disorders) of low-concentration mixtures of pesticides and a number of common environmental pollutants in children. Because of recent concerns of cognitive and neurobehavioral effects related to pesticide exposure and if proven necessary the possibility to protect future generations by regulatory measures, DENAMIC will primarily focus on possible neurotoxic effects of pesticides (e.g., organophosphate, carbamates, pyrethroids, organochlorine). One of the aims is to study perinatal and early-childhood exposure in maternal urine and cord blood, as well as breast milk and urine of the child. Data and samples from existing European cohorts with different exposure profiles, both pre-and postnatal, (Norway, Netherlands, Slovakia and Spain) are studied, which offers the possibility to distinguish between pre-and postnatal exposure effects and identify the susceptible period. Associations between chemical exposure and learning (cognitive) and neurobehavioural (ADHD, ASD, anxiety) development or disorders will be studied. The aim of this paper is to present the development of an on-line LC-MS/MS for the detection and quantification of carbamates and metabolites in urine, serum and breast milk. The developed sample prepartion method consist of a deconjugation step, and an on-line extraction, clean-up, and separation method combined with LC-MS/MS. The compounds of interested were fractionated using a Restricted Access Material (RAM) from the matrix, and then transferred to the analytical column before detection with LC-MS/MS. Different parameters for the RAM material were optimised to provide acceptable recoveries.

23 An LC/MS-MS Method for the Quantification of Metabolites of Organophosphorus Pesticides, Synthetic Pyrethroids, and Select Herbicides in Human Urine

M.D. Davis, L. Valentine-Blasini, E.L. Wade, W. Roman-Esteva, A.M. Calafate, Centers for Disease Control and Prevention / National Center for Environmental Health

Organophosphate and pyrethroid insecticides and phenoxyacetic acid herbicides represent important classes of pesticides applied in commercial and residential settings. Interest in assessing the extent of human exposure to these pesticides exists because of their widespread use and their potential adverse health effects. We have improved the sensitivity and selectivity of our analytical method for measuring 12 biomarkers of several of these pesticides for use in large biomonitoring studies. We extracted the target analytes from one milliliter of urine by a semi-automated solid phase extraction technique, separated them from each other and from other urinary biomolecules by reversed-phase high performance liquid chromatography, and detected them using tandem mass spectrometry with isotope dilution quantitation. The use

of automated 96-well plate extraction technology and the analysis of both positive and negative ions in a single injection have improved the efficiency of the method. Greater sensitivity and selectivity were achieved using an analytical column of smaller dimensions, the use of higher mass resolution for certain analytes, the use of stable isotope labeled internal standards for each analyte, and the use of confirmation ion ratios. Validation of the method showed excellent accuracy, precision, sensitivity and selectivity appropriate for trace-level analysis. The suitability of this method for the analysis of these biomarkers has been demonstrated with its application in the analysis of thousands of urine samples from different biomonitoring studies.

Wildlife Ecotoxicology

24 Ground-feeding songbirds exposed to toxic concentrations of lead in the Southeast Missouri Lead Mining District

N. Beyer, US Geological Survey / Patuxent Wildlife Research Center

The world's largest deposit of the mineral galena (lead sulfide), mined since 1721, lies in the Southeast Missouri Lead Mining District (SEMO). Mining and smelting of the ores have contaminated surrounding soils. Our work investigated the extent of exposure to lead and the associated toxic effects on ground-feeding songbirds, which are exposed to Pb mainly through ingestion of contaminated soil. Earthworms, which contain large amounts of soil in their intestines, were collected from soils with various concentrations of lead to examine one likely pathway of exposure to songbirds. Concentrations of lead in earthworms were linearly related to concentrations of lead in soils, with a slope of about a half. The lead in the soil in the intestines accounted for most of the lead in the whole earthworms. Three sites in the mining district along with reference sites were selected for studying songbirds, mainly robins (*Turdus migratorius*) and northern cardinals (*Cardinalis cardinalis*). Soils of the three study sites contained from 1000 to 3200 mg/kg of Pb. Songbirds were collected in mist nets and ground traps in spring and early summer of 2009 and 2010. Toxic effects on the songbirds was evaluated on the basis of tissue lead concentrations, inhibition of enzyme ALAD (delta-aminolevulinic acid dehydratase) and the presence of renal intranuclear inclusion bodies, which are indicative of lead poisoning. Three of 14 northern cardinals and 4 of 6 robins had lead concentrations in their livers that were indicative of systemic toxic effects (17.6 mg of lead per kg of liver tissue, dry weight). Although songbirds feeding in the canopy had low exposure to lead, many ground-feeding songbirds were exposed to toxic concentrations of lead at sites containing 1000 mg/kg or more of soil lead.

25 Metabolite profiling of Tree Swallows: a new technique for evaluating ecological impacts of contaminants on aquatic ecosystems

J.M. Davis, T.W. Collette, D.R. Ekman, USEPA / National Exposure Research Laboratory; C.M. Custer, T.W. Custer, US Geological Survey / Upper Midwest Environmental Sciences Center; P.M. Dummer, US Geological Survey; Q. Teng, USEPA / National Exposure Research Lab

Although considerable resources have been dedicated to remediating the ecological impacts of environmental contaminants on the Great Lakes and various other ecosystems, there is a need for additional techniques to monitor the effectiveness of these efforts. Because Tree Swallows (*Tachycineta bicolor*) feed on emerging aquatic insects and can exhibit levels of tissue contamination that reflect sediment contamination, they may be an effective indicator of contaminant exposure and aquatic ecosystem recovery. Other evidence has demonstrated that metabolite profiling of biota can elucidate contaminant modes of action and physiological impacts of contaminants. Thus, the combination of these methods may provide a useful tool for monitoring contaminant exposure and assessing post-remediation recovery. Here, we present results from a field-based study that profiled endogenous metabolites in Tree Swallow nestlings collected at multiple sampling locations across several Great Lakes areas of concern (e.g., Detroit, Maumee, and Raisin Rivers) and at additional reference sites exhibiting lower levels of contamination. First, we analyzed nestlings for a range of contaminants (metals, PFOs, PCBs, PCDD-F, and PBDEs). Next, we used ^{1}H-NMR spectroscopy to measure polar metabolites in livers and subsequently applied multivariate analyses. By comparing tissue contaminant levels and relative abundances of hepatic metabolites, we assessed whether metabolite profiles differed among sites and how differences may be related to contaminant levels. Results from this study indicated that metabolite profiles of nestlings collected from sites with higher levels of environmental contamination differed from those collected at reference sites. In addition, differences were partly explained by variability in contaminant loads measured in nestling tissue (e.g., PCBs, PFOs, Se, and Zn). The combination of evidence suggests that metabolite profiling of Tree Swallows may be a powerful tool for biologically-based exposure monitoring and for assessing post-remediation recovery of aquatic ecosystems.

26 A field assessment of swallows comparing population-level nesting success indicators with indicators of individual impact

D. Henshel, Indiana University; D.W. Sparks, US Fish & Wildlife Service

Population-level indicators of nesting and reproductive success, including nesting success, hatching success, and fledging success were developed for purposes of evaluating populations within the context of maintaining long-term ecosystem integrity. These metrics do not consider subtle adverse effects, in-migration of potentially less contaminated birds, nor do they provide any indication of whether the young of the population are likely to survive to reproduce successfully. For most toxicants, the most sensitive life stage is embryonic, and the most sensitive embryonic period tends to be organogenesis. Toxic effects that occur during organogenesis have the potential to have severe and long-term effects on growth, function, and survival even though no abnormalities are externally visible at hatch. The Early Embryo Teratogenesis Assay introduces a method to systematically analyze specific age-dependent indicators in embryos undergoing organogenesis by comparing developmental stage and morphometry of the organs and tissues developing at the time of observation. Using the early avian embryo, light microscopic analysis can be completed within one week of of the start of incubation, and will provide feedback on teratogenic effects on the whole embryo (length, twisting, abnormal developmental growth and shape) as well as on specific organs and tissues including heart, brain, eyes, ears, somites, pharyngeal arches and limbs. In studies on swallow embryos, field indicators of tree swallow nesting success at PCB-contaminated sites indicates that there is little difference between contaminated and reference sites. However, early embryo analysis of eggs taken from the same nests used for the field analysis indicates that heart, brain, eyes, whole embryonic morphology, limbs, and visceral arches are all significantly adversely affected at these same contaminated sites compared to the reference sites. We conclude that reproductive success field metrics developed for avian conservation are not sufficiently sensitive metrics for use in field assessment of toxicological impacts of pollution. The Early Embryo Teratogenesis Assay enables a relatively rapid assessment of toxicological effects on the most sensitive life stage, and the effects documented have the potential to have long term impacts on the growth, quality of life, reproductive success and ultimately long term survival of the affected individuals and populations.

27 In ovo exposure to BDE-99 affects the song control system of a model songbird species, the zebra finch

M.L. Eng, V. Winter, Simon Fraser University; S.A. MacDougall-Shackleton, Western University; T.D. Williams, Simon Fraser University; J.E. Elliott, Environment Canada

2,2',4,4',5-Pentabromodiphenyl ether (BDE-99) is a brominated flame retardant congener that has pervaded global food chains, being reported in avian egg and tissue samples throughout the world. Its effects on birds are not well known, but there is evidence in exposed mammals that it directly mediates and causes neurotoxicity and lowers sex steroid hormone concentrations. In birds, those processes could disrupt the song-control system and male mating behavior. Singing is an important aspect of reproduction in birds, serving to define territories and attract females. Developmental conditions that result in smaller song-control nuclei and reduced song quality in males could ultimately disrupt pair formation and lower reproductive success. In the present study, the effects of *in ovo* exposure to environmentally relevant levels of BDE-99 were assessed in a model songbird species, the zebra finch (*Taeniopygia guttata*). Embryos were exposed via egg injection to a vehicle control (DMSO), 10, 100 or 1000 ng BDE-99/g egg on the day the egg was laid. Chicks were raised to sexual maturity to investigate long-term effects of BDE-99 on the adult male song-control system and mating behavior. The volume of the robust nucleus of the arcopallium (RA), which is one of the primary song control nuclei involved in song learning and production, was significantly smaller in *in ovo* exposed birds compared to the controls, and there was a non-significant trend for other song control nuclei to decrease in volume as dose increased. This study has shown that current environmental levels of BDE-99 are high enough to have significant effects on the song control system in birds, and may therefore be impacting the

reproductive rates of free-living birds. We have previously shown that there is no effect of BDE-99 exposure during the nestling period on the song control system. Taken together, these results demonstrate that the timing of exposure can significantly alter the effects of contaminant exposure.

28 Bald Eagle (Haliaeetus leucocephalus) Population Productivity and Source-Sink Dynamics in Michigan, 1961–2010

K.L. Simon, University of Maryland / Environmental Science and Technology

The bald eagle (*Haliaeetus leucocephalus*) population in Michigan has undergone a significant recovery following the ban of the pesticide dichlorodiphenyl-trichloroethane (DDT), and its subsequent derivatives, mainly dichlorodiphenyl-dichloroethylene (*p,p'*-DDE), by the Environmental Protection Agency in the 1970s). Population productivity and recovery however, have not been even throughout the state of Michigan. Great Lakes breeding areas along the shorelines of Lakes Superior, Michigan, Huron, and Erie have historically experienced less productivity than inland breeding areas because of higher concentrations of contaminants. High quality inland areas experienced the greatest productivity until the 1990s, quickly recovering from the detrimental effects of DDT. All inland breeding areas may temporarily alleviate density dependent effects through the emigration of nonbreeding eagles to Great Lakes breeding areas. All Great Lakes Watersheds, with the exception of inland Lake Superior, experienced a productivity greater than 1.0 during Period Ten. This indicates that these watersheds continue to support an increasing breeding bald eagle population. Great Lakes breeding areas, particularly Lake Michigan and Lake Huron, are now more productive than inland breeding areas. These Great Lakes breeding pairs however, are the least efficient breeders with greater amounts of changeover between nesting pairs within one breeding area. This indicates that Great Lakes breeding pairs are less reproductively fit than inland breeding pairs. This reproductive insufficiency could be caused by chronic residual reproductive effects from *in ovo* organochlorine contaminant exposure. The demographic contribution of floaters (birds capable of breeding but not able to secure the habitat and forage base to do so) and nonbreeders may compensate for residual reproductive inability or adult mortality caused by contaminants in Great Lakes breeding areas, buffering productivity. The low reproductive fitness, yet high productivity of Great Lakes breeding areas is suggestive of a high turnover rate within the Great Lakes breeding population. A constant turnover of breeding pairs may overshadow any underlying effects caused by environmental contaminants, leading to decreased reproductive fitness in Great Lakes adults.

30 Assessing the toxic potency of Aroclor 1268 to piscivorous marine mammals using mink as a mammalian model

W. Folland, Michigan State University / Wildlife Toxicology Laboratory, Department of Animal Science; S. Winterstein, Michigan State University / Department of Fisheries and Wildlife; S. Fitzgerald, Michigan State University / Pathobiology and Diagnostic Investigation; J.L. Newsted, Cardno Entrix, Michigan State University / Wildlife Toxicology Laboratory, Department of Animal Science; S.J. Bursian, Michigan State University / Wildlife Toxicology Laboratory, Department of Animal Science; M.J. Zwiernik, Michigan State University / Wildlife Toxicology Laboratory, Department of Animal Science, College of Veterinary Medicine

Concentrations of polychlorinated biphenyls (PCBs) derived from the commercial mixture, Aroclor 1268, are elevated in aquatic biota in southeastern Georgia, USA. Total PCB concentrations in blubber samples from bottlenose dolphin (*Tursiops truncatus*) in a Georgian estuary have been reported more than 10 fold those typically observed in adjacent regional estuaries. Presently there is great uncertainty associated with quantifying the toxic potency of exposure to this highly chlorinated PCB mixture. The uncertainty originates from the lack of toxicology data combined with differing characteristics of this mixture as compared to the more studied lesser-chlorinated PCB mixtures. Aroclor 1268 is dominated by extremely hydrophobic PCBs and the mixture is essentially devoid of coplanar PCB congeners that typically drive PCB toxicity through AhR mediated toxic responses, which make biomagnification factors and relative potency difficult to predict. The American mink is the optimal model for understanding mammalian PCB toxicity and is considered to be a suitable surrogate species for cetaceans due to similarities in diet, taxonomic class, and reproductive physiology. Furthermore, mink are known to be exquisitely sensitive to PCBs, thus the application of mink toxicology data to other mammals is expected to provide a level of safety. A trial was conducted to assess and compare the effects of Aroclor 1268 on reproduction of adult female mink and on survival and growth of their offspring. Dams and kits were fed treatment diets spiked with 0, 1.7, 4.0, 10, 17, or 29 µg/g ww Aroclor 1268 or 0.0005 µg/g ww PCB 126 in an effort to determine toxic reference values for Aroclor 1268 that can be applied to marine mammals and assign a relative potency value for Aroclor 1268 with respect to 3,3',4,4',5-pentachlorobiphenyl (PCB 126). More than 150 endpoints, including reproductive, survivorship, histological, immunological, hematological, and enzyme induction parameters were correlated with dietary exposure and tissue concentrations to derive toxic reference values so that responses to Aroclor 1268 exposure in mammals may be more clearly defined.

31 Using Dose-Response Data in Wildlife Risk Assessment

R.A. Hill, B. Pyper, G. Mann, Azimuth Consulting Group

Wildlife risk assessments typically rely on point-estimate Toxicity Reference Values (TRVs) as benchmark values that denote the threshold for effects. TRVs are often not associated with any particular magnitude of effect. Furthermore, wildlife TRVs are usually based on a single study or a small number of studies. While the routine use of point-estimate TRVs may be appropriate for screening-level wildlife risk assessments, detailed risk assessments warrant rigorous consideration of all available dose-response data. Typical dose-response data sets are highly complex, spanning various species, endpoints, and durations. Hidden in this complexity can be highly useful information that will be missed if risk assessors focus only on particular endpoints or species (e.g., those appearing to be most sensitive) or particular studies (e.g., those that show strong dose-response relationships). Using example data sets for PCBs and methyl mercury, we suggest approaches for data compilation and plotting of dose-response data sets, and we review options and challenges associated with quantitative model-fitting.

Decision Analysis for Valuation in Life Cycle Assessment

32 Normalization, Weighting and Interpretation Recommendations for Life Cycle Assessment

J.C. Bare, USEPA / MS-466; V. Prado, Arizona State University / School of Sustainable Engineering and The Built Environment; T.P. Seager, Arizona State University / School of Sustainable Engineering & Built Environm

The stages of normalization, weighting and interpretation are key steps in understanding the results of an LCA. These steps convert the *data* generated by LCAs into *information* that is relevant to decision makers. Currently there is a lack of consensus in the LCA community of how to perform normalization and weighting, and in many instances, LCA practitioners truncate analysis at characterization in order to avoid the subjectivity biases associated with these latter LCA stages. However, there are subjectivity risks when presenting LCA results to decision makers without any analytic guideposts. Therefore, the areas of normalization, weighting and interpretation require more attention from the LCA community in order to achieve more transparent environmental decision making. According to the ISO standards, normalization can be performed according to an external reference (in the case of external normalization) or with respect to a baseline (internal normalization). ISO guidelines also offer broad recommendations for the weighting and interpretation stages. Nevertheless, these recommendations are brief and do not offer sufficient guidance to LCA analysts. Therefore, the goal of this study is to describe the state of practice, discuss existing knowledge gaps and opportunities for research, and provide LCA practitioners with a portfolio of options for the stages of normalization, weighting and interpretation required prior to presenting the information to decision makers.

33 Normalization approaches to select life cycle indicators to enable decision-making

G. Van Hoof, Procter & Gamble Services; M. Vieira, PRe Consultants; M. Gausman, Procter & Gamble; A. Weisbrod, Procter & Gamble (P&G), Procter & Gamble / Global Product Stewardship

With an ever increasing list of indicators, LCA practitioners face the challenge of effectively communicating results to people making decision about how, when, where or what to improve the sustainability of something. Because most decision-makers are not LCA experts, a selection process of LCA indicators is usually necessary. This is frequently limited to an arbitrary selection of indicators or single scores, value judgments are introduced, and multi-indicator information may be lost. This paper shows how three

different normalization procedures can be used to rank the list of indicators by comparing them vs. a reference system. The approaches are illustrated using a consumer product, at least one impact assessment method (e.g., ReCiPe), using Western Europe as a reference population. Indicators are ranked using midpoint normalization factors, and compared to the ranking from endpoint normalization broken down by midpoint contribution. The different approaches produce different ranking results: Endpoint Normalization shows Resources as the most relevant area of protection for this case, closely followed by Human Health and Ecosystem. The highest scoring midpoints include fossil depletion, climate change and, to a lesser extent, particulate matter formation. In contrast, Midpoint Normalization indicates different midpoints are more relevant, like Freshwater Eutrophication and Natural Land Transformation. We conclude that an approach based on Endpoint Normalization allows selection of the most relevant indicators for the system we studied. Bias, due to lack of data completeness in the reference system, is less of an issue in the Endpoint Normalization process, whereas Midpoint results are less subject to uncertainty. For this and similar case studies, we find that non-LCA expert decision makers better understand, use and communicate complex LCA results when we focus on the relevant indicators identified by the Endpoint Normalization approach and its highest scoring midpoints and key contributing unit processes.

34 Advancing Life Cycle Assessment through Stochastic Decision Analysis Methods

V. Prado, B.A. Wender, Arizona State University / School of Sustainable Engineering and The Built Environment; T.P. Seager, Arizona State University / School of Sustainable Engineering & Built Environment

Life Cycle Assessment (LCA) quantifies environmental impacts of products in raw material extraction, processing, manufacturing, distribution, use and final disposal. The findings of an LCA can be used to improve industry practices, to aid in product development, and guide public policy. Unfortunately, existing approaches to LCA are unreliable in the cases of emerging technologies, where data is unavailable and rapid technological advances outstrip environmental knowledge. Previous studies have demonstrated several shortcomings to existing practices, including the masking of environmental impacts, the difficulty of selecting appropriate weight sets for multi-stakeholder problems, and difficulties in exploration of variability and uncertainty. In particular, there is an acute need for decision-driven interpretation methods that can guide decision makers towards making balanced, environmentally sound decisions in instances of high uncertainty. We propose the first major methodological innovation in LCA since early establishment of LCA as the analytical perspective of choice in problems of environmental management. We propose to couple stochastic multi-criteria decision analytic tools with existing approaches to inventory building and characterization to create a robust approach to comparative technology assessment in the context of high uncertainty, rapid technological change, and evolving stakeholder values. Namely, this study introduces a novel method known as Stochastic Multi-attribute Analysis for Life Cycle Impact Assessment (SMAA-LCIA) that uses internal normalization by means of outranking and exploration of feasible weight spaces. Furthermore, we propose to apply the new approach to the US natural gas industry, which is currently undergoing transformative change resulting from widespread adoption of hydraulic fracturing (*fracking*), resulting in release of a complicated mixture of natural gas and liquids from so-called "tight" and shale formations. While the potential of unconventional natural gas plays in the US has resulted in a glowing forecast of US Energy Independence at the International Energy Agency, the technology has also raised serious questions with regard to water and air quality implications.

35 Comparative risk and life-cycle impact assessments: Complimentary tools for addressing emerging technologies?

T.E. McKone, University of California, University of California and Lawrence Berkeley National Laboratory / Sustainable Energy Systems Group

In order to identify, characterize, and compare opportunities for increasing the sustainable use of energy, resources, chemicals, and materials, we need reliable and informative environmental, health and economic impact assessments. In this talk I consider the different objectives and approaches used in comparative risk assessments and life-cycle impact assessments as tools to guide emerging technology decision-making. The goal of a risk assessment is to quantify the likelihood of harm in a format that assists decision makers who must act to tolerate, mitigate, or eliminate the potential harm. This goal is distinct from impact studies that strive to develop indicators of harm, measures of hazard, or ranking schemes. These latter activities focus more on characterizing the possibility of harm. Both hazard and risk relate to some measure of harm, such as number of deaths or diseases, financial loss, species loss, resource privation, etc. Life cycle assessment (LCA) has become an important tool for the environmental impact assessment of products and materials. Businesses are increasingly relying on it for their decision-making. The information obtained from an LCA can also influence environmental policies and regulations. Life-cycle impact assessment (LCIA) is the phase of LCA aimed at understanding and evaluating the magnitude and significance of the potential environmental impacts of a product system. In comparing these different approaches, I will consider the extent to which they address the possibility of harm and/or the probability of harm and whether and how the approaches confront uncertainty. I will also provide examples of where risk assessment and LCIA have impacted research directions for emerging technologies.

36 Responsible Innovation and Life Cycle Assessment

B.A. Wender, Arizona State University / School of Sustainable Engineering and The Built Environment; J. Sadowski, Consortium for Science Policy Outcomes, ASU; R. Foley, Center for Nanotechnology in Society; T.A. Hottle, Arizona State University; T.P. Seager, Arizona State University / School of Sustainable Engineering & Built Environm

Life cycle assessment (LCA) is a powerful framework to explore the environmental impacts of products, processes, and technologies because the broad boundaries called for in LCA prevent the shifting of environmental burdens from one life-cycle phase to another. Application of LCA to emerging technologies, such as nanotechnology, is increasingly called for by policy-makers as a tool to identify environmental concerns early and guide the development of emerging technologies towards decreased burden. These calls reflect the potential for LCA to contribute to Responsible Innovation (RI) through explicit identification and incorporation of broader concerns into technology development processes. However, LCA is currently ineffective at promoting the integration of social and environmental research into technological innovation for at least two reasons: 1) LCA is largely retrospective, relying heavily on data collected from mature industries with existing supply chains, and 2) LCA is narrowly focuses on environmental impacts, and thereby fails to identify institutional, social, political, and economic drivers of technology development. Regarding the first point, incorporation of future scenario development and other foresight tools into LCA enables prospective and consequential applications. Regarding the second point, there are numerous ports of entry through which social values are implied in LCAs – for example, functional unit determination, system boundary definition, and impact category selection. Explicit identification of how these values shape LCA practice and subsequent results will improve the efficacy of LCA as a tool for design. Incorporation of foresight, integration, and engagement methods from Anticipatory Governance (AG) provide a broad technology assessment framework focused on socio-technical transformations, will broaden the range of values reflected in LCA and promote the use of LCA results targeted to specific policy and decision makers. Thus, tailoring LCA to promote integration of environmental and social concerns into technology development systems presents a tangible pathway towards RI of nano- and other emerging technologies.

37 Using Life Cycle Analysis, Risk Assessment, and Other Science Information in the "Wicked" Policy Making Environment

C. Stahl, USEPA, Region III / Environmental Assessment and Innovation Division

Policy making and regulatory organizations such as the US Environmental Protection Agency are expected to utilize the best science information in analyzing potential policy alternatives. Wicked problems are those that cannot be easily defined because of many different stakeholder perspectives, there is no single right answer (only better or worse conditions), require iteration, where solutions must be discovered, have no objective measures of success, and have no clear stopping rules. In other words, policy making is about finding clumsy solutions to wicked problems. Obligations for stakeholder inclusiveness, transparency, and regulatory defensibility add complexities to the process of policy making in these organizations. Clumsy solutions must provide stakeholders with the flexibility and analytical capability to deal with multiple spatial and temporal scales and to address questions anywhere within a potentially unbounded socio-ecological system. Life Cycle Analysis,

Risk Assessment, and other approaches used in traditional science fields can provide limited (even if complex or complicated) information to analyses of wicked problems but are not in themselves, approaches that can address the wicked problem. Complexity of the data, while potentially difficult to cope technically, is a tame problem with an ultimate answer. For those interested in addressing wicked problems, it is the conceptual limitations of LCA, RA, and other similarly tame approaches that actually present the more intractable issues. Therefore, at best, these kinds of traditional approaches will not work for wicked problems or, at worst, exacerbate finding clumsy solutions. Rethinking strategies for the post-normal (wicked) science world requires stakeholder inclusiveness, transparency and trans-disciplinary learning, i.e., some first principles of good decision analysis. In this talk, we start the discussion by examining these first principles in more detail, aligning them to the elements of wicked problems, and opening the door to thinking more concretely about actionable wicked problem strategies.

38 EXPOSE-ABM Version 1.0: Evaluating the sustainability of palm oil industry using an agent-based model

Y. Manik, Harvard University; J. Leahy, University of Maine

The increasing demand of palm oil for various purposes underlines the need for expansion of the oil palm plantations. Although LCA provides a hollistic methodology in accounting for its environmental impacts, this approach alone is not sufficient. A simulation model that mimics the system complexity is important to complement the LCA limitations. To better understand the implications of land use change driven by the expansion of palm oil industry, we created the an agent-based simulation model entitled "Expansion of Palm Oil Sustainability Evaluation in Agent Base Model (EXPOSE-ABM)" developed in NetLogo platform. This model was built based on a semi-structured interviews were undertaken in September 2012 with local palm oil stakeholders in Jambi Province of Indonesia. This model considers two distinct agents (large firms and smallholders) with different land-use decision-making approaches in establishing oil palm plantation on of three possible land uses: forest on mineral soil, peat land, and grassland/shrub. Using this model, simulations have been performed to investigate the influence of the agent's behavior toward sustainability indicators. This model is helpful in predicting some impacts based on the scale and the complexity of the problem described in this model in three sustainability aspects: economics, environmental, and societal represented by the cash ratio, above ground carbon stock, vegetation covers, and employment rate. The results indicate that in order to keep the industry growth while promoting the abatement of the greenhouse gas emission, a policy instrument that could stimulate the growth of smallholders in palm oil industry is absolutely necessary.

39 Using Sustainability Return on Investment to Value LCA for Better Decisions

D. Hartter, L. Laurin, Earthshift

The Sustainability Return on Investment (S-ROI) methodology, which grew out of Total Cost Assessment, provides a flexible way to assess Life Cycle Assessment (LCA) results and other externalities within a value-based multi-criteria decision analysis system. The major drawback of any well-defined value-based system of assessing externalities is that different stakeholders have different values. As an example, a climate-change naysayer will assess a value of zero to greenhouse gas emissions, while for a citizen of the island Republic of Kiribati the value is equivalent to the loss of a homeland for their descendants. Rather than choosing one or the other of these values or some average, S-ROI encourages the use of a range of values. Further, it encourages the evaluation of impact by stakeholder. This allows any added cost of greenhouse gas mitigation to the naysayer to be assessed separately from the benefit of emissions reduction to the Kiribati citizen. When each stakeholder's values are given voice, the process becomes non-confrontational. The result does not value the Kiribati citizen over the naysayer or vice versa. All parties are working toward a win-win-win solution. Compromises may come from surprising sources, bringing more parties to the table. In various studies we have seen communities help fund corporate infrastructure requirements in return for reduced local and global emissions, safeguards put in place to secure long-term availability of bedding material for cattle farmers, and sweet potato, pig, and cattle farmers working together with an ethanol facility to ensure the livestock have the highest quality feed for a reasonable price and each stakeholder obtains a good return on their products.

Nanotechnology: Part A

40 Are sediment-dwelling organisms at higher risk for Nanoparticle exposure? Characterizing Nanoparticle exposure and effects in Hyalella azteca

H.C. Poynton, University of Massachusetts, Boston / Molecular Indicators Research, University of Massachusetts-Boston / School for the Environment; B.J. Blalock, University of Massachusetts-Boston / School for the Environment; J.M. Lazorchak, USEPA / National Exposure Research Laboratory; C.A. Impellitteri, USEPA / Water Supply and Water Resources Division; J.M. Unrine, University of Kentucky; M.E. Smith, The McConnell Group / Aquatic Toxicology Department

Nanoparticles (NPs) are expected to make their way into the aquatic environment where sedimentation of particles will likely occur, putting benthic organisms at particular risk. Therefore, organisms such as *Hyalella azteca*, an epibenthic crustacean which forages at the sediment surface, is likely to have a high potential exposure compared with water column organisms such as *Daphnia magna*. In general, *H. azteca* is more sensitive to NPs compared with *D. magna*; however, NP toxicity is particle specific. *H. azteca* is highly susceptible to Zinc Oxide (ZnO) NP exposure, with ZnO NPs demonstrating greater potency compared with the corresponding metal ion, Zn^{2+} . However, gene expression analysis is unable to distinguish between the ZnO NP exposures and Zinc Sulfate ($ZnSO_4$) exposures at equitoxic concentrations. This suggests that the NPs act as carrier for Zn^{2+}, increasing the exposure level of Zn^{2+} to *H. azteca,* and possibly other sediment dwelling organisms. Silver (Ag) NPs act differently, with a lower potency compared to Ag ions. Studies comparing the toxicity of these particles to *H. azteca* in different sediment types will help us better understand the exposure potential and susceptibility of benthic organisms to nanomaterials.

41 The influence of natural organic matter on the toxicity of nanoparticles to zebrafish embryos

K.J. Ong, University of Alberta; L.C. Felix, University of Alberta / Biological Science; J.G. Veinot, University of Alberta / Dept of Chemistry; J.D. Ede, University of Alberta / Student; G.G. Goss, University of Alberta / Biological Sciences

The biological effects of nanoparticles on aquatic organisms have been well studied in pristine experimental media, but there is a need to determine whether nanoparticles will behave in a similar manner or have comparable toxicity in natural environmental conditions. Using single walled carbon nanotubes, silver, cadmium selenide, and zinc oxide nanomaterials, we characterize the nanoparticles in water containing natural organic matter. We find that aquatic exposure to nanoparticles in different environmental conditions can alter the biological effects of the nanoparticles, including effects on zebrafish (*Danio rerio*) embryo survival, hatch, growth, morphological development, and movement over 96 hours post-fertilization. Using both physicochemical characterization and biological effects data, we determined that these effects are related to size, charge, composition, and/or polydispersity of the nanoparticles in different media. Using 5 nm Nile red containing polymer capsules, we have demonstrated that NPs are able to cross the chorion and attach to the surface of the embryo. We propose that this may be he mechanism by which nanoparticles are inhibiting hatch. Finally, we determined that while NOM associated capsule still transverse the chorion, they no longer inhibit hatch suggesting that the coating of NOM is protective in natural waters.

42 Characterizing the effect of suspended carbon nanotubes on the bioavailability of adsorbed fluoranthene to fathead minnows

E.N. Linard, Clemson University / Institute of Environmental Toxicology (ENTOX); P. van den Hurk, Clemson University / Biological Sciences; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX), Clemson University / Institute of Environmental Toxicology (ENTOX)

The introduction of carbon nanomaterials into the environment has increased exponentially in the last decade, causing environmental as well as health concerns. One concern is the interaction that such nanomaterials have with the biota in the aquatic ecosystem and the direct and indirect toxic effects that may result. Previous research has documented positive influence of dissolved organic carbon (DOC) on the stability of carbon nanotube (CNTs) suspensions in surface waters. Further, research has quantified the ability of these carbon nanomaterials to adsorb aquatic contaminants such as polynuclear aromatic hydrocarbons (PAHs). It is

important to note that both CNTs and PAHs can co-occur in wastewater treatment effluents. However, little research has characterized the bioavailability of these adsorbed PAHs in the aquatic environment. The goal of this research was to characterize the bioavailability of fluoranthene that has been adsorbed to suspended CNTs to fish. *Pimephales promelas* were exposed for 15 hrs to fluoranthene alone, fluoranthene in the presence of different DOC concentrations, and fluoranthene adsorbed to CNTs in the presence of DOC. Bioavailabile fluoranthene was quantified in each exposure through bile analysis using a fluorescence microplate reader. Through comparing the concentration of fluoranthene metabolites in the bile to the concentration of fluoranthene present in the DOC and CNT treatments we were able to quantify relative bioavailability of fluoranthene adsorbed to CNTs. Results from the inital treatments indicated that fathead minnows exhibited a dose-response to fluoranthene exposure and that while DOC enhanced the stability of CNT suspensions, in the absence of CNTs it did not influence the bioavailability of fluoranthene. Preliminary adsorption data for low concentrations of fluoranthene was best fit with a Freundlich isotherm model, suggesting that CNTs were not fully saturated with fluoranthene at the concentrations of CNTS used of 0.5 mg/L to 3.5 mg/L. Adsorption isotherms for fluoranthene and CNTs in the presence of varying DOC concentrations were further developed providing a basis for the ratio of fluoranthene to CNTs concentrations used in the CNT exposures. Results of this work will be used to develop a model to predict this complex interaction with other organic contaminants commonly present in the aquatic environment.

43 Toxicity of graphene-TiO2 nanoparticle composite to Daphnia magna

S. Li, EPA / Environmental Toxicology; X. Pan, Texas Tech University; L.K. Wallis, USEPA/Mid-Continent Ecology Division; F. Zhaoyang, Texas Tech University; D.J. Hoff, Ecotoxicology Analysis Research / Mid-Continent Ecology Division; S.A. Diamond, NanoSafe Inc. / MED

With a dramatic rise in the complexity of nanomaterials, their toxicology research needs go beyond simple forms of nanomaterials. This study investigated the toxicity of a new type of sophisticated nanomaterial, graphene-TiO_2 nanoparticle composite (GNP). Graphene, with a unique 2D structure, has several properties which could lead to increased photoactivity of nano-TiO_2, such as large surface area, high transparency, high charge transportation and separation rate. GNP was synthesized based on a hydrothermal method. Transmission electron microscope (TEM) and scanning electron microscope (SEM) images confirmed that nano-TiO_2 was anchored on the surface of reduced graphene oxide. X-ray diffraction (XRD) showed a similar XRD pattern between GNP and nano-TiO_2. Fourier transform infrared spectroscopy proved the existence of Ti-O-C bonds between graphene and nano-TiO_2. A series of 48 h standard acute toxicity tests was performed on an aquatic model organism, *Daphnia magna*, to evaluate the toxicity of synthesized GNP. Particle behavior was also monitored for both exposure media and surfaces of organisms using dynamic light scattering, UV/VIS spectrometry and SEM. Compared with the widely used photocatalytic P25 nano-TiO_2 (P25), this material exhibited a two fold increase in photocatalytic activity in preliminary studies. Currently, research is being conducted to evaluate the impact of aggregation size, simulated solar radiation spectrum, dissolved organic matter, and ultraviolet A intensity on the observed phototoxicity of GNP. This study will provide valuable data for future risk assessment of sophisticated nanomaterials.

44 Toxicity of zinc oxide nanoparticles: Role of particle dissolution and photocatalytic reactive oxygen species (ROS) generation

H. Ma, USEPA / Mid-Continent Ecology Division; L.K. Wallis, USEPA / Mid-Continent Ecology Division; S. Li, EPA / Environmental Toxicology; J.E. Cañas-Carrell, Texas Tech University / Department of Environmental Toxicology; S.A. Diamond, NanoSafe Inc. / MED

Dissolution of zinc oxide nanoparticles (ZnO NPs) to ionic zinc (Zn^{2+}) has been recognized as an important mode of action (MOA) for ZnO NP toxicity due to its high solubility and high potency of Zn^{2+} to aquatic organisms. However, toxicity of ZnO NPs associated with their photocatalytic properties (generation of reactive oxygen species [ROS] under UV radiation) has been rarely reported.The current study aims to understand the relative contribution of these two different MOAs to ZnO NP toxicity in aquatic environments. Toxicity of ZnO NPs (30-50 nm, in suspension) to *Daphnia magna* was investigated under ambient laboratory lighting and simulated solar radiation (SSR). Photocatalytic ROS generation by ZnO NP was measured by a fluorescence based assay. Time-series dissolution of ZnO NPs to Zn^{2+} within 48 h was quantified under both irradiation conditions using atomic absorption spectroscopy. Mitigation of ZnO NP toxicity by $CaCl_2$ and N-acetylcysteine (NAC) were also examined to aid in elucidating the roles of ROS generation and particle dissolution in eliciting toxicity. Concentration-dependent ROS production was measured for ZnO NPs under SSR. Dissolved zinc ions showed time-dependent increase under both irradiation conditions. At 48 h, ZnO NPs at 5 mg/L and 50 mg/L yielded maximum Zn^{2+} concentrations of 3.3 mg/L and 3.51 mg/L under laboratory lighting, and 3.76 mg/L and 5.3 mg/L under SSR, suggesting a photoinduced dissolution. Although there was no significant difference in 48-h LC50s for ZnO NPs between two irradiation conditions (0.64 mg/L [lab lighting] vs. 0.67 mg/L [SSR]), immobilization of *D. magna* was significantly greater under SSR than under laboratory lighting at different time points across the 48 h exposure period. Furthermore, alleviation of ZnO NP toxicity by $CaCl_2$ was more prominent under laboratory lighting than under SSR, suggesting that mechanisms other than particle dissolution, presumably photocatalytic ROS generation, play a role in causing toxicity. Addition of NAC decreased toxicity of ZnO NPs under both irradiation conditions. Our results suggest that as particle dissolution remains the main contributor for ZnO NP toxicity, photocatalytic ROS production under natural sunlight also plays a role in ZnO NP toxicity and should not be neglected during hazard assessment of these nanomaterials. Furthermore, the photoinduced dissolution of ZnO NPs under natural sunlight and its environmental implication warrants further investigation.

45 Transcriptomic and proteomic profiles of Caenorhabditis elegans exposed to size and surface property variant silver nanoparticles

C. Schultz, Centre for Ecology & Hydrology; K. Hollanders, I. Nelissen, VITO; N. Tran, Institut Catala de Nanotecnologia; A. Crossley, K. Jurkschat, University of Oxford / Department of Materials; O.V. Tsyusko, University of Kentucky / Department of Plant & Soil Sciences; P. Kille, Cardiff University; C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology, Centre for Ecology & Hydrology / Microbial Ecology and Ecotoxicology, NERC Centre for Ecology & Hydrology / Ecotoxicology; D. Spurgeon, Centre for Ecology & Hydrology

Silver nanoparticles (AgNP) have been shown to cause toxicity to a range of ecotoxicological endpoints in the nematode *Caenorhabditis elegans*. A key question, however, is to which extent the nature of this toxicity varies between particles of similar core composition, but different size and surface chemistry. To investigate the effect of such properties on AgNP toxicity and mode of action, exposures were conducted using size variant 70 nm and 12 nm neutrally charged Polyvinylpyrrolidon (PVP) coated particles, and coating variant 12 nm negatively charged (Mercapto-undecanoic acid coated) and 12 nm positively charged (11-Aminoundecan-1thiol coated) AgNPs. Exposures to ionic silver were carried out as controls. Initial exposures were conducted to determine reproductive EC_{30} for each NP type, revealing differences in sensitivity of *C. elegans* towards these NPs. Greatest levels of toxicity were found for the PVP coated particles (similar values for both particles), whereas negatively charged particles were less toxic and positively charged ones even less so. Characterization showed that observed differences in apparent toxicity were partly related to the particles' increased aggregation, however, differences in effect mechanisms may also be relevant. To investigate these mechanisms, we conducted exposures at the EC_{30} for each AgNP/ionic Ag followed by transcriptomic analysis. Using whole genome microarrays, a clear separation in the expression profile as response to the variant particles was evident, indicating distinct influence of size and surface charge respectively. These expression signatures showed high reproducibility between replicates, producing distinct clustering with good separation between treatments. The differently sized PVP particles induced significant differential expression of 370 genes, while different surface charges of the 12 nm particles significantly altered the expression of 353 genes. These genes were most notably associated with the sensory perception of chemicals, membrane functions, neurophysiological processes, signaling pathways and receptor activity. The expression level of key genes, e.g., related to endocytosis (rme-2, chc-1), metal handling (mtl-1, mtl-2) or general stress response (hsp-70, daf-2) was confirmed via qPCR. Additionally, protein expression and changes in sensitivity of knock-down mutants towards the tested particles will be assessed, thus providing a more complete picture of the system response.

Deepwater Horizon/MC 252 Well Incident Update – Part A

47 Corexit in GOM Sediments

M.J. Perkins, Dept. of Environmental and Molecular Toxicology; J.A. Field, Oregon State University / Environmental and Molecular Toxicology

Approximately 200 million gallons of Corexit was applied in the Gulf of Mexico in response to the Macondo oil spill of 2010. Corexit, a mixture of surfactants, is capable of reducing the interfacial tension between aqueous and oil phases, reducing oil droplet size and promoting dissolution and dispersion in the water column. It has been proposed that the surfactant components of Corexit will undergo rapid dispersion and microbially driven degradation in marine systems such as the Gulf of Mexico. Yet nearly three years after the original emergency response action, the main anionic surfactant of Corexit, bis-(2-ethylhexyl) sulfosuccinate (DOSS), has been detected in sediments near the original site of application at concentrations approaching two parts per billion. A method for the exhaustive extraction from marine sediments and the quantitative analysis of DOSS with minimal sample preparation via direct injection large volume LC-MS/MS is presented. A quantitative spatial and temporal analysis of DOSS in sediments collected during the tail end of the emergency response through the summer of 2013 from sites paralleling the path of the deep water oil plume observed during the emergency response is also presented.

48 Exposure to elutriate of sediment contaminated with Deepwater Horizon oil affects developmental responses of oyster early life stages

A. Volety, J. Vignier, J. Devine, R. Ketover, E. Nickols, L. Haynes, Florida Gulf Coast University / Department of Marine and Ecological Sciences; P. Soudant, Universite de Bretagne Occidentale / LEMAR; F.E. Chu, Virginia Institute of Marine Sciences / Department of Environmental and Aquatic Animal Health; J.M. Morris, M.W. Carney, J. Lipton, D. Cacela, M. Krasnec, Stratus Consulting Inc.

Following the BP oil spill in the Gulf of Mexico on April 20th, 2010, oil washed ashore in coastal areas, bays and estuaries in the Northern Gulf of Mexico and contaminated sediments. Oil can adsorb onto algae, detritus and sediment, creating particles that can be ingested by filter feeding organisms or slowly leach solutes into the water column exposing organisms inhabiting these areas. This study examined the effect of sediment elutriate from field-collected sediments contaminated by Deepwater Horizon oil. Sediment elutriate was obtained by mixing seawater and sediment at a ratio of 10:1 for 6 h followed by a 12h settling period. Oyster gametes, embryos and larvae were exposed to these elutriates of contaminated and control sediment at various concentrations as well as to seawater alone for 96 h. Responses including fertilization success, developmental abnormality, growth and survival of resulting larvae were recorded. Fertilization success decreased, developmental abnormality and mortality increased, and growth decreased in a dose-dependent manner upon exposure to the elutriate from the contaminated sediment.

49 Development of a chronic toxicity test methodology to determine effects of fresh and weathered MC252 oil and dispersant on Eastern oyster larvae

S.M. Pargee, C. Langdon, Oregon State University; B.A. Stubblefield, Oregon State University / Environmental and Molecular Toxicology; E. Stefansson, Oregon State University; S.M. Blunt, Hatfield Marine Science Center / Division of Earth and Ecosystem Sciences, University of Nevada Las Vegas / School of Life Sciences; S. Gage, Oregon State University

Short-term (48 hour) tests with sensitive life stages are often used as estimators of potential chronic effects; however, there are currently no standardized methods for directly testing chronic effects of test materials on bivalve mollusk larvae. We developed 10-day and 28-day toxicity test methods to estimate chronic effects for larvae of the Eastern oyster (*Crassostrea virginica*), a species native to the Gulf of Mexico, exposed to fresh and weathered Macondo (MC252) oil and Corexit 9500 (dispersant) from the Deepwater Horizon (DWH) incident. All studies were conducted using low energy, water-accommodated fractions (WAFs), which were prepared without vortex during mixing and using a 1 g:1000 ml oil-to-water loading ratio. Chemically-enhanced WAFs (CEWAFs) were prepared in the same fashion with the addition of dispersant at a 1:20 (w:w) dispersant-to-oil ratio. Both 10-day and 28-day tests were static-renewal, with renewals every 48 hours using fresh test solutions. Endpoints for the 10-day chronic test were larval survival, the proportion of larvae that were normal, and growth (for both normal larvae and normal-plus-abnormal larvae). In the 28-day test, a setting substrate was added to each test chamber at 10-14 days of exposure and the numbers of larvae that were either "eyed" (competent to set) or settled as spat were determined. The 28-day test included all endpoints determined in the 10-day test as well as survival and the proportions of larvae that were eyed or settled on day 28, based on the number of larvae initially added to the test chambers. Effect concentrations (EC10s) for growth, survival and the proportion of normal larvae in the 10-day tests were lower than those observed in previous acute studies, with growth being the most sensitive endpoint. EC10s for survival and the proportion of normal larvae in the 28-day test were comparable to those observed in 10-day tests. EC10s for the proportions of eyed and settled larvae were comparable to 10-day growth EC10s, with the proportion settled being the most sensitive 28-day endpoint. Under the test conditions in this study, weathered oils were less toxic than fresh oils, and non-dispersed oil was less toxic than dispersed oil or dispersant alone – a result consistent with those found in 48-hour acute tests with bivalve larvae.

50 DeepWater Horizon Oil Spill: Concentrations of PAH-like compounds in menhaden

C.S. Bentivegna, Seton Hall University / Department of Biological Sciences; R. Hawke, Seton Hall University; R. Hawke, Seton Hall University / Biological Sciences; A.M. Favero, S. Bonilla, T. Carluen, Seton Hall University

A blowout of the riser pipeline on the Transoceanic *DeepWater Horizon* (DWH) oil rig at the British Petroleum (BP) Macondo-1 site in the Gulf of Mexico (GOM) resulted in the largest oil spill in USA history. This project investigated the impact of the 2010 DWH oil spill by monitoring the pelagic, filtering feeding fish, menhaden, both in the GOM and along the Atlantic coast. More specifically, menhaden were collected from Barataria Bay and Vermillion Bay, LA, from Key West, FL and from Delaware Bay, NJ in fall 2010 through fall 2012. Barataria Bay was one of the most heavily oil areas during the DWH spill. Vermillion Bay received less oil, while Key West was not oiled by the DWH event. Fish in Delaware Bay, NJ have been exposed to chronic urbanization but not a recent large oil spill. Presented are results for PAH-like compounds in various tissues of menhaden as detected using fluorescent aromatic compound (FACs) spectroscopy. Data showed that levels of hydroxpyrene-like (HPY-like) PAHs in liver were actually lower in Barataria Bay compared to Delaware Bay in fall 2010. However, concentrations showed a seasonal pattern such that levels in LA and NJ fish increased in summer 2011 and 2012 and were lower in fall 2011 and 2012. Control sites (Vermillon Bay and Key West) showed lower levels of liver HPY-like PAHs than found at Barataria Bay in summer 2011. But by summer 2012, levels at Vermillion Bay were similar to those at Barataria Bay. This suggested that the migration of menhaden might be spreading the contamination. Analysis of various tissues indicated that PAH-like compounds were primarily obtained from diet as oppose to water and were circulating to organs such as spleen and gonads. This indicated that contaminants from the DWH event may ultimately affect fish health and reproduction.

51 Experimental system for exposure of pelagic fish eggs to crude oil dispersions (potential for oil dispersions to cause cardiac toxicity)

T. Nordtug, SINTEF Materials & Chemistry / Applied Environmental Biology and Chemistry; B. Hansen, K.M. Hammer, I.B. Overjordet, I. Andreassen, SINTEF Materials and Chemistry / Applied Environmental Biology and Chemistry; P.A. Wold, A.J. Olsen, Norwegian University of Science and Technology / Department of Biology; D. Altin, BioTrix; B.A. Stubblefield, Oregon State University / Environmental and Molecular Toxicology; A.J. Edgington, Clemson University / Biological Science and CU-ENTOX, Oregon State University

Controlled lab-scale exposure of organisms to oil dispersions is challenging due to the inherent affinity of oil to surfaces, and the formation of surface oil films. Oil droplets in the ocean will rise towards the sea surface at a rate determined by their density and diameter as well as the vertical turbulence in the water. Micro-droplets with a diameter less than 50 µm have a slow rising velocity in the range of centimeters per hour and are expected to have long residence times in the water column. Pelagic fish eggs, if present, may thus be exposed to dispersed oil from subsurface oil spills for periods of several days. In the present work exposure systems were constructed to expose pelagic fish eggs to controlled concentrations of mechanically dispersed micron-sized oil droplets (median diameter 15 – 20 µm based on volume) and water soluble fractions of oil. Fish eggs of the pelagic spawner Northeast Arctic cod (*Gadus morhua*) were used as a model species. In nature cod

spawn at depths from 50 to 200 meters and the eggs are slightly positive buoyant and will slowly drift towards the sea surface where they hatch. In order to achieve a controlled exposure scenario for the eggs we built a custom-made flow-through system where eggs were kept sub-surface while exposed to highly controlled concentrations of dispersed oil with defined oil droplet sizes. Two 4-day exposure experiments were performed on cod eggs 1-5 days post fertilization (dpf) and 7-11 dpf, respectively. Egg survival, cardiac development, developmental stage, and PAH body burden were determined at the end of each exposure period. After exposure, eggs were put in clean sea water for recovery until hatch. Post exposure mortality, heart rate and development were recorded in the recovery period, and after hatching fish larvae were sampled for analyses of growth, cardiac development and PAH body burden.

52 Advancement in Deepwater Horizon Oil Spill Toxicity Testing: Development of an Innovative Bioassay System for use with Pelagic Fish Embryos and Larvae

J. Stieglitz, University of Miami – RSMAS / Division of Marine Biology and Fisheries; E. Mager, University of Miami / Rosenstiel School of Marine and Atmospheric Science / Division of Marine Biology & Fisheries; R. Hoenig, University of Miami, RSMAS / MBF Division; D. Benetti, University of Miami, RSMAS / Division of Marine Biology and Fisheries; M. Grosell, University of Miami / Division of Marine Biology and Fisheries

A novel 96-hr acute toxicity bioassay system has been developed for toxicity testing embryo and larval stages of pelagic marine fish. Accurate determination of toxicity to embryo and larval stages of such species requires the use of scientifically acceptable bioassay protocols and systems. Due to key differences in the sensitivity and developmental process of pelagic marine fish embryos, traditional 96-hr bioassays yielded inconsistent test performance results, often with less than desired control survival. Development of a novel exposure system, the pelagic embryo-larval exposure chamber (PELEC), was necessary to conduct consistently successful bioassays on embryo/larval life stages of pelagic marine fish. By utilizing upwelling hydrodynamics in replicated recirculating chambers, it was possible to significantly improve control survival in pelagic marine fish embryo bioassays. Results indicate that control performance of mahi-mahi (*Coryphaena hippurus*) embryos in the PELEC system (89.8% ± 2.12), measured as percent survival after 96-hrs, consistently outperformed agitated static exposure (76.8% ± 4.49) and traditional static exposure (67.5% ± 4.79) systems. The PELEC system has also been used for testing the effects of crude oil from the Deepwater Horizon oil spill on yellowfin tuna (*Thunnus albacares*) embryos, and preliminary results of these trials will be presented. Utilization of this bioassay system in ecotoxicology allows for controlled and accurate testing of fish embryos and larvae from challenging pelagic species such as mahi-mahi and yellowfin tuna, providing a possible method to quantify the effects of the Deepwater Horizon oil spill on the early life stages of these valuable marine resources.

53 Combined effects of Deepwater Horizon oil exposure and natural stressors on the swimming performance of juvenile mahi-mahi (Coryphaena hippurus)

E. Mager, University of Miami / Rosenstiel School of Marine and Atmospheric Science / Division of Marine Biology & Fisheries; A. Esbaugh, University of Texas Marine Science Institute / Rosenstiel School of Marine and Atmospheric Science, University of Texas – Austin, Marine Science Institute / Department of Marine Science; J. Stieglitz, University of Miami – RSMAS / Division of Marine Biology and Fisheries; R. Hoenig, C. Bodinier, University of Miami, RSMAS / MBF Division; J. Incardona, NOAA Fisheries / Env. Conservation Division; N.L. Scholz, NOAA Fisheries / Northwest Fisheries Science Center; D. Benetti, University of Miami, RSMAS / Division of Marine Biology and Fisheries; M. Grosell, University of Miami / Division of Marine Biology and Fisheries

To investigate the potential impacts that the 2010 Deepwater Horizon (DWH) incident may have on the physiological fitness of pelagic fish species, mahi-mahi (*Coryphaena hippurus*) were acutely exposed to water accommodated fractions (WAFs) of DWH crude oil as embryos/larvae or as juveniles and then subjected to an incremental velocity test to obtain measures of critical aerobic swimming speed (U_{crit}) and aerobic scope. Juveniles that were exposed for 48 h to 1.2 ± 0.6 μg L^{-1} ΣPAHs (geometric mean ± SEM) as embryos/larvae demonstrated a 37% decrease in U_{crit} compared to controls. Additionally, a 24 h exposure of 30 ± 7 μg L^{-1} ΣPAHs (geometric mean ± SEM) to mahi-mahi during the juvenile stage reduced U_{crit} by 22%. WAF-exposed larvae from the 48 h exposure exhibited increased incidence of pericardial and yolk sac edema (4.5-fold), injury indicative of a hallmark cardiotoxic syndrome associated with tricyclic PAH exposure. Respirometric analyses and cost of transport calculations point to a reduction in swimming efficiency as a potential alternative or contributing mechanism to one which limits aerobic scope (e.g., cardiac output) as an underlying cause for the observed decreases in U_{crit}. The effects of combining natural environmental stressors likely to be encountered in the Gulf of Mexico (e.g., hypoxia, UV exposure, temperature) with WAF exposures to mahi-mahi will be reported.

Integrating Human and Ecological Toxicology Chemical Hazard Assessment via Adverse Outcome Pathways

54 Overview of an internationally harmonized program for adverse outcome pathway development

D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; S.W. Kennedy, Environment Canada, University of Ottawa / Department of Biology; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory; K. Tollefsen, NIVA / Ecotoxicology and Risk Assessment, Norwegian Institute for Water Research (NIVA); Norwegian University of Life Sciences (UMB) / Ecotoxicology; Centre for Environmental Radioactivity (CERAD CoE); K.M. Crofton, USEPA / Integrated Systems Toxicology Division, NHEERL; R. Kavlock, USEPA / Office of Research and Development; M. Whelan, European Comission, DG Joint Research Centre / European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM); A. Gourmelon, OECD / Environment Health nd Safety Division

Adverse outcome pathways (AOPs) are critical frameworks for organizing knowledge concerning the scientifically credible predictive linkages between toxicological observations made at molecular and cellular levels (e.g., via molecular screening assays, biomarker responses, or chemical-biological interactions predicted from quantitative structure activity relationships [QSARs]) and adverse outcomes that occur at levels of organization relevant to risk assessment (e.g., impacts on survival, growth, reproduction in the case of ecotoxicology). The Organization for Economic Cooperation and Development (OECD) is fostering the development of AOPs to (1) inform test guideline development, particularly relative to more mechanistically-oriented and/or efficient alternatives to traditional whole animal tests with apical endpoints; (2) support application of Integrated Approaches to Testing and Assessment; and (3) complement the use of the OECD QSAR toolbox for grouping chemicals. To aid the development of high quality AOPs suitable for regulatory application, the OECD published a guidance document and template for developing and assessing AOPs and is implementing a knowledge-base for storing, organizing, and disseminating AOP knowledge. The guidance and template were designed to aid experts in assembling and presenting the weight of evidence supporting predictive relationships represented in the AOP. In an effort to help coordinate and harmonize international AOP development efforts, individual experts and organizations are invited to submit AOP project proposals to OECD's Extended Advisory Group on Molecular Screening and Toxicogenomics (EAG MST). This presentation will describe the purpose and process for submitting AOP project proposals, developing AOPs in a format compatible with the AOP knowledge-base and suitable for scientific review and obtaining endorsement of the AOP by OECD's expert groups and regulatory authorities. The goal of this presentation is to foster participation from both the ecotoxicology and human health research communities in developing AOP knowledge that will provide a foundation for new approaches to risk assessment and regulatory decision-making. *The contents of this abstract neither constitute nor necessarily reflect USEPA Policy nor the views of OECD member countries.*

55 Web-Based Adverse Outcome Pathway (AOP) Visualization Tool

T. Habib, BTS / Environmental Laboratory; K.A. Gust, ERDC-EL-EP-P / Environmental Laboratory, US Army, Engineer Research and Development Center; M.S. Wilbanks, US Army Engineer Research and Development Center; D.R. Johnson, Us Army Engineer Research & Development Center / Environmental Laboratory, US Army / CEERD-EP-R / Environmental Laboratory; P. Gong, Enivronmental Services, Badger Technical Services LLC; N. Vinas, Mississippi State University; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory

Adverse Outcome Pathway (AOP) is a conceptual construct that delineates the documented and testable processes by which a chemical induces molecular perturbations that can cause effects at the sub-cellular, cellular, tissue,

organ, whole animal, and population levels of observation. In collaboration with the Organization of Economic Co-operation and Development (OECD) AOP wiki project, we have developed an online AOP knowledge management and visualization tool in an attempt to capture the adverse effects thought to occur when exposed to a known chemical. The content is organized in a relational database to give users the contextual details such as upstream cause, downstream effect, associated pathways, species specific, experimental methods (in vivo, ex-vivo, etc.) in a web browser. Chemicals, genes, and species entries in the knowledgebase also use external information from National Center for Biotechnology Information (NCBI) and ACToR database. The knowledgebase can be searched based on chemical initiator, key events, or adverse outcome. Search results will also provide likely paths between chemical to adverse effects as linked pathways. These links are established based on the upstream cause and the downstream effect of the chemical. The upstream cause of the AOP is the molecular initiating event that represents the chemical-induced perturbation of the biological system, while the downstream effect of the AOP is the adverse biological effect. Since adverse effects can be measured at any level of biological organization, multiple upstream causes may link to multiple downstream effects in an AOP. All of these paths link back to the original data in the source article. The search results can be exported and viewed as network using the open source tool, Cytoscape (http://www.cytoscape.org/). All data will be exportable as tab delimited files for use in other programs. HTML with CSS, JavaScript, and PHP was used to implement the dynamic web pages and relational database MySQL was used to host the data. We also expect the tool to become an anchor point to OECD AOP wiki primarily to extract the information to view in a graphical mode, and as an investigative source to improve adverse pathways.

56 AOPs & Biomarkers: Bridging High Throughput Screening and Regulatory Decision Making

S. Edwards, USEPA / Integrated Systems Toxicology Division – NHEERL

As high throughput (HTS) toxicity testing plays a larger role in toxicity testing, computational toxicology has emerged as a critical component in interpreting the large volume of data produced. As computational models for this purpose become increasingly sophisticated, additional data sources are required to complement the HTS testing results. Biomarkers of effect can provide measurable data connecting the magnitude of perturbation from the *in vitro*system to a level of concern at the organism or population level. The adverse outcome pathway (AOP) concept provides an ideal framework for combining these two complementary data sources. This talk will survey recent international efforts to formalize the AOP concept and demonstrate how biomarkers linked to the AOP framework can provide tools for evaluating key events downstream of the toxicity pathway in humans and thereby provide a bridge between HTS toxicity pathway perturbations and the regulatory endpoints of concern. [This is an abstract or a proposed presentation and does not necessarily reflect EPA policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.]

57 Development of Adverse Outcome Pathways for Endocrine Disruption in Daphnia magna

K. Tollefsen, Ecotoxicology and Risk Assessment, Norwegian Institute for Water Research (NIVA); Norwegian University of Life Sciences (UMB) / Ecotoxicology; Centre for Environmental Radioactivity (CERAD CoE); M. Cronin, Liverpool John Moores University; O. Evensen, Norwegian School of Veterinary Sciences; F. Falciani, University of Liverpool; T. Iguchi, National Institute for Basic Biology; E. Perkins, US Army Engineer Research and Development Center; B. Salbu, Norwegian University of Life Sciences (UMB); I. Sylte, University of Tromsø; N. Vinas, Mississippi State University

A number of exogenous compounds have the potential to interfere with the endocrine system of animals and potentially perturb vital endocrine processes to a degree causing an adverse effect (outcome) on ecologically relevant endpoints such as growth, development and reproduction. These endocrine disrupting (ED) effects have been well characterised in aquatic vertebrates and mammals due to a well-defined endocrine system and substantial research effort the last decades, but knowledge of ED effects in a large range of species are still poorly characterised. Lack of knowledge of ED effects in non-vertebrates is a major limiting factor to properly perform risk assessment of endocrine disrupting chemicals (EDCs) across taxa. The present project aims to develop and evaluate adverse outcome pathways (AOPs) for ED in the freshwater crustacean *Daphnia magna* by linking responses at the molecular level with adverse outcomes relevant for regulatory processes. Computational and experimental methods will be used to identify possible ED targets in *D. magna* and identify novel EDCs in crustaceans by targeted *in vitro* bioassays. ED mode of action will be investigated in *in vivo* studies by un-biased toxicogenomics, functional assays and standardised regulatory tests to link perturbations of key molecular events to adverse outcomes of regulatory relevance. The data obtained in the project aim to expand the knowledge of ED effects in Daphnids and expand AOPs for future implementation within regulatory frameworks. Acknowledgements: The authors thank the Norwegian Research council for funding, project NFR-221455 "Adverse Outcome Pathways for Endocrine Disruption in *Daphnia magna*, a conceptual approach for mechanistically-based Risk assessment". More information can be found at www.niva.no/EDRISK.

58 Development of an AOP for neurodevelopment in larval fish to predict effects of contaminants across multiple ecologically relevant fish taxa

C.A. Murphy, Michigan State University / Fisheries and Wildlife; M.J. Carvan, University of Wisconsin-Milwaukee / School of Freshwater Sciences; N. Vinas, Mississippi State University; F.X. Mora, University of Wisconsin – Milwaukee; P.W. Dornbos, Department of Fisheries and Wildlife; N. Basu, McGill University / Department of Environmental Health Sciences; J. Head, University of Michigan / Cooperative Institute of Limnology and Ecosystem Research; R. Goetz, NOAA

The highly synchronized and regulated processes involved in neurodevelopment are known to be particularly sensitive to contaminants. To date, assessment of the impact of contaminants on the neurodevelopmental processes has been restricted to limited species and to measurements of adverse effects that are difficult to extrapolate to higher levels of biological organization. These limitations make ecological risk assessment and extrapolation to other species challenging. We suggest that an adverse outcome pathway framework approach may overcome these shortcomings and we propose to demonstrate this by linking molecular perturbations in developing zebrafish larvae brains after exposure to contaminants to changes in behavior and to overall cohort survival and growth using RNA-sequencing, reverse engineering, behavioral assays and individual-based modeling techniques. Once our framework is developed, we propose to construct fathead minnow and yellow perch AOPs in parallel and to use these AOPs to determine information vital for cross-species extrapolation.

59 Using Adverse Outcome Pathway Focused Effects-Based Monitoring to Evaluate Potential Impacts of Complex Pollutant Mixtures at Field Sites

J.P. Berninger, USEPA; D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; N. Garcia-Reyero, Mississippi State University / Institute for Genomics Biocomputing and Biotechnology; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory; T.W. Collette, D.R. Ekman, USEPA / National Exposure Research Laboratory

Effects-based monitoring (EBM) is an effective tool for evaluating environments with complex mixtures of pollutants. Our current approach uses caged fish assessed via a suite of measured apical endpoints, targeted QPCR, metabolomics, and transcriptomics, coupled with in vitro bioassays to determine potential effects. Part of strength of the methodology comes from employing endpoints associated with adverse outcome pathways (AOPs). The focus of our current program of EBM is on adverse reproductive pathways. Impacts along the reproductive pathway are among the most well established and ecotoxicological AOPs; a long history of laboratory and field research has linked impacts occurring at multiple levels of biological organization with population level effects. This AOP-focused, EBM approach has been developed and applied at several field sites where chemicals of emerging concern, particularly endocrine active compounds, were purported to be a major constituent of the site contamination profile. At these sites, in vitro screening of water samples for estrogenicity (T47D) and androgenicity (MDA-kb2) provides the first level of connection with the AOP. Many of the sites, particularly waste water treatment plants (WWTPs) exhibit significant levels of estrogenicity, establishing a potential for molecular initiating events within the reproductive AOP. Apical endpoints, plasma hormone and vitellogenin concentrations and ex-vivo steroidogenesis, provide a link at the tissue/organ level of biological organization. Fish exposed at several WWTP associated sites exhibited elevated hormone levels; particularly male

concentrations 17β-estradiol and female ex-vivo steroid production. Targeted QPCR was used to evaluate gene regulation associated with key events along the AOP. The '-omics' approaches are less targeted at the specific reproductive AOPs, but by evaluating impacts on metabolites, genes or gene pathways, in the context of AOP knowledge putative links to responses observed at field sites can be established,. Overall, AOP-focused, effects-based monitoring provides a means of defining potential biological consequences of exposure to complex mixtures of environmental pollutants.

60 Potential adverse outcome pathways related to exposure to ionic silver and silver nanoparticles on the fathead minnow

N. Vinas, Mississippi State University; A.J. Kennedy, US Army Engineer Research and Development Center, CEERD-EPR; L. Escalon, US Army, Engineer Research & Development Center; T. Habib, BTS / Environmental Laboratory; J.D. Goss Laird, US Army Engineer Research & Development Center / SpecPro Incorporated; J.A. Steevens, US Army Engineer Research & Development Center / Environmental Laboratory; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory

Nanoparticles are compounds of emerging concern with largely unknown risks for human and ecological health. It is crucial to evaluate their potential biological impact to prevent unintended adverse effects on human health and the environment.We analyzed the transcriptional effects of polyvinylpyrrolidone-coated silver nanoparticles (PVP-AgNPs) and silver nitrate ($AgNO_3$) on the fathead minnow (*Pimephales promelas*) to understand their potential toxicity and adverse outcomes. Fathead minnow females were exposed to either 4 µg/L of $AgNO_3$ or 70 µg/L of PVP-AgNPs for 96h. Microarray analyses were performed on liver and brain. Functional analysis identified potential toxicity pathways and molecular initiating events (MIEs) that were confirmed with functional assays. Data suggested that $AgNO_3$ and PVP-AgNPs had both common and distinct transcriptional effects. The nanoparticles were linked to neurotoxicity and oxidative stress, and identified as a dopamine receptor antagonist. Silver nitrate was also identified as a potential neurotoxicant and was confirmed as adrenergic and cannabinoid receptors antagonist.While silver nitrate and PVP-AgNPs were both potential neurotoxicants, they appeared to act through different MIEs.

61 Investigations into the Development of an Adverse Outcome Pathway for Histological Jaw Lesions in Mink: A sentinel species

J.L. Newsted, Cardno Entrix, Michigan State University / Wildlife Toxicology Laboratory, Department of Animal Science; M.J. Zwiernik, Michigan State University / Wildlife Toxicology Laboratory, Department of Animal Science, College of Veterinary Medicine; S.J. Bursian, Michigan State University / Wildlife Toxicology Laboratory, Department of Animal Science

Mink (*Mustela vison*) exposed to 2,3,7,8,-tetrachlorodibenzodioxin (TCDD) and other TCDD-like compounds, such as 3,3',4,4',5-pentachlorobiphenyl (PCB 126), develop an invasive jaw lesion characterized histologically as mandibular and maxillary squamous cell proliferation. Histologically, this lesion has been characterized as mandibular and maxillary squamous epithelial proliferation that form nests and cords adjacent to the teeth. These squamous cells invade the adjacent alveolar bone leading to severe osteoporosis. Gross observations reveal red, swollen gums and bleeding around the teeth, as well as crooked, displaced and lost teeth. Gross lesions similar to those induced by TCDD-like chemicals in mink have also been described in marine mammals such as Baltic grey seals as well as terrestrial mammals including rats and raccoons. It has been hypothesized that the development of this lesion is mediated by the AhR via the chemical-induced stimulation of the epithelial cells in the rests of Malassez, which produces a bone-resorbing factor. In rats, it has been hypothesized that Ah receptor may indirectly related to abnormal tooth development and bone demineralization through the signaling the epidermal growth factor receptor. To better understand the nature of this lesion in mink and its possible association to similar lesions observed in other mammalian species, we have conducted a review of all pertinent literature including an evaluation of unpublished study results with mink that has been used to develop a research plan to identify and investigate adverse outcome pathways (AOP) involved in the development of this lesion. The goal of this effort provides the basis for the development of predictive models to evaluate novel environmental chemicals as well as to aid in the extrapolation of toxicological results to other mammalian species.

Mercury Characterization and Contaminated Site Remediation: Methods, Challenges, and Lessons Learned Part A

62 Mercury: Binding, Methylation and Demethylation

J. Smith, Oak Ridge National Laboratory

The fate of mercury deposited in the environment depends on the molecular-level physical interactions and chemical reactions, mostly involving mercury interacting with water, various ligands, and proteins. Using the tools of molecular biophysics and computational chemistry we will examine the fundamental underpinnings of several environmentally-important mercury transformations. Mercury (II) preferentially binds to thiol groups; we will examine why this is the case (the traditional explanation fails here). We will also outline how chemical concepts guided our recent discovery of the bacterial genes responsible for producing toxic methylmercury in the environment. Finally, mechanisms used by proteins to detoxify methylmercury mercury-resistant bacteria will be explored.

63 Mercury-cell surface interactions on mercury methylation by Geobacter sulfurreducens PCA

H. Lin, Oak Ridge National Laboratory / Environmental Sciences Division; B. Rao, Oak Ridge National Lab / Environmental Sciences Division; R.A. Hurt, D.A. Elias, Oak Ridge National Lab / Biosciences Division; L. Liang, B. Gu, Oak Ridge National Lab / Environmental Sciences Division

Microbial methylation of mercury (Hg) to methylmercury (MeHg) has received extensive interest due to trophic transfer and bioaccumulation of the neurotoxic MeHg in biota. MeHg is produced primarily by certain anaerobic bacteria, such as *G. sulfurreducens* PCA. Although the genetic basis of bacterial Hg methylation is recently discovered, the mechanisms of Hg uptake and biochemical pathways are not fully understood. Here, we examine the interactions between Hg and *G. sulfurreducens* PCA cells to gain insight into how microbes affect redox transformation of Hg and whether cell association of Hg may influence Hg uptake and methylation. We show that PCA cells are capable of not only reducing Hg(II) but also oxidizing dissolved elemental Hg(0), depending on the ratio of Hg to cell concentrations, the presence or absence of specific Hg-complexing ligands, and specific mutant strains. Under conditions of a low cell to Hg ratio, Hg(II) reduction was dominant, whereas at a high cell to Hg ratio, reoxidation of Hg(0) to Hg(II) occurred. The latter reaction was enhanced by certain thiol ligands, such as cysteine. The *c*-type cytochrome deficient mutant strain, Δ*omcBESTZ*, is impaired in Hg(II) reduction. The mutant showed a higher Hg-cell association and MeHg production than the wild type. However, the methylation deletion mutant, Δ*hgcAB*, showed the highest Hg(II) reduction. These results show interesting correlations on Hg(II) reduction, cell surface association, and its uptake and methylation: reduction of Hg(II) decreases the availability of Hg to microbial methylation, whereas increasing Hg(0) oxidation and Hg-cell association increase methylation. Our results also suggest that the reduction of Hg(II) to Hg(0) is likely an alternative detoxification mechanism when the methylation pathway is blocked.

64 Methylmercury Photodegradation Affected by the Presence of Organic Ligands

Y. Qian, B. Gu, B. Rao, L. Liang, Oak Ridge National Laboratory

Photolytic degradation of methylmercury (MeHg) is a major sink in many surface waters although detailed rates and mechanisms of MeHg photodegradation are not well understood. In this study, we investigated the effects of organic matter on MeHg photodegradation by using different sources of naturally dissolved organic matter (DOM) and model organic ligands with various known molecular structures under both natural and simulated lighting conditions. We show that DOM and organic ligands all enhance photodegradation of MeHg under UV-A irradiation. The most significantly enhanced MeHg degradation was observed in the presence of aromatic thiols, such as thiosalicylate. Not only the sources and concentrations of DOM affected the degradation rates, but also the oxidation states of the DOM. The reduced DOM was three times more effective than the oxidized DOM in degrading MeHg, and we attribute this result to weakening the methyl-Hg bond by complexation of MeHg with thiolate ligands in DOM. Furthermore, we found that degradation rates were strongly correlated to light quality, with the irradiation of shorter wavelength much more effective than the longer wavelength. Under natural sunlight conditions, UV-A is the

major driver, whereas visible light shows little influence on the photodegradation of MeHg. We collected fresh water samples from a contaminated creek for photodegradation study under nature daylight and observed a high MeHg degradation rate of up to $3.71*10^{-3}$ m^2 E^{-1}, which suggests that photodegradation could be an important sink for MeHg in this ecosystem.

65 Characterization of Mercury Interactions with Individual Molecular Species in Natural Organic Matter

B.F. Mann, Oak Ridge National Laboratory / Environmental Sciences Division; R.L. Hettich, L. Liang, B. Gu, Oak Ridge National Laboratory

Natural organic matter (NOM) is known to form stable complexes with mercury (Hg) as well as to participate in redox transformations that affect Hg speciation in the environment. These interactions determine the bioavailability of Hg and consequently, the rate at which it is taken in and converted to highly toxic methylmercury by methylating bacteria. NOM has frequently been treated as a singular substance or a few operationally defined fractions in studies of the speciation and sequestration of Hg. However, the fate of the exposed NOM, including the molecular transformations of the individual compounds therein, has rarely been characterized because NOM is one of the most complex and analytically challenging sample mixtures known. Nonetheless, these measurements are now possible with high-performance liquid chromatography and high-resolution mass spectrometry. In this study, we explore the interactions of NOM with both Hg(0) and Hg(II) by exposing them to NOM samples of both terrestrial and aquatic origins. Taking full advantage of the quantitative capabilities of these high-throughput analytical approaches, we have employed multivariate statistics such as principal component analysis to describe the interrelated transformations of compounds in NOM following exposure to Hg. We discuss the identification of the key molecular species that participate in Hg complexation and redox reactions with important implications to the bioavailability and microbial methylation of Hg in the environment.

66 Profiling mercury binding thiol groups in natural organic matter and methylating bacteria using fluorescent spectroscopy

B. Anandha Rao, Oak Ridge National Laboratory / Environmental Sciences Division; C. Roop, University of Tennessee / Chemistry; B. Mann, H. Lin, L. Liang, B. Gu, Oak Ridge National Laboratory

Recent studies have emphasized the crucial role of organic thiols in controlling the fate of mercury (Hg) species, including highly toxic methylmercury, in both biotic and abiotic systems. Reactions of inorganic Hg species with dissolved organic matter (DOM) result in both reduction of Hg(II) and oxidation of Hg(0), depending on the Hg to DOM ratios. These observations suggest the involvement of two competing mechanisms: reduction of Hg(II) by reduced semiquinone moieties and oxidation of Hg(0) by DOM-thiols via oxidative complexation. Similar phenomena of Hg reduction, oxidation, and surface binding have been observed in methylating bacteria. As a key aspect of studying these processes, a robust and sensitive analytical approach for quantifying the organic thiols present in DOM and bacteria is needed but is not currently available. To that end, we utilized a chemical probe with thiol-specific fluorogenic labeling and systematically evaluated the optimum labeling conditions to ensure high sensitivity (nanomolar levels) and selectivity. Application of our method to measure thiols in *Geobacter sulfurreducens* cells resulted in a value of 0.07 μmoles $g^{-1}$and a value of 3.8 μmoles g^{-1} for a complex soil humic DOM sample. The measured thiol values are in good agreement with those predicted values based on experiments with Hg species. Furthermore, we have developed an HPLC-fluorescence method, with detection based on the described fluorogenic labeling procedure, by which the individual thiols in DOM can be resolved from other components of these complex mixtures, thereby allowing us to characterize the individual thiolated compounds. A clearer understanding of the diversity (or lack thereof) of the major thiols in DOM can be useful for predicting the impact of Hg-DOM complexation, as it pertains to Hg uptake by methylating bacteria, as well as other mechanisms of Hg transformation in the environment.

67 Comparison of Hg and MeHg cycling in a contaminated creek with uncontaminated systems

C. Miller, Environmental Science; A. Riscassi, Oak Ridge National Lab / Environmental Sciences Division; S. Brooks, Oak Ridge National Laboratory; X. Yin, Oak Ridge National Lab

To examine factors controlling Hg cycling total mercury (Hg) and methylmercury (MeHg) concentrations in East Fork Poplar Creek (EFPC), a Hg contaminated system, were compared to uncontaminated systems where atmospheric deposition is the dominant source of Hg. EFPC currently receives a point source Hg discharge (~1000 ng/L) to its headwater and inputs from heterogeneously distributed Hg in creek sediments and bank and floodplain soils. Dissolved organic matter (DOC) concentrations in EFPC are low (< 3 mg C/L) under baseflow conditions, which is similar to other systems with low wetland (< 3%) basin cover. There are strong correlations between dissolved Hg and DOC in most uncontaminated systems with Hg and DOC increasing downstream as inputs from the terrestrial environments increase. This relationship is not observed in EFPC as a result of the high Hg load and low wetland area. An increase in MeHg downstream, which is observed in EFPC, has been observed in both contaminated and uncontaminated systems and this increase is often coupled with an increase in DOC concentration. In uncontaminated systems with DOC concentrations similar to EFPC, MeHg concentrations are often less than 0.05 ng/L but in EFPC concentrations range from 0.01-0.56 ng/L. Higher than expected MeHg concentration in EFPC which contains elevated Hg from non-atmospheric sources suggests that the concentrations or source of Hg can change result in higher MeHg concentration than observed in uncontaminated systems. Sediment Hg concentrations in EFPC are ~1000 times higher than uncontaminated sediments but MeHg concentrations are only 10-100 times higher. Relationships between reduced iron and sulfur and MeHg and mercury methylation potentials (MMP) in EFPC are similar to other systems. MMP in EFPC are similar to those measured in uncontaminated systems but predicted MeHg concentrations, based on MMP and Hg concentration, are higher than observed. Possible explanations for this are increased demethylation or a lower fraction of bioavailable Hg in EFPC compared to uncontaminated creeks. Caution is needed when data from uncontaminated systems is used to explain the cycling of Hg in contaminated creeks. For example, in EFPC parallels between sediment MeHg relationships with redox status can be drawn with uncontaminated systems but relationships with water column DOC and Hg and MeHg differ.

68 Spatial and temporal variations in prey fish mercury concentrations in a reservoir

Z. Dong, Harvard School of Public Health / Environmental Health; L.A. Schaider, Harvard School of Public Health / Department of Environmental Health; R.A. Lynch, University of Oklahoma Health Sciences Center / Occupational and Environmental Health; J.P. Shine, Harvard School of Public Health / Department of Environmental Health

Human exposure to mercury (Hg) through fish consumption, primarily in the form of methylmercury (MeHg), is a public health concern, with thousands of fish consumption advisories issued in the US Understanding factors controlling Hg in fish and whether Hg changes over space and time is important for assessing both human and ecological health risks, and for evaluating efficacy of Hg reduction strategies. Compared to higher trophic level fish, prey fish at lower trophic levels may more closely reflect spatial and temporal variations in MeHg production. To study spatial and temporal variations in their Hg concentrations, prey fish and young-of-year predator fish samples were collected at Grand Lake, northeastern Oklahoma, USA, a reservoir located within 100 km of six coal-fired power plants, as part of a community-based participatory research project on Hg exposure among residents who consume local fish. In addition to surveying Hg concentrations in commonly-consumed fish species, we measured total Hg concentrations in over 350 prey fish, collected throughout the watershed from April to September, 2012, and MeHg in a subset of the samples. Stable isotopes ($d^{13}C$ and $d^{15}N$) were analyzed for six major species (i.e., shad, sawfin shiner, fathead minnow, brook silverside, black bass and bluegill), to evaluate observed changes in Hg concentrations from the perspective of food web dynamics. The results showed little spatial variations in total Hg across sampling locations, while different prey fish species exhibited different temporal trends, with total Hg concentrations varying up to five-fold over time. These results provide insight into the magnitude of temporal and spatial variations in fish Hg at lower trophic levels, as well as the extent that Hg concentrations in fish reflect localized variations in MeHg production.

69 Water Quality Improvements Linked to Reduced Methylmercury Concentrations in Onondaga Lake Water and Fish

B. Henry, M. Murphy, Anchor QEA; S. Todorova, Cardno Entrix; C. Driscoll, Syracuse University; D. Matthews, S. Effler, Upstate Freshwater Institute; D. Babcock, Parsons

Onondaga Lake in Syracuse, NY has been impacted by a long history of municipal and industrial activity, including two mercury cell chlor-alkali plants. The 2005 Record of Decision requires sediment dredging, capping, and monitored natural recovery, as well as potential addition of oxygen or nitrate to the hypolimnion to decrease methylmercury release from sediment. Upgrades at the Metropolitan Sewage Treatment Plant, including ammonia and phosphorus controls in 2004 and 2005, respectively, have resulted in increased nitrate concentrations and concomitant decreases in methylmercury in lake water. Nitrate addition to the lake is the subject of a 3-year pilot test that started in 2011. Dredging began in 2012. Monitoring was conducted in 2008-2012 to document current conditions. Water samples were collected from multiple depths from April through November and analyzed for total mercury, methylmercury, and ancillary water quality parameters. Mercury in tributaries, zooplankton, and fish was also monitored. Fish, benthic invertebrates, and zooplankton were analyzed for stable carbon and nitrogen isotopes to assess the food web. Methylmercury concentrations in the epilimnion of Onondaga Lake now average 0.05–0.15 ng/L. They are considerably lower than current concentrations in tributaries to the lake and are within the range of concentrations observed at lakes in New York, Wisconsin, Minnesota, and Sweden that lack point sources of mercury contamination. Concentrations in the hypolimnion peaked at 0.23 ng/L in 2012, compared to 15 ng/L in 1992 during summer stratification. Much of the decrease in methylmercury concentration is attributed to increased nitrate concentrations and diminished electron acceptor demand in the hypolimnion during summer anoxia. Length normalized mercury concentrations in smallmouth bass have recently decreased after remaining stable from 1996 through the mid-2000s, and are close to background concentrations reported for the northeastern US Stable isotope analysis showed a shared carbon signature for plankton and predator fish (and thus a pelagic food chain), so decreases in methylmercury concentrations in surface water are expected to be reflected in fish tissue concentrations. The results indicate that water quality improvements can have profound effects on methylmercury concentrations in water and fish irrespective of sediment contamination or remediation.

Urban Impact on Aquatic Biota from Stream Water and Sediment Quality

70 Urban headwater hypoxia: the effect of hydrologic and climatic conditions on dissolved oxygen concentrations in urban streams

M. Lutz, US Geological Survey-WRD; S.R. Corsi, US Geological Survey / Water Resources Division

Urban streams and their biota are subject to a variety of stressors. Frequently, compromised biological communities in these environments are assumed to be responding directly to the pronounced hydrologic fluctuations or influx of anthropogenic compounds. The effects of depressed oxygen concentrations, however, have been largely overlooked. The current study was developed based on observations of hypoxic conditions in urban headwater streams from data collected in conjunction with a stream toxicity study conducted from 2005 to 2007; of the 27 streams studied there, 15 showed hypoxic conditions. Three of the streams showing overall depressed oxygen levels were selected for further study here. The current study focused on seven sites in three watersheds in the Milwaukee, Wis., metropolitan area. In 2010 and 2011, from May to September each year, continuous water quality measurements were collected at all seven sites. Hypoxic conditions were observed at all seven sites, with six of the sites exhibiting such conditions for considerable proportions of the overall record. Oxygen concentrations responded strongly to hydrologic conditions. During low flow conditions, dissolved oxygen concentrations frequently followed a steady increasing or decreasing trend, with generally increasing diurnal amplitude. Hydrologic events frequently disrupted these trends, and reset concentrations to a new norm. Further, storms frequently acted to temporarily decrease the amplitude of the diurnal concentration cycle. In 2011, metabolism studies were conducted at six of these sites in order to address reach-scale questions about oxygen production and consumption. Dissolved oxygen concentrations decreased along each reach. Results showed production-to-respiration ratios of less than 0.25 percent at all reaches, indicating all of them to be strongly heterotrophic.

71 White Roads, White Trees and White Stripes: Stream Conductivity and Road Salt Relationships to Land Use and Fish Assemblages in Maryland Streams

R. Morgan, UMCES / Appalachian Laboratory

Stream conductivity reflects both landscape and anthropogenic interactions, although increasing chloride inputs from road salt application in eastern North America is also important. Employing a spatially extensive database derived from the Maryland Biological Stream Survey (MBSS), relationships of stream conductivity (as a chloride surrogate) to stream MBSS fish metrics, abundance, biomass, and assemblages were determined to estimate potential effects. Background stream conductivity for the MBSS strata and Maryland L3 ecoregions ranged from 51 to 150 µS/cm, with the Piedmont having the highest background conductivity (145 – 160 µS/cm). For MBSS sites there were strong relationships of stream conductivity and chloride with both impervious surface and road density, with 0.26% of the MBSS sites exceeding the USEPA acute chloride criterion and 1.5% the chronic chloride criterion. For the Maryland Piedmont, observed conductivity values between 230 – 540 µS/cm caused important alterations in the biotic community as measured by the series of fish metrics, and thus affecting fish species assemblages associated with chloride concentrations between 33 to 108 mg/L. Maryland Coastal, Coldwater, and Highland species assemblages displayed variable responses of conductivity to fish metrics, abundance and biomass. If regional conductivity and chloride levels continue to increase or remain high from road deicing due to increasing urbanization in a watershed and/or climatic changes, differences in stream fish assemblages and therefore fish diversity may become more apparent. Because of the linkage between salt usage and impervious road surfaces resulting from urbanization, it is important to manage effectively the use of road salt to protect biotic resources.

72 Inorganic and Organic Contaminants in Sediments from an Urban Playa and Associated Toxicity among Hyalella azteca

D.R. Faust, Mississippi State University / Wildlife, Fisheries, and Aquaculture; N. Knowles, E. Magruder, I-20 Wildlife Preserve and Jenna Welch Nature Study Center; D. Haukos, Texas Tech University / Department of Natural Resources Management; G.P. Cobb, Baylor University / Department of Environmental Science; J.D. Maul, Texas Tech University / The Institute of Environmental and Human Health / Department of Environmental Toxicology; T. Anderson, Texas Tech University; P.N. Smith, Texas Tech University / Environmental Toxicology

Playa wetlands are important components of the Southern High Plains landscape as they are the major aquatic surface feature. Chemical contaminants associated with playas have been documented, particularly for grassland and agricultural watersheds, but not for playas in urban settings. The objectives of this study were to determine concentrations of inorganic and organic contaminants in sediments from an urban playa within the I-20 Wildlife Preserve and Jenna Welch Nature Study Center in Midland, TX, and evaluate toxicity of these sediments to *Hyalella azteca*. Concentrations of most trace elements were below sediment quality guidelines with exceptions of lead, cadmium, and arsenic. Concentrations of organic contaminants, particularly PAHs, DDT, DDE, and malathion, were above sediment quality guidelines at various locations within the playa. Decreased survival was observed among *H. azteca* exposed to sediment from a single location when compared those exposed to reference sediments. This location also produced maximum observed concentrations for six of eight trace elements, potentially due to its location at the lowest elevation within the playa. This study documented concentrations of contaminants in sediments of an urban playa associated with past and present land uses in its urban setting, including those from automotive emissions and historical pesticide use.

73 From Streets to Streams: assessing the toxicity potential of urban sediment by particle size

B. Selbig, USGS

Urban sediment can act as a transport mechanism for a variety of pollutants to move towards a receiving water body. The concentrations of these pollutants oftentimes exceed levels that are toxic to aquatic organisms. Many treatment structures are designed to capture coarse sediment but do not work well to similarly capture the fines. This study measured concentrations of select trace metals and PAHs in both the silt and sand fraction of

urban sediment from four sources: stormwater bed, stormwater suspended, street dirt, and streambed. Concentrations were used to assess the toxic potential of sediment based on published sediment quality guidelines. All sources of sediment showed some level of toxic potential with stormwater bed sediment the highest followed by stormwater suspended, street dirt, and streambed. Both metals and PAH concentration distributions were highly correlated between the four sampling locations suggesting the presence of one or perhaps only a few sources of these pollutants which remain persistent as sediment is transported from street to stream. Comparison to other forms of combustion- and vehicle-related sources of PAHs revealed coal tar sealants to have the strongest correlation, in both the silt and sand fraction, at all four sampling sites. This information is important for environmental managers when selecting the most appropriate Best Management Practice (BMP) as a way to mitigate pollution conveyed in urban stormwater from source to sink.

74 Adult Coho Salmon Exposed to Road Runoff Exhibit Pre-spawn Mortality Symptomology

J.A. Spromberg, Ocean Associates Inc. Contractor with NOAA Fisheries / Northwest Fisheries Science Center; D.H. Baldwin, NOAA Fisheries / Northwest Fisheries Science Center; J.K. McIntyre, School of Aquatic and Fishery Sciences / School of Aquatic & Fishery Sciences; S. Damm, US Fish and Wildlife Service; J. Davis, US Fish & Wildlife Service; N.L. Scholz, NOAA Fisheries

Several urban streams in the Seattle area were the focus of habitat restoration projects in the 1990s. Post-project effectiveness monitoring surveys revealed anomalous behaviors among adult coho salmon returning to spawn in these restored streams. Behaviors included erratic surface swimming, gaping, fin splaying, and loss of orientation and equilibrium. Affected fish died within hours, and female carcasses showed high rates (> 90%) of egg retention. This phenomenon was termed coho pre-spawn mortality (PSM). From 2002-2010, rates of coho PSM ranged from ~ 30-90% in monitored urban streams. The severity of PSM was closely associated with both the timing and amount of fall rains. Coho also showed evidence of exposure to metals and petroleum hydrocarbons, both of which commonly originate from motor vehicles. The weight of evidence suggests that an as-yet unidentified toxic contaminant or contaminant mixture in urban stormwater runoff is killing coho spawners. Geospatial analyses point to urban land uses, impervious surfaces and specifically road density to be correlated related to the levels of PSM across watersheds. During the autumns of 2011 and 2012, we exposed adult coho recently returned to freshwater to various mixtures of PAHs and metals to simulate vehicle runoff, or to collected road runoff. The PAH and metal mixtures tested did not produce PSM symptomology. Road runoff collected from an elevated highway produced typical PSM symptomology in all exposed fish within 4h. The results suggest that while the contaminants in the simulated runoff mixtures cannot be ruled out as playing a role in the causation of coho PSM, the tested mixtures alone were not sufficient to cause the phenomenon. Contaminants sufficient to cause PSM are however, present in the road runoff, but have not yet been identified. Tissue samples from experimentally exposed fish and field collected symptomatic fish will be used to identify the physiological impairments leading to PSM symptomology.

75 Ecosystem structure and function as ecotoxicological responses to pharmaceutical and personal care products

E.J. Rosi-Marshall, H. Bechtold, A. Shogren, Cary Institute of Ecosystem Studies; M. Rojas, Loyola University Chicago; T.V. Royer, University of Indiana Bloomington; D.D. Snow, University of Nebraska; J. Kelly, Loyola University Chicago

Pharmaceutical and personal care products (PPCPs) are ubiquitous in surface waters throughout the world. We discuss the various scales of inquiry that can be employed to measure the effects of PPCPs on stream ecosystem structure and function. For example, our recent studies indicate that primary production and respiration of stream biofilms are sensitive to common PPCPs, but that the two processes can respond differently to particular compounds. We also find that biofilm responses to PPCPs are variable across land use gradients. In urban streams, microbial function is less sensitive to PPCP exposure than in forested streams. These streams also span a gradient of PPCP concentrations that may result in resistant communities of organisms. We measured the structure of microbial communities using 16SrRNA pyrosequencing and found that bacterial community structure both of which appear to be sensitive to exposure to PPCPs at environmental levels. Our data indicate that exposure to PPCPs may affect stream ecosystem structure and function. Further investigations employing these and other ecosystem approaches may shed new light on the ecotoxicological consequences of PPCPs in aquatic ecosystems.

76 Organic Waste Compounds in Great Lakes Tributaries: Occurrence and Aquatic Toxicity in Different Flow Regimes and Land Uses

A.K. Baldwin, S.R. Corsi, P.L. Lenaker, L. De Cicco, D.J. Sullivan, US Geological Survey-WI

Elevated concentrations of organic waste chemicals (OWCs) in some Great Lakes tributaries indicated a high potential for adverse impacts on aquatic organisms. OWCs used in agriculture, industry, and households make their way into surface waters through runoff, leaking septic-conveyance systems, regulated and unregulated discharges, and combined sewage overflows, among other sources. During 2011-13, as part of the Great Lakes Restoration Initiative, 250 samples were collected at 17 tributaries in Minnesota, Wisconsin, Michigan, Ohio, Indiana, and New York, representing a range of land uses from forested to agricultural to urban. Samples were collected during high and low-flow conditions and analyzed for a suite of 69 OWCs, including herbicides, insecticides, polycyclic aromatic hydrocarbons (PAHs), plasticizers, antioxidants, detergent metabolites, fire retardants, non-prescription human drugs, flavors/fragrances, and dyes. Concentrations of many of the compounds showed a clear urban gradient, with concentrations increasing as urban area in the drainage basin increased. These include most of the insecticides, PAHs, fire retardants, human drugs, flavors/fragrances, and dyes. High-flow and low-flow sample concentrations were generally similar, except at urban sites where high-flow concentrations were higher than low-flow for many compounds, including herbicides, insecticides, PAHs, human drugs, and dyes. Urban runoff and storm-related leaks of sanitary/septic systems may be important sources of these and other compounds to the streams. Aquatic-toxicity benchmarks were exceeded for a number of compounds with known benchmarks. The compounds with the greatest benchmark exceedances were the PAHs, both in terms of exceedance frequency and magnitude. In the majority of samples from the most urban sites, PAH concentrations showed high potential for adverse impacts to aquatic life.

77 The Stream Pollution Trends (SPoT) Program: Monitoring Toxicity and Contaminants of Emerging Concern in California

K. Siegler, University of California – Davis / Environmental Toxicology; B.M. Phillips, University of California – Davis / Environmental Toxicology / Granite Canyon; B.S. Anderson, University of California – Davis / Department of Environmental Toxicology; J. Voorhees, Univeristy of California – Davis / MPSL – Environmental Toxicology; L.L. Jennings, University of California – Davis / Enviromental Toxicology; M. Petersen, University of California – Davis; R.S. Tjeerdema, University of California – Davis / Environmental Toxicology; K. Larsen, R. Breuer, California State Water Resources Control Board

The Stream Pollution Trends (SPoT) monitoring program conducts statewide surveys of California stream water quality. As part of the Surface Water Ambient Monitoring Program (SWAMP), SPoT is designed to detect trends in contamination and toxicity in major watersheds of California. Integrative measurements of sediment toxicity and a suite of pesticides, trace metals, and industrial compounds have been conducted annually since 2008. Sediment toxicity was fairly consistent from 2008-2012 using the 10d *Hyalella azteca* test. The prevalence of pyrethroid pesticide detections increased from 55% in 2008 to 85% in 2010. Correlation studies showed that concentrations of most measured pollutants increased with urban land cover in the watersheds. In 2013, a collaboration with the California Department of Pesticide Regulation (CDPR) includes intensive monitoring in urban watersheds to investigate the effectiveness of new policies to reduce urban pyrethroid loading. Intensive monitoring will also characterize the occurrence and concentrations of fipronil, a contaminant of emerging concern, in urban areas. Cyanotoxins (Microcystin L-R) are an emerging contaminant of concern in California and will be measured in collaboration with California State University, Monterey Bay using an enzyme-linked immunosorbent assay (ELISA). SPoT data on these contaminants of emerging concern are used by regulatory agencies both to evaluate long-term water quality trends and to develop policy recommendations.

Endocrine-disrupting Chemicals and Pharmaceuticals in the Environment: Part A

78 Enantioselective analysis of chiral pharmaceuticals in wastewater and environmental water samples

E. Sanganyado, University of California Riverside / Environmental Science; J. Gan, University of California Riverside / Environmental Science

Chiral human pharmaceuticals including atenolol, fluoxetine, ibuprofen and tramadol are environmental pollutants of emerging concern. Normal-phase and reversed-phase chiral liquid chromatography (HPLC-UV) methods were developed to resolve stereoisomers of atenolol, fluoxetine, ibuprofen and tramadol. In normal phase, enantiomeric resolution (R_S) of tramadol was 7.95 and ibuprofen was 1.66, obtained using Chiralpak AD-H and Chiralcel OJ columns, respectively. The mobile phases were n-hexane/2-propanol/diethylamine (90:10:0.1, v/v/v) and n-hexane/2-propanol/trifluoroacetic acid (98:2:0.1, v/v/v) for the enantioseparation of tramadol and ibuprofen, respectively. Enantiomers of atenolol and fluoxetine were resolved using Chirobiotic V column and a mobile phase constituting methanol, 15 mM ammonium acetate and 0.005% formic acid. The R_S of atenolol and fluoxetine were 2.40 and 2.24, respectively. The methods were subsequently used to evaluate changes in enantiomeric composition of these compounds in river water and wastewater, as well as sediments. Results from these experiments will be discussed at the meeting.

79 Determination of antidepressants in biosolids destined for land application and the potential for bioaccumulation in terrestrial organisms

M.M. Schultz, College of Wooster / Department of Chemistry; L.M. Niemi, College of Wooster / Chemistry; M. Dawaher, College of Wooster / Biochemistry & Molecular Biology; M. Hall, College of Wooster / Chemistry

Antidepressants are one of the most widely dispensed classes of pharmaceuticals in the United States. As wastewater treatment plants are a primary source of pharmaceuticals in the environment, the use of biosolids as fertilizer is a potential route for antidepressants to enter the terrestrial environment. A microsolvent extraction method, utilizing green chemistry, was developed for the extraction of the target antidepressants and degradation products from biosolids, or more specifically lagoon sludge. Liquid chromatography tandem mass spectrometry was used for the quantitative determination of antidepressants in the lagoon sludge extracts. The method was applied to biosolids destined for land application. A suite of antidepressants was consistently detected in the lagoon sludge samples collected over nine months. Thus, antidepressants are being introduced to terrestrial environments through the land application of these biosolids. Sertraline, citalopram, and norsertraline were the most commonly detected antidepressants and degradation products in the biosolid samples. Detected, individual antidepressant concentrations ranged from 8.5 ng/kg (norfluoxetine) to 420 ng/kg, wet weight (norsertraline). Studies are underway to measure the concentrations of antidepressants in the biosolid-amended soil over time, and to investigate the bioaccumulation of antidepressants in earthworms residing in the biosolid-amended soil.

80 Artificial sweeteners as tracers of wastewater contamination

T. Sultana, Trent University; C.D. Metcalfe, Trent University / Environmental & Resource Studies

Artificial sweeteners (AS) are used as table top sweetners and as food additives in low calorie foods and drinks and in some personal care products. Sucralose (SCL) and the potassium salt of acesulfame (ACS) are two AS which are not metabolized in the human body. Therefore, these compounds have a high potential to be carried in sewage into municipal wastewater. Because of the concern about contamination of surface water and drinking water by discharges from wastewater treatment plants (WWTPs), the present study was aimed at monitoring the occurrence of AS in surface water along the Grand River in Ontario, Canada as a tracer of wastewater contamination. An analytical method was developed using liquid chromatography and tandem mass spectrometery (LC-MS/MS) for the simultaneous trace quantification of SCL and ACS in grab samples of water, as well as from passive sampling using the Polar Organic Integrative Sampler (POCIS) with HLB sorbent. Pre-concentration of water samples using MCX solid phase extraction (SPE) cartridges after pH adjustment to 1.5 yielded satisfactory recoveries of >75% for the target compounds. Deuterated surrogates were spiked before SPE to aid in analyte quantitation. Monitoring in the Grand River and in the intake of a drinking water treatment plant downstream of the discharge from a WWTP indicated that both ACS and SCL were present at low ug/L concentrations and were very persistent in the river. The time weighted average concentrations estimated from 2 week deployments of POCIS were consistent with grab sample results for SCL, but not for ACS. Therefore, work is underway to develop a more efficient sorbent for passive sampler monitoring of ACS.

81 Evaluation of the transport and potential risks of the nonylphenoletoxylates in the industrial site

A. Pasanen, Geological Survey of Finland, Eastern Finland Office; M. Lyytikainen, University of Eastern Finland / Department of Biology; L. Solismaa, Geological Survey of Finland, Eastern Finland Office; J. Keronen, University of Eastern Finland / Department of Biology; J. Kankkunen, S. Backnas, Geological Survey of Finland, Eastern Finland Office; J.V. Kukkonen, University of Jyvaskyla / Department of Biology

Nonylphenoletoxylates are synthetic, organic compounds with excellent surfactant properties. They are harmful for the environment especially because of their decomposition product, nonylphenol. Nonylphenol is considered to be an endocrine disruptor due to weak ability to mimic estrogens. It may also cause respiratory toxicity, decrease the growth of organisms and inhibit differentiation of cells. Nonylphenoletoxylates and nonylphenol are considered as hazardous substances for the water environment in the Finnish and the European legislations. Therefore, environmental quality standard has been set for nonylphenoletoxylates and nonylphenol. The aim of the study was to I) evaluate maximum concentrations and harmful consequences for the environment of the use of nonylphenoletoxylates, 2) evaluate the transport, environmental load and decomposition of the nonylphenoletoxylates and 3) compare measured concentrations with the modeled concentrations and evaluate the environmental risks. In addition, the analysis methodology developed for the nonylphenoletoxylates and nonylphenol is described. The study area is located in the municipality of Siilinjärvi in Eastern Finland and consists of industrial and mining site and surrounding water bodies. Extensive water and sediment sampling was performed. The sampling included measuring the physical parameters of water using a field surveying device. The analyses showed that concentrations of nonylphenoletoxylates were high in the industrial and mining site but were diminished in the nearby lake. This diminution was thought to be caused by adsorption of nonylphenoletoxylates to tailings and mixing of nonylphenoletoxylates bearing and clean waters. Nonylphenol was not detected in the analysis. Environmental quality standard is exceeded in the industrial and mining site but the concentrations are below it in the lake waters. Maximum environmental load of the nonylphenoletoxylate to lake was estimated to 1080 kg/a and to the western side of the tailings pond 27 kg/a. According to the modeling, the risk for the environment in the lake area is insignificant. Further, the infiltration of the nonylphenoletoxylates to ground water was estimated to be minor. It is suggested that the concentrations of the nonylphenoletoxylates should be monitored regularly and the emissions should be reduced to protect the environment in the industrial area.

82 Estimation of County-Level Hormone Contributions to Surface Water from Humans, Livestock, Wildlife and Fish

C.D. Hassinger, ARCADIS-US; P.D. Anderson, Arcadis US, Inc.; K. Gehl, T. Negley, ARCADIS; W. Stiteler, Arcadis US, Inc.

The presence of hormones such as estrogen and testosterone in water bodies of the United States (US) has recently received increased scrutiny due to their demonstrated effects on aquatic species at concentrations as low as 1 ng/L. Hormones in surface water can originate from multiple sources including humans, livestock, wildlife, fish, and plants; however little is known about the relative contribution of individual sources across the US As a result, resources allocated to reducing hormone contributions to surface water have the potential to be misallocated. This paper builds upon previous research presented by Anderson, et al. which estimated the relative hormone contributions to surface water from humans, livestock, wildlife and fish at a continental-level (coterminous US). The research highlighted a critical need to better understand the significance and spatial distribution of hormone releases from both animal feeding operations and fish relative to other hormone sources. Publically available data were used to refine previous continental scale estimates to a county-level resolution. Human population estimates were based on 2010 US census data stratified by sex and pregnancy status. Livestock population data for cattle (beef and dairy), pigs and poultry species were obtained from the US Department of Agriculture. Wildlife

population estimates were derived by applying the standard 10% assimilation efficiency between trophic levels to net primary productivity data. Fish hormone contributions were estimated from fish biomass data for lakes and rivers and county-level surface water surface area data. The total estrogen and testosterone mass excreted daily by each contributor group was estimated for each county. Excretion estimates were then normalized by county area and the daily mass of hormone transported to surface water for each group was estimated by applying derived species-specific attenuation factors. The relative hormone contributions to surface water at the county-level were analyzed and displayed in a Geographic Information System (GIS). Case studies are presented that illustrate regions where hormone contribution profiles are unique. Results help to quantify the relative importance of various hormone sources and provide useful information to focus risk management on the most significant hormone sources in key regions of the US.

83 Effects of Conjugated Estrogens on a Microcrustacean, Moina macrocopa

W. Pruksarojanakul, P.D. Cooper, The Australian National University / Research School of Biology, ANU College of Medicine, Biology & Environment; T. Pewnim, Silpakorn University / Department of Chemistry, Faculty of Science

Endocrine-disrupting chemicals (EDCs) of estrogenic nature found in the environment may come in different forms such as estrone (E_1), 17β-estradiol (E_2), ethinyl estradiol (EE_2), diethylstilbestrol (DES), 4-nonylphenol (NP), 4-octylphenol (OP), or bisphenol A. While the first four compounds are estrogenic drugs used for different purposes ranging from estrogen replacement to being a component of contraceptive pills, the latter three compounds are not steroid in nature but have structures similar to estrogens. NP and OP are transformed products of alkylphenol polyethoxylate surfactants commonly used as detergents, while bisphenol A often comes with certain plastic materials. We are interested in using a microcrustacean species, namely, *Moina macrocopa* as a bio-monitor for the presence of estrogenic compounds in aquatic environment, thus investigated the effects of conjugated estrogens present in an estrogen replacement drug on *Moina macrocopa*. It was found that conjugated estrogens at 10^{-5} M and 10^{-3} M induced the formation of resting eggs (ephippia) in *Moina macrocopa*. The average rate of animals bearing ephippia/group of 10 animals examined were 1.6 ± 0.5 and 4.7 ± 0.6 (mean ± sd), respectively. All animals in the control group had no ephippia. Scanning electron microscope study on released ephippia revealed a unique pattern on the surface of the structure enclosing the ephippia.

84 A lab-scale investigation into the potential interactions between polybrominated diphenyl ethers (PBDEs) and other secondary contaminants

A.P. Daso, University of Pretoria; E. Rohwer, University of Pretoria / Chemistry; J.O. Okonkwo, Tshwane University of Technology / Environmental, Water and Earth Sciences; P. Forbes, University of Pretoria / Chemistry

Several studies have focussed on the evaluation of the concentrations of polybrominated diphenyl ethers in various biological and environmental matrices. Considering the complex nature of matrices in which these contaminants are commonly found, their potential interactions with other secondary contaminants, especially those present at elevated concentrations need to be investigated. So far, the influence of total organic carbon (TOC) on PBDE levels in different environmental matrices has been reported in a limited number of publications. Other complex chemical reactions that could ultimately influence the availability of these emerging contaminants in a given medium are yet to be fully explored. Significantly, the potential of PBDEs to act as ligands for certain trace metals are yet to be investigated. In our on-going study, these possibilities are being investigated and the likelihood of certain anions to compete as ligands for these trace metals in the presence of PBDEs is also being investigated. The findings of these studies will be presented.

85 Assessing the degradation of 11 pharmaceuticals in wastewater treatment plant effluents using UV disinfection and advanced oxidation process

R.R. Singh, University at Buffalo; D.S. Aga, University at Buffalo / Department of Chemistry; K. Linden, University of Colorado at Boulder

The presence of pharmaceutical contaminants in the environment can potentially cause adverse ecological effects. Therefore, a process to efficiently remove recalcitrant pharmaceuticals in wastewater effluents is imperative to prevent unwanted effects of these chemicals. A number of wastewater treatment plants (WWTPs) employ ultraviolet (UV) disinfection to remove pathogens prior to discharging treated effluents into the surface waters. While this process is not directly aimed at removing pharmaceuticals, the addition of an advanced oxidation process could lead to the degradation of many pharmaceuticals. Therefore, this study is aimed at assessing and comparing UV disinfection and advanced oxidation in the degradation of 11 target pharmaceuticals using reversed phase liquid chromatography tandem mass spectrometry (LC-MS/MS). Detection was performed using a triple quadrupole mass spectrometer with electrospray ionization (ESI) source, under positive multiple reaction monitoring (MRM) mode. For each compound, the transition resulting to the highest signal of a fragment ion was used for quantitation, while the second most abundant ion was used as qualifier. Deuterated standards were used as surrogates and for quantitation using isotope dilution. Eleven pharmaceuticals were successfully monitored including Acetaminophen, Iopromide, Caffeine, Trimethoprim, Sulfamethoxazole, Erythromycin, Carbamazepine, Naproxen, Diclofenac and Ibuprofen with concentrations found in the part per trillion levels. The extent of removal varied between WWTPs, UV flux, and nutrient levels. The method is therefore able to assess the extent to which UV disinfection and advanced oxidation can degrade PPCPs in WWTP effluents.

Use of Freshwater Mollusk Toxicity Data for Improved Conservation of Water and Sediment Quality

86 Using Traditional and Emerging Endpoints to Assess Endocrine Disruption in Freshwater Mussels Exposed to the Synthetic Estrogen 17α-Ethinylestradiol

J. Leonard, NC State University / Department of Environmental and Molecular Toxicology; W.G. Cope, North Carolina State University / Department of Environmental and Molecular Toxicology; C. Barnhart, Missouri State University / Biology; R.B. Bringolf, University of Georgia / Interdisciplinary Toxicology Program

Surface water concentrations of the synthetic estrogen 17α-ethinylestradiol (EE2) as low as 5 ng/L have been shown to cause adverse reproductive effects in fish under acute and chronic conditions. However, much greater concentrations or prolonged exposure periods are required to elicit detectable effects in freshwater mussels (order Unionida), possibly due to different windows of susceptibility during reproductive periods. In addition, little is known of the underlying mechanisms leading to reproductive toxicity in Unionids. To address this knowledge gap, we designed a series of acute (4 d), sub-chronic (14 d and 28 d), and chronic (180 d) exposures of EE2 on *Lampsilis fasciola* and *Elliptio complanata* at environmentally relevant (5, 50 ng/L) and high concentrations (1, 500 μg/L) to examine EE2 effects on traditional endpoints such as larval (glochidia) mortality and adult behavior, reproduction, and energy reserves, as well as high throughput analysis of gill metabolites using emerging metabolomics technology. Larval mortality increased relative to the control for both species, while conglutinate (packets of glochidia) condition decreased, especially in *E. complanata* exposed for a longer period (28 d vs. 14 d). A greater number of immature eggs were released in EE2 treatments, suggesting rapid shunting of eggs from gonads to marsupial gills and EE2-induced spawning behavior in females. EE2 altered female mantle lure display behavior of *L. fasciola* in a concentration dependent manner, indicating possible complications in attracting suitable fish hosts in wild populations. While macronutrients like carbohydrates, lipids, and proteins appeared to vary only seasonally, metabolomics analysis revealed significant sex-specific differences even at environmentally-relevant concentrations, especially in metabolites related to neuroendocrine functions. Results of our research suggest that using traditional toxicity endpoints that detect significant effects mainly at higher concentrations may underestimate the endocrine disrupting potential of compounds like EE2.

87 Closer to Reality: Incorporating Components of the Benthos in Lethal and Sublethal Experiments of Thermal Sensitivity in Freshwater Mussels

J.M. Archambault, North Carolina State University / Department of Biology; W. Cope, North Carolina State University / Department of Toxicology; T.J. Kwak, United States Geological Survey / North Carolina Cooperative Fish and Wildlife Research Unit, North Carolina State University / Biology

Recent research has elucidated the acute lethal effects of elevated water temperature to glochidia (larvae), juvenile, and adult life stages of freshwater mussel species (Order Unionida), but evaluation of their thermal sensitivity

has never been conducted in sediment, and few studies have focused on sublethal effects of thermal stress; to our knowledge no one has done so with juveniles. Determining whether the presence of sediment and other ecological factors affect the thermal sensitivity of these benthic organisms is a necessary step in building upon the water-only standard method for testing, on which the regulatory framework for potential thermal criteria are currently based. Therefore, we evaluated the effects of elevated temperature on survival, byssus (attachment filaments) production (in juveniles only), and burrowing behavior of five species (juveniles of *Amblema plicata, Lampsilis abrupta, Lampsilis cariosa,* and *Lampsilis siliquoidea*, and adults of *Lampsilis fasciola*) in acute (96-h) laboratory experiments in sediment, with two acclimation temperatures (22 and 27 °C) and two experimental water levels (watered and dewatered treatments) as proxies for flow regime. In additional experiments, we added a vertical temperature gradient to the sediment and evaluated the thermal sensitivities of two species (*Lampsilis abrupta* and *Lampsilis radiata*). LT50s ranged from 29.9 to 37.2 °C, and results from sediment tests were generally similar when compared to tests conducted using the water-only standard method of testing, indicating that the presence of sediment alone does not alter thermal sensitivity. Increasing temperature significantly reduced burrowing in all species tested, and the dewatered treatment (a proxy for drought conditions) reduced burrowing in all but *Amblema plicata*. Production of byssal threads was affected most drastically by flow regime, with the probability of byssus presence in *L. abrupta, L. cariosa,* and *L. siliquoidea* reduced by 93 – 99% in the dewatered treatment, compared to the watered treatment (a proxy for low flow conditions). Increasing temperature alone reduced byssus by 17 – 35% in all species evaluated. Our findings suggest that rising stream temperature and altered hydrologic flow from climate change and other anthropogenic factors may directly impact freshwater mussel diversity by causing mortality, and may impact populations via sublethal effects.

88 Interactive effects of copper and dissolved organic matter on juvenile freshwater mussel physiology

M. Mussoi Giacomin, Instituto de Ciências Biológicas; P.L. Gillis, Environment Canada / Aquatic Contaminants Research Division; A. Bianchini, Fundação Universidade / Instituto de Ciências Biológicas, Universidade Federal do Rio Grande / Instituto de Ciências Biológicas; C.M. Wood, McMaster University / Department of Biology

Freshwater mussels are especially sensitive to a variety of contaminants including metals, but the toxic mechanisms of action are not fully understood. Also, our current understanding of the protective effects of dissolved organic matter (DOM) is undergoing revision, since recent studies have found that DOM, in addition to its well known ability to reduce metal bioavailability through complexation, may also directly affect organism physiology. The present study investigated the physiological effects of acute waterborne Cu exposure and the protection offered by DOM to juvenile freshwater mussels (*Lampsilis siliquoidea*). Mussels (6 – 12 months old) were acutely (24 and 96 h) exposed to a range of environmentally relevant Cu concentrations alone and in combination with several different concentrations of natural DOM (collected from a terrigenous source). In order to evaluate the physiological effects of Cu and DOM exposure, the following parameters were analyzed: unidirectional Na^+ influx, whole-body ion concentrations (Na^+, K^+, Ca^{2+} and Mg^{2+}), enzyme (Na^+K^+-ATPase, H^+-ATPase and carbonic anhydrase) activities, copper bioaccumulation, reactive oxygen species (ROS) and antioxidant capacity against peroxyl radicals. Indices of oxidative stress exhibited no clear trends. However exposure to DOM alone caused a marked concentration-dependent increase in unidirectional Na^+ influx but led to no changes in Na^+K^+-ATPase activity, and to a decrease in v-type H^+-ATPase activity. When mussels were exposed to Cu alone at the highest concentration (12 µg Cu/L) tested, Na^+K^+-ATPase activity and unidirectional Na^+ influx rate were inhibited, although no alterations in whole-body Na^+ were observed. Animals exposed to Cu in combination with DOM had their influx rates re-established back to control levels. In general, our results indicate that Cu is a strong ionoregulatory toxicant to freshwater mussels, as reported for several other freshwater species. Also, DOM at the concentrations tested (3 and 6 mg C/L) appears to be protective against ionoregulatory disruption and metal bioaccumulation. These findings suggest that natural DOM can promote changes in membrane permeability, as well as in transport functions, and that the protective effects against metal toxicity are partly related to direct positive alterations in animal physiology. (CRC/IRC Program, IDRC, CNPq).

89 The Effects of Metals on the Freshwater Pulmonate Snail, Lymnaea stagnalis: State of the Science and Future Perspectives

K.V. Brix, University of British Columbia / Biology, University of Miami, RSMAS; M. Grosell, University of Miami / Division of Marine Biology and Fisheries

Historically, freshwater snails have not generally been considered particularly sensitive to contaminants. However, chronic toxicity studies conducted over the past decade indicate that freshwater snails are often among the most sensitive taxa tested to date for many metals. Chronic studies on Co, Cu, Pb, and Ni with the pulmonate snail, *Lymnaea stagnalis*,all result in significant growth inhibition of juvenile snails at concentrations near, and in some cases below, USEPA water quality criteria. Studies on the mechanisms underlying *L. stagnalis* hypersensitivity to Co, Cu, Pb, and Ni all suggest Ca^{2+} homeostasis is disrupted, but is probably not the result of direct competition by metals at Ca^{2+} uptake pathways. Indeed, *L. stagnalis* is relatively insensitive to Zn, a known antagonist of Ca^{2+} uptake pathways. There is also generally an inhibition of feeding rates when snails are exposed to metals, though this effect occurs at concentrations higher than the EC20 for Ca^{2+} uptake and growth for all metals studied to date. Overall, available data suggests these metals may be exerting toxicity via a common, but currently unknown mechanism of action. Specific areas needing further research including development of full life cycle test methods, Biotic Ligand Models, and additional mechanistic studies will be discussed.

90 Glochidia toxicity testing: the viability endpoint and standardizing test duration

R.B. Bringolf, University of Georgia / Interdisciplinary Toxicology Program; A.K. Fritts, University of Georgia / Forestry & Natural Resources; M.C. Barnhart, Missouri State University / Department of Biology; W. Cope, North Carolina State University / Department of Toxicology

The parasitic glochidial (larval) stage of freshwater mussels is becoming increasingly popular for toxicity testing because it is the most sensitive life stage to many contaminants, juveniles can be difficult and expensive to produce, and adults collected from the wild have inherent limitations. Glochidia toxicity data may play a pivotal role in development of water quality criteria for some contaminants but little is known about the relevance of the primary endpoint, viability, and questions remain about how to standardize test duration across glochidia of different species. Therefore, our goals were to determine if viability is an ecologically relevant endpoint and to inform development of a standardized test duration. To better understand the viability endpoint, we tested the relationship between viability (shell closure response to sodium chloride) and infectivity (the ability to attach to a host fish and successfully metamorphose to the juvenile stage) for seven species. Viability and infectivity were strongly correlated when viability was >90% but when viability dropped below 90% (typically after 24 h), the endpoint was not always a strong predictor of infectivity. We also compared the viability and infectivity of glochidia from three species after a 24-h exposure to a toxicant (sodium chloride or copper). Viability decreased in a dose-dependent manner following the toxicant exposure so we used a standardized number of viable glochidia (4000/L) to test infectivity. Infectivity (i.e., metamorphosis success) did not differ among viable glochidia across treatments, indicating that no other modes of toxicity were evident for these toxicants (to reduce metamorphosis success). With these toxicants a decrease in recruitment was attributable to a smaller number of viable glochidia available to attach to host fish, rather than a decrease in metamorphosis success. Our results indicate that viability is an ecologically relevant endpoint for glochidia toxicity tests and support the criteria in current ASTM guidelines for glochidia toxicity tests, which specify >90% viability in control treatments. Glochidia of various species are viable for different durations once released from the female, so a uniform toxicity test duration is not warranted but standardization of test duration should be based on maintenance of >90% viability in controls.

91 Development and validation of OECD test guidelines on mollusk reproductive toxicity tests

V. Ducrot, INRA / Ecotoxicology and Quality of Aquatic Ecosystems; D. Azam, INRA; R. Brown, AstraZeneca; S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutive Biology; H. Holbech, University of Southern Denmark / Department of Biology; L.L. Lagadic, INRA / Ecotox & Quality of Aquatic Environ; P. Matthiessen, Independent Consultant; J. Oehlmann, Johann Wolfgang Goethe-Universitat Frankfurt am / Aquatic Ecotoxicology; L. Weltje, BASF SE / Agricultural Centre; T. Hutchinson, School of Biological Sciences, Plymouth University

Validated test guidelines in line with the OECD Conceptual Framework for the Testing and Assessment of Endocrine Disrupting Chemicals (EDTA) have recently been developed for rodents, fish and amphibians. Apical test methods with aquatic invertebrates are also being considered in this assessment framework. The comparison of endpoints relevant for reproduction in invertebrates often shows a much higher sensitivity in mollusks vs., e.g., daphnids. The OECD test guideline program has thus been extended to cover reproductive toxicity in mollusks. Therefore, a mollusk reproduction test guideline is being developed describing partial life-cycle test protocols with apical endpoints in these species for the assessment of any type of chemical, including endocrine disruptors (as level 4 and 5 assays of the EDTA Conceptual Framework). The guideline project is led by a consortium of experts (Germany/United -Kingdom/France/Denmark) from academia, industry and government. Existing mollusk toxicity test protocols have been reviewed in an OECD Detailed Review Paper that identifies two candidate species for developing freshwater tests: *Potamopyrgus antipodarum* and *Lymnaea stagnalis*. However, this review did not clarify which toxicity test design/conditions are the most appropriate for chemicals assessment. To date, expert knowledge has been gathered and formed the basis of draft standard operating procedures (SOPs) for the culture and test implementation of the partial life-cycle tests in both species. Pre-validation of these SOPs consisted in two ring-tests involving 10 partner labs with different level of expertise in mollusk tests. Effects of cadmium on the individual reproductive outputs were studied over 28-d in *P. antipodarum* and over 56-d in *L. stagnalis*. Pre-validation tests were successful in both species and underlined the need to have well-defined laboratory cultures of animals for optimal results. Toxicity tests provided consistent results among laboratories for each species (e.g., homogenous and non-significantly different NOECs, LOECs and EC50 values for most partner labs). The draft SOPs are currently under optimization (e.g., statistical tests showed that the duration of the test with *L. stagnalis* can be shortened to from 56-d to 35-d) and further tested for various types of reproductive toxicants including mammalian endocrine disruptors. A broader scale ring-test will be conducted in 2014 for both species based upon the consolidated SOPs.

92 Comprehensive mollusk acute toxicity database improves the use of interspecies extrapolation models to predict toxicity of untested mussel species

S. Raimondo, USEPA / Gulf Ecology Division, National Health and Environmental Effects Laboratory; L. Lee, Student Contractor to USEPA; C.R. Jackson, USEPA; M.G. Barron, USEPA / Gulf Ecology Division; T. Augspurger, US Fish and Wildlife Service / Ecological Services; C. Bauer, USEPA; B.K. Shephard, USEPA / Office of Env. Assessment (OEA-095); N. Wang, DuPont / Haskell Global Centers for Health and Environmental Sciences; C.G. Ingersoll, US Geological Survey / Columbia Environmental Research Center

Interspecies correlation estimation (ICE) models extrapolate acute toxicity data from surrogate test species to untested taxa. A suite of ICE models is available on the US Environmental Protection Agency's web-based application, Web-ICE (http://epa.gov/ceampubl/fchain/webice/). A recent Federal Insecticide, Fungicide, and Rodenticide Act Science Advisory Panel review of ICE models for application in pesticide registration and development of Ambient Water Quality Criteria (AWQC) determined that currently available Web-ICE models are limited in their ability to predict toxicity to freshwater mussels, a taxa of concern for both endangered species risk assessment and for minimum data requirements for AWQC. We developed an expanded database of mollusk acute toxicity test endpoints to increase the number of ICE models that can be used to predict toxicity to mussel species. Over 1400 mollusk toxicity values, greater than 50% of which were for unionid mussels (glochidia and juvenile), were added to the existing Web-ICE database. The new mollusk database consists of 103 species for 78 chemicals. Following a rigorous data quality screen, over 75 additional ICE models were developed to predict acute toxicity to unionid mussels, compared to 2 ICE models from the original suite of Web-ICE models. An evaluation of uncertainty using model cross validation identified the surrogates that produced the most accurate predictions for various mussel species of concern. These models predicted mussel toxicity within 5-fold of the measured value with high confidence. The mollusk toxicity addendum to the Web-ICE database considerably increases the number of models that predict toxicity to freshwater mussels, adding to the utility of Web-ICE in endangered species risk assessment and development or evaluation of AWQC. Results of ongoing studies testing different families or tribes of freshwater mussels or snails with 10 to 20 chemicals with a range of toxic modes of action will provide additional data for developing and applying Web-ICE models for freshwater mollusks.

93 Use of Mollusk Data in Final Aquatic Life Ambient Water Quality Criteria for Ammonia – Freshwater (2013)

L.F. Huff, USEPA / Office of Water; C.G. Delos, USEPA / Mail Code 4304; J.R. Beaman, Environmental Protection Agency / Toxicologist; T.K. Linton, Great Lakes Environmental Center; K.K. Taulbee, Great Lakes Environmental Center / Research Scientist; D.R. Mount, USEPA / ORD; R.J. Hockett, USEPA / Mid-Continent Ecology Division

The US Environmental Protection Agency (EPA) has published final revised national recommended ambient water quality criteria for the protection of aquatic life from the toxic effects of ammonia in fresh water. Aquatic life criteria are intended to protect aquatic ecosystems and are developed using data on the distribution of the most sensitive genera. The 1999 recommended criterion maximum concentration was based on toxicity to rainbow trout, and the criterion continuous concentration was based on toxicity to early life stages of bluegill sunfish. Subsequent to the 1999 revision, EPA became aware of new toxicity studies indicating high sensitivity of freshwater mussels to ammonia, and began to update the 1999 criteria to reflect this new information. In 2009, following external peer review, EPA published draft updated recommended ammonia criteria, with differing values (bifurcated) for waters with and without freshwater mussels. The 2009 draft "mussels present" criteria were driven by the acute and chronic sensitivity of freshwater unionid mussels. As noted in the 2009 draft ammonia criteria document, available data indicated that another freshwater mollusk taxon, non-pulmonate (gill-bearing) snails, is also sensitive to the effects of ammonia. Following publication of the draft 2009 ammonia criteria, additional toxicity testing has validated information on the chronic effects of ammonia on sensitive non-pulmonate snail species. In 2013, EPA finalized the updated ammonia criteria that are applicable nationally, taking into account the latest toxicity information for freshwater species, including sensitive unionid mussels and non-pulmonate snails. Because freshwater mussels and non-pulmonate snails are broadly distributed across the US, EPA decided to recommend a single set of criterion values (non-bifurcated) based on data meant to represent the freshwater community as a whole (EPA's standard approach for national recommended aquatic life criteria). The 2013 criteria magnitudes are more stringent than the previously recommended 1999 criteria.

Impacts of Resource Mining on Inland Environments: Oil and Gas Extraction and Coal Mining

94 Life cycle greenhouse gas emissions and freshwater consumption of North American shale gases

I.J. Laurenzi,

Natural gas is produced from a variety of shale plays in the US, including the Barnett in Texas and the Marcellus in West Virginia and Pennsylvania. As these resources have been developed, there has been an increasing interest in their environmental impacts, including greenhouse gas (GHG) emissions and water consumption. Much attention has focused upon hydraulic fracturing, however, environmental impacts associated with shale gas may also be associated with activities including the treatment and processing of "raw" shale gas, pipeline transportation, and importantly, end use, e.g., power generation. Regional variability complicates the assessment of environmental impacts associated with shale gas: Geological properties of shale reservoirs differ among and within plays, resulting in differences among the compositions of shale gases, the depths and maturities of reservoirs, etc. These differences may in turn introduce technological variability, particularly in the designs of wells, practices for well completion, operation of gas gathering systems and treatment and processing plants. To address this variability

explicitly, we have quantified the carbon footprints and life cycle water consumption associated with several North American shale gases. Our results show that both GHG emissions and water consumption associated with North American shale gases are about 50% lower than those of coal. Our results also show that the ultimate recovery of gas from a well is the primary determinant of the variability among wells. We conclude that substantial GHG reductions and freshwater savings may result from the replacement of coal-fired power generation with gas-fired power generation, and that the environmental characterization of shale gas should be conducted on a regional basis.

95 Continuous vs. point-in-time sampling of conductivity as a predictor of aquatic invertebrate community structure in salinized headwater streams

A.J. Timpano, Virginia Water Resources Research Center; D.J. Soucek, Institute of Natural Resource Sustainability / Illinois Natural History Survey; S.H. Schoenholtz, Virginia Tech / Virginia Water Resources Research Center; C. Zipper, Virginia Tech / Dept. of Crop and Soil Environmental Sciences

Salinization is of growing concern as an aquatic-life stressor in Appalachian headwater streams influenced by coal mining. Consequently, it is desirable to improve understanding of associations between salinity and biological effects. Such analyses are often conducted using temporally discrete water samples collected during seasonal benthic macroinvertebrate surveys. Because salinity is not temporally static in such streams, discrete seasonal salinity measures are often inadequate to describe life-cycle exposures. Our research compares a continuous water-quality sampling approach to traditional discrete-sampling methods as means for evaluating salinization effects on benthic macroinvertebrate community structure. We used automated dataloggers to record conductivity continuously for ~21 months in 27 headwater streams spanning a gradient of salinity (specific conductance ~20 – 2,000 mS/cm) where non-salinity stressors were not evident. We found that models using salinity measures derived from continuous conductivity data described the salinity-biota association with greater certainty than models using discrete salinity measures as predictors of genus-level macroinvertebrate community metrics. Our results suggest that predictive models using continuous conductivity data from time periods prior to macroinvertebrate sampling provide more accurate predictions of biotic effects compared to models constructed using discrete conductivity data collected at the time of biological sampling.

96 Understanding the effect of calcium to bicarbonate ratio in saline mine discharge on a species of Ephemeroptera

V. Nanjappa, S. Vink, The University of Queensland / Centre for Water in the Minerals Industry, SMI; J. Dunlop, Queensland Government

Changes in salinity and ionic composition in freshwater ecosystems have significant influence on the health of aquatic organisms. Ephemeroptera is known to be sensitive to salinity. Acute toxicity tests with two ionic compositions based on the mine water composition on mayflies have revealed difference in toxicity. The LC50 for Leptophlebiidae was 6.9 mS/cm and 7.6 mS/cm for two different artificial mine water ionic compositions. This difference was postulated to be due to the variation in calcium to bicarbonate ratio. This project evaluates the effect of increase in calcium to bicarbonate ratio on a species of Ephemeroptera using 96h acute toxicity tests in a test diluent representative of mine water. Initial tests reveal there is significant influence of calcium concentration on Ephemeroptera. The results of these tests will be beneficial for the management of the site specific discharges and protection of the aquatic ecosystem.

97 Trophic transfer and bioaccumulation of selenium through a food chain in the mountaintop removal coal mining-impacted Mud River, WV

M.C. Arnold, T. Lindberg, Duke University / Nicholas School of the Environment; Y. Liu, Duke University / Pratt School of Engineering; H. Hsu-Kim, Duke University / Department of Civil & Environmental Engineering; L. Friedrich, Stantec Consultants Ltd.; V.P. Palace, Department of Fisheries & Oceans Canada, Stantec Consultants Ltd.; R.T. Di Giulio, Duke University / Nicholas School of the Environment

Mountaintop removal/valley fill coal mining (MTR/VF) is the process of blasting non-coal rock layers off the tops of mountains to access underlying coal seam with resulting overburden being dumped into headwater stream valleys. MTR/VF has been shown to significantly alter stream chemistry and increase the concentrations of contaminants of concern in streams receiving effluent from the fills. One such contaminant, selenium, is mobilized during the mining process and afterward through errosional processes active in the fill material. Native creek chubs (*Semotilus atromaculatus*) and green sunfish (*Lempomis cyanellus*) were collected using electroshocking from the MTR/VF-impacted main stem of the Mud River (MR7) and the non-impacted Left Fork Mud River (LFMR) in Boone County, WV. Natural biofilms were grown on Plexiglas plates and collected after six weeks from each site. Skinless fillet, ovary, and liver were analyzed for selenium concentrations and speciation. Skinless fillets from MR7 fish were significantly higher (α=.05) for both creek chubs and green sunfish at 6.45±1.44 and 8.78±1.84 mg Se / kg dw respectively compared to LFMR fish (2.03±0.29 and 3.49±0.90 mg Se/kg dw). Creek chub livers contained significantly higher (α=.05) average selenium of 32.87±15.01 mg Se/kg dw compared to 11.65±5.41 mg Se/kg dw found in LFMR creek chub livers. Analysis of fish ovary tissues from both MR7 and LFMR is ongoing. X-ray absorption near edge spectroscopy (XANES) revealed that all fish tissues analyzed contained predominantly organic species of selenium. Laser ablation-ICPMS studies are currently being conducted on the otoliths of sampled fish to determine lifetime exposure histories. In an effort to understand the trophic transfer of inorganic contaminants through a food chain, natural biofilms were brought back to the laboratory where they were fed to adult female fathead minnows (*Pimephales promelas*). Fathead minnows fed on MR7 biofilms showed increased selenium and manganese compared to LFMR biofilm-fed fish, indicating inorganic contaminants from MTR/VF-impacted streams are able to move through a simulated food chain into higher trophic organisms.

98 Toxicity of Produced Water in Freshwater Systems: Separating the Effects of Common Ions and Dissolved Gases

D.A. Pillard, AECOM / Environmental Toxicology; R.B. Naddy, AECOM Environment

In addition to elevated concentrations of dissolved gases, such as methane and ethane, produced water (PW) may contain high concentrations of certain common freshwater ions such as sodium, bicarbonate, calcium, chloride and sulfate. A series of laboratory studies were conducted to assess the effects of both common ions and dissolved gases on the freshwater toxicity of produced water from Fidelity Exploration and Production's coal-bed natural gas (CBNG) wells in southern Montana near the Tongue River. The tests focused on *Ceriodaphnia dubia* (Cladocera), since tests with this species sometimes failed the permit-required limits (LC_{50}< 100% PW), while tests with the fathead minnow (*Pimephales promelas*) always passed (LC_{50}>100% PW). Both methane and ethane were found in measurable concentrations in untreated PW, although ethane concentrations were always much lower than methane. Aeration tests and methane injection studies demonstrated that concentrations of methane equal to, or greater than, actual PW concentrations were not high enough to cause toxicity to *C. dubia*. However, toxicity of mock (synthetic) effluents prepared to match the concentrations of seven major effluent ions was usually the same or greater than effluent toxicity, suggesting that total dissolved solids (TDS) alone, and in particular bicarbonate, were responsible for the observed toxicity. Addition of methane to mock effluents did not consistently increase *C. dubia* mortality, nor did complete removal of methane eliminate toxicity. Variability in *C. dubia* survival among tests, even when TDS concentrations were similar, is believed to be due to small but significant differences in organism condition at test termination, thus blurring the lines between dead and live organisms. Tests on water collected from the Tongue River immediately downstream of an outfall were not acutely or chronically toxic, reflecting substantial, nearly instantaneous, dilution of the PW.

99 Evaluating the effect of background water chemistry on the aquatic toxicity of major ion salts

D.R. Mount, USEPA / ORD; R.J. Erickson, USEPA / Mid-Continent Ecology Division; T.L. Highland, USEPA / ORD, NHEERL, Mid-Continent Ecology Division; R.J. Hockett, USEPA / Mid-Continent Ecology Division; D.J. Hoff, Ecotoxicology Analysis Research / Mid-Continent Ecology Division; T.J. Norberg-King, USEPA / ORD, NHEERL, Mid-Continent Ecology Division; K. Peterson, c/o USEPA

Increased concentrations of major ions (Na, K, Ca, Mg, Cl, SO_4, HCO_3) in surface waters are a consequence of many land uses, including extraction of oil, gas, and several other minerals. Several existing studies have indicated that the toxicity of excess concentrations of these ions can depend on

characteristics of the underlying water chemistry. As an example, literature data have indicated that the toxicity of sodium chloride increases as water hardness decreases. As such, toxicity of waters enriched with major ions can depend not only on what ions are elevated, but also on which ions are present in low concentrations. The current study was focused on surveying a full range of major ion salts, and identifying the degree to which the toxicity of these salts varied with differences in the underlying water chemistry. This was accomplished by conducting many series of exposures in which dilution water chemistry was manipulated in different ways. For example, in some series, concentrations of all the ions in the dilution water might be raised or lowered simultaneously, such that all ions remain in the same ratios as concentrations change. In others, only certain ions were manipulated and others held constant; an example of the latter would be comparing waters with the same hardness (i.e., Ca + Mg is constant), but with varying ratios of Ca to Mg. By interpreting results of multiple experiments together, one can infer which water quality parameters most influence toxicity of major ions. The most extensive testing was completed with *Ceriodaphnia dubia*, with more limited testing using *Daphnia magna* and fathead minnows. The data show that effects of water hardness on the toxicity of NaCl and Na_2SO_4 are driven primarily by Ca specifically, not by overall water hardness. An even greater influence of Ca was found for $MgSO_4$ and $MgCl_2$. Toxicity of potassium salts was also sensitive to background water chemistry, but these effects were associated with variation in Na concentration rather than Ca. Chloride:sulfate ratios and alkalinity/pH seemed to have comparatively little influence on toxicity of major ion salts when tested over ranges common to natural waters. *This abstract does not necessarily reflect EPA policy.*

100 Assessment of acute and full life-cycle toxicity of major ions to Centroptilum triangulifer using a laboratory cultured diet

D.J. Soucek, Institute of Natural Resource Sustainability / Illinois Natuiral History Survey; A. Dickinson, Illinois Natural History Survey

A number of field studies have shown that mayflies (Ephemeroptera) tend to be more sensitive than other benthic macroinvertebrate taxa to elevated levels of total dissolved solids in streams impacted by mining. Until relatively recently, difficulties with culturing have precluded the use of mayflies as laboratory toxicity testing organisms; however, other research groups have 1) found parthenogenic species that readily reproduce under laboratory conditions, and 2) developed laboratory cultured diets for mayflies that will help to move toward standardization of methods. The goal of the present study was to build on these efforts and develop a method for conducting full life-cycle toxicity tests with the mayfly *Centroptilum triangulifer* using a modification of the previously mentioned laboratory cultured diet. We used the USEPA *Ceriodaphnia dubia* chronic test as a model, with one < 24-h-old organism placed into each of ten replicate beakers per treatment. Organisms were fed scrapings of live biofilms consisting of two known diatom species cultured in the laboratory. Endpoints included survival to pre-emergent nymph (PEN) stage, number of days to PEN stage, percent emergence, pre-egg-laying live weight, and number of eggs produced. Initial tests were conducted with sodium salts of chloride, sulfate, and nitrate. In acute (96-h) exposures, LC50s for *C. triangulifer* were approximately one half to two thirds of those generated in our laboratory with *Ceriodaphnia dubia*. The full life-cycle test with sodium chloride had excellent control survival to PEN stage (100%), and robust weight (mean > 3 mg per adult) and reproduction (mean > 1400 eggs per adult) results. In this chloride test, the most sensitive endpoints were survival to PEN stage, and mean number of days to PEN stage, with a maximum allowable toxicant concentration (MATC) of 267 mg Cl^-/L. Measured in terms of conductivity, the MATC was 1,178 µS/cm, which was surprisingly similar to the XC95 for this genus (1,092 µS/cm) documented in the USEPA conductivity benchmark despite the difference in ionic composition.

101 The Acute or Chronic Toxicity of Sodium Bicarbonate to Aquatic Organisms: Laboratory and Field Exposures

D.D. Harper, US Geological Survey / CERC; A.M. Farag, US Geological Survey

Sodium bicarbonate is a major constituent of water produced during coal bed natural gas extraction in the Powder and Tongue River watersheds of Wyoming and Montana. Concentrations of sodium bicarbonate in produced waters can exceed 3,000 mg $NaHCO_3$/L, and aquatic toxicity data are limited for this constituent. We conducted laboratory experiments to determine the acute toxicity of sodium bicarbonate to 13 aquatic species exposed in simulated Powder and Tongue River waters. Cladoceran (*Ceriodaphnia dubia*), unionid mussel (*Lampsilis siliquoidea*), pallid sturgeon (*Scaphirhynchus albus*), African clawed frogs (*Xenopus laevis*), and fathead minnow (*Pimephales promelas*) were the top five most sensitive species tested, with 48-96 hour LC50s of 989-1,749 mg $NaHCO_3$/L. Additional experiments were performed in the laboratory to determine chronic effects of sodium bicarbonate to early life-stage fathead minnow, the cladoceran, white sucker (*Catastomus commersoni*), and the unionid mussel. Chronic effect concentrations ranged from 450 to 800 mg $NaHCO_3$/L (IC20 based on survival, growth and reproduction effects). Sublethal investigations during chronic studies revealed a decrease in the activity of Na/K ATPase, an enzyme involved in ionoregulation. Field experiments were conducted with larval fathead minnows and pallid sturgeon to assess in situ survival in Powder River Basin streams composed primarily of produced waters. Survival of both fathead minnows and pallid sturgeon were significantly reduced at experimental sites compared to the control site. As was observed in the laboratory, 2-day post hatch fathead minnows were more sensitive to sodium bicarbonate than 4-day post hatch fish.

Emerging Halogenated Phenolic Chemicals in Biotic and Abiotic Environments

102 Identification of (halogenated) phenolic chemicals in polar bear plasma by Effect-Directed Analysis

E. Simon, BioDetection Systems; M. van Velzen, S. Brandsma, Institute for Environmental Studies, VU University; E. Lie, K. Loken, Norwegian School of Veterinary Science; J. Bytingsvik, B. Jenssen, Norwegian University of Science and Technology; J. Aars, Norwegian Polar Institute; J. de Boer, T. Hamers, Institute for Environmental Studies, VU University; M. Lamoree, Chemistry & Biology, Institute for Environmental Studies, VU University

Recent *in vivo* and *in vitro* studies demonstrated that in addition to other endocrine systems the thyroid hormone (TH) system is also vulnerable for environmental contaminants. TH-disrupting compounds (TDCs) can bioaccumulate and biomagnify in adipose tissue and blood of top predators and are capable of competitive binding to transthyretin (TTR), which is one of the transport proteins of the thyroid hormone, thyroxine (T4). Effect-Directed Analysis (EDA) was performed on polar bear plasma samples to identify the compounds responsible for the elevated thyroid hormone (TH)-disrupting potency measured in the radioligand T4*-TTR binding assay. A set of initially target analyzed transthyretin (TTR)-binding compounds explained ~40% of the measured activities. In order to detect the compounds causing the remaining activity, a generic identification strategy was developed. The extracts were analyzed by high resolution time-of-flight mass spectrometry coupled to liquid chromatography (LC-ToF-MS). Library-based identification was applied to the full-scan data screening for accurate mass- and isotope pattern-match between the compound lists and the data files. The libraries were compiled based on different selection criteria, such as TTR-binding-, blood accumulating potency and environmental occurrence of the compounds. Out of the tentatively identified 23 suspects nonylphenol was confirmed chemically by evaluating its chromatographic and mass spectrometric behavior after simultaneous injection of its analytical standard and the sample extracts on the LC-TOF-MS. Then, Isotope Cluster Analysis (ICA) was applied to the original LC-ToF-MS data enabling specific screening of halogenated isotope patterns. This strategy led us to the successful identification and confirmation of three (di-)hydroxylated octachlorinated biphenyls. As no pure standards were available, these compounds were successfully synthesized from their corresponding methoxylated form prior to analytical and toxicological confirmation. All analytically confirmed, identified compounds (i.e., nonylphenol and (di)OH-octaCBs) showed TTR-binding potency in the bioassay and could explained another ~35% of the total measured TTR-binding activities. However, the contribution to the total measured activity of nonylphenol compared to the (di)OH-octaCBs is negligible due to its weaker TTR-binding affinity its plasma concentration level is remarkable (2.5-6.2 µg/L).

103 Effects of PBDEs and Hydroxylated PBDE Metabolites on Thyroid Hormone Metabolism in Cultured Astrocytes

S. Roberts, H.M. Stapleton, Duke University / Nicholas School of the Environment

Exposure to polybrominated diphenyl ether (PBDE) flame retardants has been linked to neurodevelopmental deficits in children, but a clear mechanism has not been established. PBDEs have been shown to alter thyroid hormone (TH) metabolism in animal exposure studies, and subtle changes in TH levels in the brain could lead to neurodevelopmental impairments. The objective of this study is to assess the effects of PBDEs and their hydroxylated metabolites (OH-PBDEs) on the activation (Type 2 deiodinase (DIO2) activity and mRNA expression), clearance (sulfotransferase (SULT) activity and mRNA expression), and active transport (membrane transporter mRNA expression) of THs in cultured human astrocytes, which act as a metabolic barrier to protect neurons and regulate TH levels in the brain. H4 astrocytoma cells were cultured in the presence of 3 PBDE congeners (BDE 47, 99, and 153), 2 OH-PBDEs (6-OHBDE 47 and 5'-OHBDE 99), Aroclor 1254 (positive control), and DMSO (vehicle control). After 6 hours, the cells were harvested, and the in vitro conversion of the prohormone thyroxine (T4) to the active hormone triiodothyronine (T3) by DIO2 was evaluated using liquid chromatography with tandem mass spectrometry (LC/MS/MS). DIO2 activity decreased in the cells treated with BDE 99 (66% decrease at 1 μM and 83% decrease at 20 μM; $p< 0.05$). The expression of DIO2 mRNA also decreased in the cells treated with BDE 99 (1.3 fold decrease at 1 μM, 2.9 fold decrease at 10 μM, and a 2.6 fold decrease at 20 μM). DIO2 activity decreased in cells treated with OHBDE 99 (250 nM; 67% decrease; $p< 0.05$) and BDE 153 (1 μM; 66% decrease; $p< 0.05$). In contrast, DIO2 activity increased in the cells treated with BDE 47 (1 μM; 44% increase), OHBDE 47 (1 μM; 8% increase), and Aroclor 1254 (approximately 1 μM; 114% increase; $p< 0.05$). The results indicate that PBDEs interfere with the regulation of T3 in brain cells, which could represent a potential mechanism of PBDE neurodevelopmental toxicity. Ongoing analyses will provide dose-response relationships for DIO2 and SULT activity and will evaluate changes in the mRNA expression of DIO2, SULT, and membrane transporters for all of the tested compounds. Determining the mechanisms of PBDE toxicity will help regulators make decisions regarding the management of existing reservoirs of PBDEs (e-waste, used furniture, etc.) and emerging structurally similar replacement flame retardants.

104 Characterizing the PPAR γ ligand binding potential of several major flame retardants and their metabolites

M. Fang, Duke University; H.M. Stapleton, Duke University / Nicholas School of the Environment; T.F. Webster, Boston University School of Public / Dept. Environmental Health

Recent studies have showed that prenatal exposures to "environmental obesogens" may increase odds of obesity in children. Many of these chemicals compounds appear to act via a mechanism that includes activation of peroxisome proliferator-activated nuclear receptors (PPARs), leading to increased adipogenesis. In a recent study, pre-and postnatal exposure to the flame retardant mixture Firemaster 550 (FM550) resulted in a 20-30% weight gain in male and female rats relative to controls. Here, we build upon this study by exploring the potential ligand binding of FM 550 components (and their metabolites) with PPARγ. We also investigate the potential binding of other ubiquitous flame retardants and their metabolites. Furthermore, PPARγ binding activities of house dust samples containing those chemicals were also examined. A commercial high throughput assay, (PolarScreen™ PPARγ-competitor assay kit, Invitrogen) was used to investigate the binding potency. The tested compounds included several PBDE congeners (BDE47 and BDE99) and their metabolites [hydroxylated BDEs (OH-BDEs) and halogenated phenols], halogenated analogues of bisphenol A, FM550 and related components [triphenyl phosphate (TPP), isopropylated triaryl phosphates (ITP), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) and bis(2-ethylhexyl)-tetrabromophthalate (TBPH)] as well as three major FM 550 metabolites [diphenol phosphate (DPP), tetrabromo benzoic acid (TBBA) and tetrabromo mono(2-ethylhexyl)phthalate (TB-MEHP)]. Extracts of dust samples (n = 12; 3 mg dust/mL concentration were also tested for ligand binding potential. We found that many of the contaminants can competitively bind with PPARγ LBD in a dose-response manner. Within FM 550, TPP was found to be a potent ligand of PPARγ (IC_{50}: 38 microMolar) and is likely the major contributor to the binding potential of FM550. OH-BDEs were found to be potent ligands. The ligand binding of 3-OH-BDE47 was calculated to be 96% of the potency of the well-known agonist rosiglitazone. Halogenated phenols were also found to be PPARγ ligands and the relative potency increased in the order TFP.

105 Polychlorinated biphenyls and their hydroxylated metabolites (OH-PCBs) in the Baikal seals and implication from a toxicogenomic approach

K. Nomiyama, Center for Marine Environmental Studies (CMES), Eh; A. Eguchi, National Institute for Environmental Studies / Center for Environmental Health Sciences; S. Hirakawa, C. Kanbara, Ehime University / Center for Marine Environmental Studies (CMES); E. Kim, Kyung Hee University / Department of Life and Nanopharmaceutical Science; H. Iwata, Ehime University / Center for Marine Environmental Studies; S. Tanabe, Ehime University / Center for Marine Environmental Studies (CMES)

In Russia, environmental pollution is a serious problem due to rapid industrial activities since the 1960s and inadequate environmental management in the former Soviet Union. As a result, Lake Baikal was exposed to a variety of anthropogenic contaminants. Baikal seals (*Pusa sibirica*), a species endemic to the Lake Baikal basin, were reported to be particularly vulnerable to POPs because of high biomagnification resulting through the food web. In fact, analyses of Baikal seals tissues demonstrated high contamination by polychlorinated biphenyls (PCBs) and dioxins. The objective of this study was to investigate the contamination levels of PCBs and hydroxylated metabolites (OH-PCBs) in the liver of Baikal seals collected in 2005. In addition, using the microarray, we attempted to monitor the hepatic gene expression profiles in individual seals and to relate the levels of OH-PCBs to the altered gene expression. Concentrations of total OH-PCBs in Baikal seal livers were in the ranges of 950–6060 pg g^{-1} (median: 1900 pg g^{-1}) in males and 464–4790 pg g^{-1} (median: 1550 pg g^{-1}) in females. Among all the identified OH-PCB isomers, 4′OH-CB101/120 was dominant, followed by 3′OH-CB138, 4OH-CB134, 3OH-CB153, 4OH-CB97, 4OH-CB146, 4OH-CB163 and 4OH-CB187 in that decreasing order. Microarray data analyses suggested that various gene classes relating to xenobiotic-metabolizing enzymes, receptors, immune function, antioxidants, and glycoproteins were significantly correlated with concentrations of hepatic OH-PCB level and OH-PCB/PCB ratios. Moreover, multiple regression analyses identified some significant models in which certain genes showed expression levels altered by OH-PCBs levels and ratio of OH-PCBs/PCBs. The regression models explained associations with wound response, protein catabolic process, proteolysis, ferritin complex, fibrinogen complex, oxidoreductase activity, and metal ion transport. These results suggest that OH-PCBs exposure might have potential repercussion on the repair of wound, inflammation and infection.

106 Quantitative Analysis of OH- and MeO- BDEs in Human Breast Milk

D.S. Aga, University at Buffalo / Department of Chemistry; D.M. Butryn, University at Buffalo / Chemistry; M.S. Gross, University at Buffalo / Department of Chemistry; J. Olson, B. McGarrigle, University at Buffalo / Pharmacology and Toxicology

Polybrominated diphenyl ethers (PBDEs) are consumer flame retardants found in furniture, electronics, and plastics that have been shown to be endocrine disruptors in humans and animals. The congeners, as well as their metabolites, are persistent, bioaccumulative, and toxic, and have been linked to impaired behavioral development in toddlers and children. Due to their non-polar, lipophilic properties, these environmental contaminants tend to accumulate in fat and a major, direct source of PBDE exposure to infants is through human breast milk. This project aims to achieve a non-labor intensive method to quantitatively analyze PBDEs and their metabolites in breast milk of pregnant women. Denaturation of lipids was followed by solid-phase extraction to isolate the analytes. After clean-up with sulfuric acid activated silica columns, derivatization of the hydroxylated analogs to their methylated species was performed in order to allow the compounds to become more volatile when subjected to gas chromatography. Selective reaction monitoring (SRM) mode was operated to target the fragmented ions using a triple quadrupole gas chromatography tandem mass spectrometry (QQQ-GC-MS/MS) for high selectivity. Quantification of the metabolites along with their methylated compounds was calculated by using an external calibration curve, along with a mass labeled internal standard. The aim of this study is to identify and quantify the hydroxylated and methoxylated BDE metabolites in human milk that will ultimately better inform future mechanistic and epidemiological studies investigating the potential of PBDEs and their metabolites in producing neurodevelopmental disorders.

107 Analysis of anthropogenic and naturally occurring brominated phenols in the blood of Japanese pet animals

H. Mizukawa, Hokkaido University / Graduate School of Veterinary Medicine; K. Nomiyama, Center for Marine Environmental Studies (CMES), Ehime University / Center for Marine Environmental Studies (CMES); S. Nakatsu, Nakatsu Veterinary Surgery; M. Ishizuka, Hokkaido University / Graduate School of Veterinary Medicine; S. Tanabe, Ehime University / Center for Marine Environmental Studies (CMES)

The present study determined the concentrations and residue patterns of bromophenols (BPhs) in the whole blood of pet cats and pet dogs collected from a veterinary hospital in Japan. To estimate the dietary BPhs exposure levels of pet animals, BPhs in selected dry and wet pet food samples including seafood flavored food were also analyzed. Concentrations of BPhs in the blood of cats were significantly higher compared to the dog blood. Among the congeners, 2,4,6-tribromophenol (TBP) was accounted for a large proportion (> 90%) of BPhs detected in both species. 2,4,6-TBP have been identified as a natural compound formed by polychaete worms and algae, and also known as the metabolite of PBDEs and other brominated flame retardants. Beside 2,4,6-TBP, 2,4,5-TBP, 2,3,4,6-tetrabromophenol (TeBP), 2,3,5,6-TeBP and pentabromophenol (PBP) were also found in dogs, and 2,4,5-TBP, 2,3,5-TBP, 2,3,4,6-TeBP, 2,3,5,6-TeBP, 2,3,4,5-TeBP and PBP were found in cats. These differences might be due to the differences in the metabolism of PBDEs, binding affinity to TH transport proteins (e.g., transthyretin and thyroxine-binding globulin), and/or extent of exposure to parent compounds. Median concentrations of BPhs in dry dog food, wet dog food, dry cat food, and wet cat food were 12 pg g^{-1}, 20 pg g^{-1}, 110 pg g^{-1} and 270 pg g^{-1}, respectively. The most abundant congener in all pet food was 2,4,6-TBP. This profile is quite similar to the blood samples of the pets, suggesting that diet might be an important exposure route for BPhs in pet animals. BPh concentrations in cat food were significantly higher compared to dog food. These results suggest that pet cats' major intake of BPhs was through cat food containing seafood materials. However, it is also reported that 2,4,6-TBP was present in house dust, and it might be from the brominated flame retardants in household materials. In further studies, we need to analyze house dust collected from the houses of the custodians of the pets. To demonstrate the metabolic pattern of PBDEs, *in vitro* biotransformation experiments of PBDEs using cat and dog liver microsomes were conducted. After exposure to PBDEs, 2,4,6- and 2,4,5-TBP were found in dog liver microsomes, but only 2,4,6-TBP was found in cat liver microsomes. On the other hand, hydroxylated PBDEs were detected neither in cats nor in dogs. This result suggests that between dogs and cats, there are species-specific metabolic capacities for PBDEs.

108 Hydroxylated Polychlorinated Biphenyls (OH-PCBs) in sediment from a Lake Michigan waterway and original commercial Aroclors

R.F. Marek, University of Iowa / Civil & Environmental Engineering, IIHR-Hydroscience and Engineering; A. Martinez, University of Iowa / Department of Civil & Environmental Engineering,and IIHR-Hydroscience and Engineering; K.C. Hornbuckle, University of Iowa / Civil and Environmental Engineering, IIHR-Hydroscience and Engineering

Hydroxylated polychlorinated biphenyls (OH-PCBs) were measured in surficial sediment from Indiana Harbor and Ship Canal (IHSC), East Chicago, IN and five original Monsanto Aroclors. These compounds were measured using gas chromatography with tandem mass spectrometry (GC-MS/MS) and certified standards that allowed us to identify 65 individual or co-eluting congeners. Concentrations in the sediment ranged from 0.20 to 26 ng/g dry weight. Profiles of most samples were similar and were dominated by mono- to penta-chlorinated OH-PCBs. Interestingly, most of the samples strongly resembled the OH-PCB profiles of Aroclors 1221, 1242, 1248 and 1254, yet 25% of OH-PCBs measured in the sediment were not detected in Aroclors. A strong positive correlation was found between ΣOH-PCB and ΣPCB ($p < 0.0001$) and also between many individual OH-PCB:PCB pairs ($p < 0.05$). Analysis of OH-PCB:PCB pairs suggest PCB degradation is unlikely as a source of OH-PCBs in IHSC sediment. We report levels of OH-PCBs in sediment and Aroclors, and our discovery is significant because it is likely that OH-PCB contamination exists in sediment anywhere that PCB contamination from Aroclors is present.

109 The optimization of the mobile phases and the column in LC-MS/MS analysis of BPA analogues and derivatives in human urine

W. Zou, CDPH – ELAP; A. Aditham, J. She, California Department of Public Health

Background: Targeted screening of BPA derivatives and analogues in urine provides a sensitive indication of human exposures. In the present study, BPA, bisphenol AF (BPAF), bisphenol B (BPB), bisphenol F (BPF), bisphenol S (BPS), bisphenol A diglycidyl ether (BADGE) were analyzed. These compounds were selected for study base on their large-volume production/importation; detection in consumer products, dust, or biomonitoring studies; in vitro or in vivo endocrine activity; or persistence. Methods and results: We used an Agilent 1290 UHPLC coupled with an Agilent 6460 triple quadruple mass spectrometer. We optimized mobile phases and column to reach lowest LOD for each compound. We tested four different mobile phase combinations: 1) A: ammonium acetate 6.5 mM in water, pH 5.5, B: acetonitrile; 2) A: ammonium acetate 6.5 mM in water, pH 5.5, B: methanol; 3) A: water, B: acetonitrile; 4) A: water, B: methanol. The results show that the aqueous phase with or without ammonium acetate buffer did not affect the signal to noise ratio significantly; therefore, ammonium acetate buffer was chosen as the aqueous phase due to its capacity to maintain repeatable retention times. Methanol was significantly better than acetonitrile at reducing background noise and improving signal to noise ratio dramatically, therefore, methanol was chosen as the organic phase. Then, four different columns were compared: Kinetex phenylhexyl column (150 x 2.1 mm, 1.7 micron), Kinetex PFP column (150 x 2.1 mm, 1.7 micron), ACE phenyl column (150 x 2 mm, 2.1 micron), and ACE PFP column (150 x 2 mm, 2.1 micron). The results show that the ACE PFP column helped to generate nice-shaped peaks at sub-ppb levels. Conclusion: In summary, the combination of column optimization and mobile phase optimization improved LOD ~100 x for each analyte.

Helping Contaminants Emerge: Non-targeted and Effect-directed Environmental Analysis

110 Finding "Unknown Unknowns" in the Environment

R.A. Hites, Indiana University / School of Public & Environmental Affairs

How do compounds become listed as environmental chemical of concern? In some cases, compounds were listed because of a widely-reported environmental contamination incident and because of their known or suspected persistence or toxicity. For example, chlorinated dioxins are listed because of incidents in Seveso, Italy, and Times Beach, Missouri. In other cases, the list has evolved serendipitously. For example, DDT was specifically targeted as a result of Carson's *Silent Spring*, polychlorinated biphenyls were discovered during the routine analysis of DDT in fish, and polybrominated biphenyls were discovered during the analysis of PCBs in dairy cow feed. Is this a sustainable approach? Do analytical methods need to focus on previously unsuspected compounds (so-called unknown unknowns)? Do analytical methods need to be developed for specific compounds with high production volumes and chemical stabilities – assuming such knowledge is available? Do analytical methods need to be directed by one or more biological effects? In the past, all of these approaches have played a role, and this presentation will give examples of each.

111 GC/ECNI-MS-SIM nontarget screening of polyhalogenated compounds in dolphin blubber from Australia

C. Hauler, W. Vetter, University of Hohenheim

Polyhalogenated organic compounds comprising various classes of anthropogenic contaminants along with halogenated natural products are serious environmental contaminants whose analysis is a high priority task in environmental and food chemistry. While a range of methods has been developed for the quantification of known organohalogens, the discovery and identification of "unknowns" requires the use of nontarget GC/MS methods e. g. GCxGC-TOF has recently been shown to be a suitable method for this purpose. Likewise, a quadrupole-based nontarget GC/EI-MS-SIM method has been introduced by Rosenfelder *et al.* which is based on the fact that the molecular mass of polyhalogenated compounds is usually directly linked with the GC retention time. Since the molecular ion usually provides the most relevant structural information, the method focussed on the mass range >300 Da. The retention time range was divided into three mass ranges of 112 u (i.e., *m/z* 300-412, *m/z* 350-462, and *m/z* 450-562) which were screened in eight GC runs consisting of 15 consecutive SIM ions. In this

study, we transferred this nontarget SIM strategy to the GC/ECNI-MS mode. Previously, GC/ECNI-MS has been mainly used for the detection of e. g. polybrominated compounds by means of the abundant bromide isotope ions *m*/*z* 79 and *m*/*z* 81. Since GC/ECNI-MS is more sensitive than GC/EI-MS we assumed that switching to GC/ECNI-MS-SIM would allow the detection of the molecular ions of the polyhalogenated compounds. Applied to a dolphin sample from Australia, this new method enabled the detection of more than 400 polyhalogenated compounds, which was more than two-fold the number of compounds detected by the corresponding nontarget-GC/EI-MS-SIM method. Many of these compounds have not been detected before in any other sample analyzed by us.

112 Non-target analysis of Harbor Seal and Mussel Tissue Samples from the San Francisco Bay Region for New Compounds

J.R. Kucklick, National Institute of Standards & Technology / Chemical Sciences Division/ Hollings Marine Laboratory; J. Murray, National Institute of Standards and Technology; N. Rosenfelder, National Institute of Standards and Technology / Chemical Sciences Division/ Hollings Marine Laboratory; M.D. Sedlak, San Francisco Estuary Institute; D.J. Greig, The Marine Mammal Center

The accumulation of legacy persistent organic pollutants (POPs) in marine fauna is well documented and concentrations are generally declining due to controls on use. However, many chemicals or chemical bi-products in use today share characteristics with legacy POPs (hydrophobicity and stability) leading to bioaccumulation in marine organisms. The goal of this work is to use two gas chromatography (GC) techniques that utilize electron ionization mass spectrometry (EI-MS) for the detection of new compounds in harbor seal blubber and mussel tissue samples collected from the San Francisco Bay region. The first technique, multidimensional GC time-of-flight/MS (GC x GC TOF/MS) combines the high peak capacity of GC x GC with full-scan spectra derived from TOF/MS. The spectra obtained can be compared to EI mass spectral libraries such as the NIST Mass Spectral Library. The second technique, sequential scanning GC/MS, collects mass spectral data over a given mass range that is incrementally changed over several runs proportionally to the expected elution of compounds of a given mass from a GC column. Harbor seal blubber and mussel tissue samples were extracted using pressurized fluid extraction, cleaned up by size exclusion chromatography and then fractionated into different polarity fractions using silica/alumina column chromatography. Extracts were first analyzed by GC x GC TOF/MS. "New" compounds appearing in multiple samples were purchased as neat compounds (if available) and made into calibration solutions. The calibration solutions were processed through the method above along with a new sample subset. Extracts were further analyzed by GC-MS/MS for compound confirmation. Harbor seal blubber samples have been analyzed by GC x GC TOF/MS, and "new" compounds are in the process of being confirmed by GC-MS/MS. Mussel tissues are in the process of being prepared for analysis by GC x GC TOF/MS. Both harbor seal blubber and mussel tissue fractions will be further analyzed by segmented scanning GC/MS as an additional non-targeted analytical approach. Results from this project will help inform water managers in the San Francisco Bay region about previously undescribed compounds in the San Francisco Bay marine food web.

113 Comparing the profile of halogenated organic compounds in two ecotypes of Southern California bottlenose dolphins using non-targeted analysis

N.J. Shaul, Scripps Institution of Oceanography; E. Hoh, San Diego State University / Graduate School of Public Health; L. Aluwihare, University of California San Diego; N. Dodder, K. Maruya, Southern California Coastal Water Research Project; D. Weller, Southwest Fisheries Science Center, NOAA

Searching for unknown chemicals that are outside of the standard list of pollutants (i.e., PCBs, PBDEs, pesticides, etc.) requires a non-targeted analytical approach. Blubber samples (2 g) from mature male bottlenose dolphins (*Tursiops truncatus*) corresponding to coastal and offshore ecotypes were extracted and analyzed by two dimensional gas chromatography and time of flight mass spectrometry (GCxGC/TOF-MS). For the initial dolphin sample, chosen at random from the pool of samples from both ecotypes, the LECO ChromaTOF® software identified a total of 17,038 peaks at a signal-to-noise ratio of 10 that were searched against NIST's 2011 mass spectral library. The spectra of each peak were then scrutinized to identify the halogenated compounds in the sample by recognizing the halogenation patterns for Br and Cl. This subset of halogenated peaks was then used to create a reference data processing method that was used to search the remaining 7 samples. Any unique compounds in those 7 samples were added to the compounds from the first analyzed sample, and a library was created using R statistical computing software from the exported mass spectra and related identification information for the 361 unique halogenated organic compounds in the 8 samples. The majority of compounds belong to well-known classes of persistent organic pollutants (POPs), including pesticides and brominated flame retardants and several interesting contaminants such as terphenyls and DDT degradation products. An initial comparison across samples suggests that there are fewer POPs in offshore ecotypes. We were also able to identify several classes of brominated and mixed halogenated compounds that are suspected to be of natural origin, and of these compounds the brominated dimethyl bipyrroles had the most diverse profile (21 congeners), followed by brominated methyl bipyrroles (7 congeners) and methoxy PBDEs (5 congeners). The semi-quantitative abundances of each compound relative to an internal standard will be catalogued for the individual samples and for each habitat, and selected compounds will be quantified using authentic standards to see if any differences exist in the HOC profile between the 2 ecotypes. The reference library resulting from this work will be used to identify and compare bioaccumulative contaminants in other environmental samples, including sediments, fish, and other marine mammal species.

114 Bioanalytical assays: functional link between emerging contaminants and adverse outcome pathways

N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology

Evaluating toxicity of emerging contaminants is important for risk assessment. Of special concern are chemicals that may be additive (or synergistic) in their molecular activities and which may activate adverse outcome pathways, leading organisms towards decreased growth, decreased reproduction, increased susceptibility to disease or even death. In the past evaluation of toxicity has been accomplished by life cycle toxicity assessments, one chemical at a time. With the myriad of chemicals now identified in surface waters it is important to develop more effective toxicity tests that are high throughput and that link from the molecular initiating event to adverse outcomes in exposed organisms. Global gene expression and proteomics methods have been used to define molecular pathways that lead to adversity and to identify their molecular drivers. This information is being utilized to develop high throughput assays for easier screening and monitoring of contaminants. While still in their infancy, bioanalytical methods can be used together with analytical chemistry methods to monitor surface waters and eventually may be used in risk assessment.

115 Formation of mutagenicity during advanced oxidation process (AOP) treated drinking water

A. Jia, University of Arizona; B. Martijn, PWN Technologies; M. Park, S. Merel, University of Arizona; S.A. Snyder, University of Arizona / Chemical and Environmental Engineering

With the increasing population and limited availability of groundwater, an increasing demand for the treatment of surface water as drinking water are highly expected these days. Advanced Oxidation Processes (AOP) have been proven to effectively remove organic contaminants from pre-treated natural water, and a growing number of utilities are considering the use of UV and/or UV-based oxidation disinfection processes. Although knowledge about practical application of UV technology is already operational, additional information is needed about possible side effects of this technology and how these side effects could be eliminated. In this study, water samples are collected from 5 continuously MP UV/H_2O_2 reactors which receive pretreated surface water at two different locations in The Netherlands. Final effluents from the activated carbon filters which installed at the end of the reactors are also collected. After SPE extraction, the water extracts are screened by AMESII mutagenicity test and TA98 and TAmix strains are both tested with the absence and presence of liver enzyme S9. No mutagenic response was obtained with the TAmix, but TA98 strain showed an increasing mutagenicity after the MP UV/H_2O_2 treatment in both locations, while BAC and GAC treatment cannot effectively remove those activities. The results indicate that MP UV/H_2O_2 treatment may lead to the formation of mutagenic by-products, and it brings the request for the identification of the possible causal compounds. Parallel laboratory collimated beam study showed that linear relationship existed between the nitrite concentration with the UV

dose increase, while ubiquitously natural organic matter (NOM) in surface water possibly result in the formation of various nitrogen-compounds. Since AMESII test is based on histidine supply, various possible nitrogen-compounds including histidine are analyzed for these samples through mass spectrometry. The results showed 2-10 ng/L of histidine present in these MP UV treated samples by LC-MS/MS analysis, while further test on histidine effect at such level showed there is no mutagenicity response due to the histidine presence. High resolution QTOF mass spectrometry is then used for the screen of all nitrogen-compounds presented in the samples and mass fragments in treated samples that are significantly up regulated as compared to the influent profile are processed for structural identification.

116 Towards higher throughput in Effect-Directed Analysis

M. Lamoree, Chemistry & Biology, Institute for Environmental Studies, VU University

In the past decades, Effect-Directed Analysis (EDA) – in which chemical analytical techniques are combined with (in vitro) bioassays to identify environmental contaminants capable of causing adverse effects – has developed into a promising tool for investigative monitoring. Research in this field was rekindled in the late nineties and initially focused on the identification of compounds that caused estrogenic effects in fish. By the implementation of assays covering other (endocrine disruption) endpoints, such as (anti-) androgenicity and thyroid hormone disruption, the scope of EDA was widened. In addition, technological innovations in the field of analytical chemistry have contributed significantly to the potential to find, identify and quantify unknown compounds present in the environment. The application of accurate mass spectrometric techniques (time-of-flight, Orbitrap) in environmental analysis has been shown to facilitate the identification of unknown toxicants to some extent, but no major breakthrough in the identification of (known) unknowns has been achieved. In this presentation, examples of successful EDA studies will be given revealing the identity of (emerging) pollutants in sediments, surface waters and polar bear plasma. Despite these modest successes, the application and acceptance of EDA as a viable approach for the identification of compounds causing adverse effects has been delayed due to the laborious and time-consuming nature of the EDA work flow. When problems related to the relatively low throughput can be addressed, EDA may find its application in various fields, such as investigative water/sediment quality monitoring to support the (inter) national policies, process control for, e.g., biomass raw material treatment for biobased synthesis of commodity chemicals, for human and environmental exposomics, etc. In this presentation, solutions will be presented for the realization of higher throughput in EDA, such as the use of two-dimensional LCxLC-MS approaches to improve the bioactivity-to-identity correlation, the implementation of micro-fractionation in combination with miniaturized bioassays and improved high resolution mass spectrometric identification strategies.

117 Receptor-affinity extraction coupled to high-resolution mass spectrometry to characterize xenoestrogens in a wastewater-impacted coastal environment

K.A. Stencel, G.J. Getzinger, Duke University / Nicholas School of the Environment; L.K. Shaw, University of South Carolina / Biochemistry & Chemistry; L. Ferguson, Duke University / Department of Civil and Environmental Engineering

We have applied a novel effect-directed analysis method to the isolation and characterization of xenoestrogenic micropollutants within an estuarine environment impacted by municipal wastewater. Specifically, we have utilized hormone receptor-affinity extraction to isolate compounds from water and wastewater that bind to the human estrogen receptor prior to qualitative and quantitative analysis by high-resolution (LTQ-Orbitrap) tandem mass spectrometry. Receptor affinity extraction utilizes hexahistidine-tagged, recombinant human estrogen receptor (alpha isoform) ligand binding domain (ERα-LBD) to bind trace estrogenic compounds in solution prior to co-purification of the ERα-LBD-xenoestrogen complex using immobilized metal-affinity chromatography. This method allows for the selective determination of xenoextrogens in complex environmental samples by combining the specificity of biological activity and the analytical power of non-targeted, high resolution mass spectrometry. Samples of effluent and surface water were collected for analysis from a municipal wastewater plant and wastewater irrigated golf course retention ponds on Kiawah Island, SC over a period of two weeks. Targeted, structurally diverse (xeno)estrogenic compounds were quantified in samples by HPLC-Orbitrap MS/MS including 17β-estradiol, ethinylestradiol, estriol, estrone, octylphenol, nonylphenol, BPA, and genistein at levels ranging from < 1 to > 1000 ng/L. Non-targeted analysis of micropollutants isolated by receptor affinity extraction revealed the presence of several previously uncharacterized xenoestrogens, including anti-inflammatory and anti-viral prescription drugs and perfluorinated acids. Our results will be discussed in context of the removal and appearance of selected xenoestrogens throught the process of wastewater storage, application for turf irrigation, and discharge/transport through estuarine receiving waters.

Life Cycle Assessment for Sustainable Practices

118 A Life-Cycle Perspective for Emerging Energy Technologies

W. Morrow, Lawrence Berkeley National Laboratory; T.E. McKone, University of California, University of California and Lawrence Berkeley National Laboratory / Sustainable Energy Systems Group; J. Greenblatt, C. Scown, R. Sathre, A. Gopal, A. Shehabi, Lawrence Berkeley National Laboratory; H. Breunig, University of California, Berkeley / Civil and Environmental Engineering; D. Millstein, Lawrence Berkeley National Laboratory

Societies have close, complex relationships with energy-resource consumption. Infrastructure systems have formed around the most convenient energy sources available, often with significant negative ecological impacts. Low-carbon technologies offer paths to energy independence that reduce the risk of catastrophic climate change and improve human and ecological health. However, achieving the goals of a growing global population will demand rapid development of these new technologies. At even the earliest stages of development, expected cost, performance, infrastructure compatibility, and environmental externalities can prevent a technology from progressing toward commercialization. Life-cycle assessment (LCA), the primary analytical method for assessing the full environmental and economic impacts of products and services, must be integrated into technology development so that potential issues can be identified and addressed early in the process. The importance of emerging technology assessment is obvious, and we have identified some major methodological challenges. These insights are derived from the efforts of the newly formed Emerging Technology Assessment (ETA) Team at Lawrence Berkeley National Laboratory, whose members have worked closely with researchers in six areas: vehicles, biofuels, buildings, carbon capture, carbon sequestration, and photovoltaics. ETA researchers use analytical methods to assess the potential large-scale energy, climate, health, and cost impacts of low-carbon technologies under development. The goal of these early-stage assessments is to identify promising new technologies, and potential barriers that must be overcome to facilitate successful and sustainable scale-up. Three key challenges associated with this analysis are: defining appropriate metrics, anticipating scale-up, and managing system boundaries and uncertainty. Most of these challenges are familiar to LCA analysts evaluating existing technologies, but we are evaluating them in a new context as we anticipate the behavior of novel technologies as they emerge in a dynamic and uncertain future. By helping basic science researchers create successful technologies, we hope to achieve global-scale energy, environmental, and human health goals. We invite the science and LCA communities to help develop this forward-looking LCA perspective for guiding scientific research.

119 Sustainable Lighting: Energy Efficient Lighting and the Rebound Effect

A. Hicks, Department of Civil and Materials Engineering; T.L. Theis, University of Illinois

The consumption of energy for the production of light in the United States consumes a significant portion of the national energy budget, amounting to 694 terawatt-hours (TWh) of energy (7.4 quads of primary energy) equivalent to 7.6% of total energy consumption and 18.8% of total electricity consumption. Improvements in the energy efficiency of residential lighting are projected to significantly decrease the amount of energy used for lighting with the transition from incandescent to compact fluorescent (CFL) and finally to light emitting diode (LED). However, if due to increased efficiency and a lower ownership cost of light, consumers actually use more light, then there is the potential for the projected energy savings to be negated. This has occurred previously for lighting in the United Kingdom and in other industries. Life cycle assessments (LCA) have shown the majority of the environmental impact of lighting to occur during the use phase. Agent

based modeling (ABM) is coupled with LCA to determine population level impacts for the adoption of energy efficient lighting with regards to the potential rebound effect. ABM is a type of modeling where the actions of individuals contribute to overall macro scale impacts and trends, such as population level light and energy consumption. The use phase dependent on consumer adoption of lighting types and consumption of light are modeled using a cellular ABM informed with survey data and probabilistic adoption. The integration of ABM allows all three parameters of sustainability theory to be incorporated, environmental, social, and economic, with respect to how agents evaluate lighting options. The use phase results are then coupled with raw materials and manufacturing impacts derived using standard LCA methods. Preliminary results suggest that while the rebound effect has to potential to reduce the projected energy savings due to the adoption of more energy efficient lighting, it will not cause a large increase in energy consumption for lighting. Results of different scenarios, including policy interventions and potential energy rebound, will be presented and compared for the use phase and the overall population.

120 An assessment of temporality and spatiality problems in Life Cycle Assessment using the GREET 2 model

J.M. Pryshlakivsky, Ryerson University / Environmental Applied Science & Management

While the shortcomings of Life Cycle Assessment are reasonably well documented, there has been little in the way of research to quantify and assess the effects of temporality and spatiality within this field. Life Cycle Assessment seeks to assess the impacts of processes and services in order to facilitate informed decisions regarding environmental impacts and resource usage. However, the results of Life Cycle Assessment studies are centred in a specific time and place. Both temporality and spatiality arise in Life Cycle Assessment from variation with the input parameters and, as well, the resultant outputs of manufacturing processes. This may be differentiated from uncertainty in that uncertainty arises from a lack of data, or data that is either incorrect or ambiguous. However, modeling the effects of temporality and spatiality requires attaching a number of caveats to the available data. To test the potential impacts of temporality and spatiality, this research utilizes the GREET 2 (Greenhouse gases, Regulated Emissions, and Energy use in Transportation) model from the Argonne National Laboratory (US Department of Energy). GREET 2 tracks the impacts of the manufacturing phase of automobiles from raw material acquisition to final disposal. Variation in embodied energy (sometimes termed energy intensity, gross energy requirements [GER], or specific energy consumption [SEC]) of materials used in the manufacture of the GREET 2 automobiles will be used as the primary vehicle for testing the temporality and spatiality hypotheses. This variation will be modeled utilizing a classical Monte Carlo simulation. Materials studied here are those typically found in a modern internal combustion vehicle and include aluminum, steel, copper, lead, polyethylene terephthalate (PET), high density polyethylene (HDPE), polypropylene (PP), glass, paint and rubber.

121 Building a policy framework for environmental management of nanotechnologies using Life Cycle Assessment (LCA) and Risk Assessment (RA)

E. Alvarado-Diaz, CIMAV, S.C. / Environmental Science and Technoloy; G. Gonzalez-Sanchez, CIMAV; M. Miseljic, Technical University of Denmark / Management Engineering, Quantitative Sustainability Assessment; S.I. Olsen, Technical University of Denmark

The rapid growth of nanotechnologies developments concerning to the science and technology fields needs to be directed within a sustainable framework, where environment, society and economy meet together in order to reach the human progress. In the last ten years, the Mexican government has considered nanotechnology as a strategic sector within the last National Plans of Development, which has promoted the implementation of public policies for the nanotechnologies transfer from the laboratories to the production plant and finally to the market. This also has promoted the collaboration between public and private institutions like research centres, universities and companies .All this efforts being accomplished by public and private funds. Besides the engineered nanomaterials (ENMs) has better physical and chemical performance in different applications, the potential environmental and human health impacts of the technology developments based on this ENMs, must be assessed in order to build a policy structure which guide their transition throughout their life cycle stages, from the raw material extraction until the end of life. Regarding the explanation above, this case study is focused on the environmental assessment of the production of plastic additives with ZnO, $Mg(OH)_2$ and Ag nanoparticles incorporated as fillers. Moreover, this case study reflects on, from the Mexican perspective, how Life Cycle Assessment (LCA) and Risk Assessment (RA) can be used as complementary decision making tools for the environmental management of a company, regarding to their nanotechnology based products at the early stages of development. As well hotspots and opportunity areas are identified.

122 Integrating Occupational Health into Life Cycle Assessment

K.A. Scanlon, Department of Environmental and Occupational Health; S. Lloyd, Consultant; G. Gray, R. Francis, P. LaPuma, The George Washington University

Conclusions from life cycle assessment (LCA) studies can be applied to support decisions regarding product design or public policy, therefore, all relevant inputs and outputs to the product system should be evaluated to estimate impacts and inform tradeoff analyses. However, work-related impacts to human health often are not considered in LCA. Integrating occupational health into LCA will provide opportunities to prevent shifting of impacts between the work environment and the environment external to the workplace. Three separate, yet related, efforts are highlighted in this presentation. First, we introduce the work environment disability-adjusted life year (WE-DALY), one portion of a characterization factor used to express the magnitude of impacts to human health from the work environment. The WE-DALY equation requires input in the form of the number of fatal and nonfatal injuries and illnesses that occur in the industries relevant to the product system in the study, the age of the worker at the time of the fatal or nonfatal injury or illness, the severity of the injury or illness, and the duration of time lived with the outcomes of the injury or illness. Second, we present a methodological approach for integrating occupational health aspects into LCA; the steps to develop work environment characterization factors (WE-CF) and ways to structure life cycle inventory data to successfully employ the WE-CFs. Third, the WE-CF is demonstrated in a work environment characterization model built within an existing LCA model. Specifically, the new characterization model is applied to update LCAs of landfill and waste-to-energy municipal solid waste management methods. In this application, human health impacts from environmental aspects and human health impacts from work environment aspects are assessed concurrently. The presentation will conclude with suggestions for WE-CF refinement and additional case studies to qualify this approach.

123 Data and LCA, from theory to practical application with industry

J. Chargoy; N. Suppen Reynaga, Center for Life Cycle Assessment and Sustainable Design (CADIS)

Life Cycle Inventory Analysis (LCI) is the phase of Life Cycle Assessment (LCA) that involves the compilation and quantification of inputs and outputs for a product throughout its life cycle. This phase of the analysis is the most demanding of all. Due to the large amount of information that is required to collect, especially in areas where there are no databases that represent consistently production processes, as in the case of Mexico. This presentation describes the main findings in data collection and database development process according CADIS experience. Also shows how CADIS has become a link between theory and practice through case studies from both public and private sector that applied LCA methodology using CADIS inventories. During the last decade, the Center for Life Cycle Assessment and Sustainable Design (CADIS) has developed life cycle data for key sector in Mexico as electric energy, petro chemistry, building materials, water, municipal solid waste treatment, among others. These inventories were developed with funding from the National Council of Science and Technology (CONACYT) and with the collaboration of the Federal Electricity Commission (CFE), PEMEX, building material manufacturers and social housing developers. Once LCI was concluded it was necessary to develop an appropriate way to share this information with LCA practitioner in the region. According to this, CADIS now is working in a regional database, in order to increase LCA studies confidence with accurate LCI data.

124 A proposal for Characterization step in social life cycle assessment: wastewater facilities as a case study

A. Padilla, P. Guereca, J. Morgan-Sagastme, UNAM

In Social Life Cycle Assessment (SLCA), a further problem in the investigation is normalization and characterization when social impacts are evaluated; this step is very controversial since it involves value judgments. In order to strengthen the valuation step, this work establishes an alternative method, which includes normalization and characterization. The proposed methodology permits normalization and weighting in SLCIA to be defined from a mathematically strengthened approach. Methods. For this study, impact categories are presented by descriptive summaries. While applying Subcategory Assessment Method (SAM) for the wastewater treatment technologies, the methodology of valuation proposed, consist of the integration of subcategories in a global category indicator. This work integrates eight subcategories of stakeholders' workers in 3 steps; the evaluation impact, the acquisition of a partial indicator (distance to target approach), and the treatment of this partial indicator for application in workers 'stakeholders . The equation of Ecoindicator 95 adapted to the multi-attribute value theories and then adapted to social issues is considered as the starting point. Eight workers were evaluated, for the proposal methodology. Workers is the only stakeholder considered for this work. Scoring system is utilized for interpreting positive and negative impacts. By ranking and calculating the data, significant issues might be discovered. Results. Impact subcategories' results show that the maximum value is 1.67 while the minimum score is 0.8. There is not very negative effect in the different subcategories. But the value of fair salary impact subcategory is 0.8, which indicatesBy analyzing stakeholder categories' results, it is found that workers stakeholder categories' result is 6.2. In this case distance to target approach is a way to deal systematically with unsharp figures, which better represent the social reality that WWTP having negative impacts on the wage aspect. WWTP have high positive impact on training (1.6), and have positive effects in subcategories of child labor and force labor. The significant issues are fair salary and working hours.

Nanotechnology: Part B

125 Impact of UV Irradiation on the Surface Chemistry, Structure, and Nanoparticle Release of Multiwall Carbon Nanotube Epoxy Nanocomposites

E.J. Petersen, National Institute of Standards & Technology; T. Lam, J. Gorham, K. Scott, D. Stanley, R. Sharma, A. Liddle, NIST; B. Pellegrin, Université de Toulouse; T. Nguyen, NIST

One of the most promising applications of nanomaterials is as nanofillers to enhance the properties of polymeric materials. However, the effect of nanofillers on polymers subject to typical environmental stresses, such as ultraviolet (UV) radiation, high humidity, or elevated temperatures, is not well understood. In particular, the degradation mechanisms of nanocomposites and the effect of the nanofiller on surface chemistry and the potential for nanoparticle release are unclear. In this study, multiwall carbon nanotube (MWCNT) epoxy nanocomposite materials were prepared and exposed to carefully controlled UV doses (equivalent of up to ≈ 4 years in Florida) under conditions of elevated temperature and humidity. The samples were then analyzed using a suite of techniques to assess changes occurring in the bulk material such as mass (gravimetric analysis), surface chemistry (Fourier transform infrared spectroscopy and X-ray photoelectron spectroscopy), and surface and sub-surface morphology (scanning electron microscopy, atomic force microscopy, and energy filtered transmission electron microscopy) of the UV-irradiated samples. Nanoparticle release was assessed by scratch lithography and by scanning for released MWCNTs using electron microscopy. Overall, the photodegradation process of the epoxy matrix was retarded by the presence of the MWCNT filler suggesting that the MWCNTs may enhance the lifetime of the nanocomposite material. Multiple microscopic and spectroscopic techniques also showed an accumulation of MWCNTs on the nanocomposite surface that grew with increasing UV dose. The presence of MWCNTs on the nanocomposite surface may be significant with regard to the potential risk of MWCNT release during the nanocomposite lifetime. Preliminary tests performed using scratch lithography suggested that the exposed MWCNTs remained attached to the nanocomposite surface. MWCNT release was also not observed using electron microscopy.

126 Variations in carbon nanomaterial structure and surface chemistry impact the genomic response of Daphnia magna over multiple generations

D.A. Arndt, University of Wisconsin Milwaukee / Great Lakes Water Institute; M. Moua, University of Wisconsin Milwaukee / Department of Chemistry and Biochemistry; J. Chen, University of Wisconsin-Milwaukee; R. Klaper, University of Wisconsin-Milwaukee / School of Freshwater Sciences

Previous work in our laboratory indicates that carbon nanomaterials vary in their toxicity with alterations in surface chemistry. Although the toxicity of carbon nanomaterials to daphnids has been demonstrated, the mechanism of toxicity is still unclear, and the impact of surface chemistry on the mechanism of toxicity is also unclear. Some studies indicate that nanomaterials are toxic because of oxidative stress, but it is likely that nanomaterials induce toxicity by other mechanisms, as well. In this project, we investigated how the adverse outcomes seen with these same nanomaterials are related to the expression of key genes associated with stress, reproduction, growth, and xenobiotic metabolism. In addition to genetic pathways involved in oxidative stress, pathways important in DNA damage, heat shock, reproduction, and chitin metabolism will also be examined. This research will improve our understanding of the underlying mechanisms of nanomaterial toxicity, including how variations in core structure and functionalization can influence particle toxicity. Our previous research also indicated that some carbon nanomaterials induce multi-generational toxicity to *Daphnia*. In this study, the genomic response of a nanomaterial-exposed parent generation (F_0) and that of the next three unexposed generations (F_1, F_2, and F_3) were also investigated. *Daphnia* are emerging as a promising ecotoxicogenomic model organism, and knowledge of adverse outcome pathways in exposed and future generation daphnids will improve the predictive power of ecotoxicology.

127 Behavior as an Indicator of Impacts of Carbon Nanomaterials on Daphnia Magna

A.J. Nikolaus, R. Klaper, University of Wisconsin-Milwaukee / School of Freshwater Sciences

As the use of carbon nanomaterials across multiple industries grows so too does the need to understand the possible impacts that they may have on freshwater organisms. More specifically, understanding the impacts on behavior that may occur in response to nanomaterial exposure is important as deviations in standard behavior can lead to changes in predation and in feeding rate. Most studies have shown low acute toxicity but few have been done on low-level impacts for other responses such as the behavior of organisms and the ones that have looked primarily at vertical migration. Our previous studies have found that exposure to both functionalized and unfunctionalized carbon fullerenes caused changes in hopping behavior and heart rate in the model organism *Daphnia magna*. This study we focused on the horizontal behavioral movement of *Daphnia* in response to exposure to several different forms of carbon nanomaterials at multiple concentrations and tests were carried out both with and without an external light source to simulate both a day and night cycle. These daphnia were exposed for lengths 2 and 6 hours with recordings of their behavior made before and after exposure to track changes. Significant differences were found between the control groups and those that were exposed in both average velocity and total time spent mobile. Single walled carbon nanotubes with an attached carboxyl group (SWNT-COOH) or polyethylene glycol coating (SWNT-PEG) as well as hydroxylated carbon fullerenes (C60-OH) all showed increased effects with time and concentration. Differences in the effects depending on the light cycle were also seen. These results are in line with other studies that found that even acute exposures to other chemicals could produce significant locomotive and phototactic behavior.

128 Elucidating the Mechanisms by Which Particles Enter Cells and the Influence of Specific Protein-Particle Interactions

R.R. Garner, Clemson University / Institute of Environmental Toxicology (CU-ENTOX); W.S. Baldwin, Clemson University / Department of Biological Sciences; A.M. Rao, Clemson University; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX)

There are currently over 1000 consumer products on the market that contain or utilize nanomaterials, and while these materials have been shown to cross cell membranes, little research has examined the processes by which this occurs. The goal of this project was to characterize the influence of particle-protein interactions on the uptake of gold nanoparticles by A549 carcinomic human alveolar cells, and the processes by which this occurs. Particle uptake decreased as cells were exposed in media supplemented with

increasing concentrations of fetal bovine serum (FBS) (R^2=0.87, p< 0.0001). Subsequently, particle uptake increased as cells were exposed in media supplemented with increasing concentrations of bovine serum albumin (BSA) (R^2=0.89, p< 0.0001). These data suggest that albumin, one of a mere vast array on components in FBS, is integral in the transport of particles across membranes. Cells were then exposed to known inhibitors of endocytotic pathways to determine the mode of particle uptake. Specifically, cells were exposed to filipin and nystatin to inhibit caveolin-dependent endocytosis, chlorpromazine and phenylarsine oxide to inhibit clathrin-dependent endocytosis, and cytochalasin D and ethyl-isopropyl-amiloride to inhibit macropinocytosis. A novel toxicity identification evaluation (TIE) approach was then utilized to elucidate what components of FBS assist in particle uptake through first determining those that deter uptake. Following a Phase II TIE, certain classes of compounds were removed through the purchase of specialty FBS. Specifically, cells were exposed to particles in media supplemented with charcoal stripped FBS to remove lipophilic non-polar materials such as viruses, growth factors, hormones, cytokines, and steroids. Cells were further exposed to particles in media supplemented with dialyzed FBS to remove hormones and cytokines, as well as with ultra-low IgG FBS to examine their effect on uptake. A Phase III TIE was then utilized to examine the effect of fetuin, transferrin, apolipoprotein, and hemoglobin on uptake through their addition to media supplemented with particles and BSA. This research provides a fundamental foundation upon which to accurately understand biological interactions with nanoparticles and will facilitate future risk assessment endeavors through the utilization of this rapid high throughput assay that may be used to predict particle-cell interactions.

129 Effects of TiO2 and polystyrene nanoparticles on C. elegans: Imapact of Size, Light Exposure and PAH Interaction

W. Ahlf, TU Hamburg-Harburg / Environmental Technology & Energy Economics; B. Hegemann, University Konstanz; J. Angelstorf, Hamburg University of Applied Sciences

C. elegans is an ideal surrogate to represent the behavior and effect of particles in a whole organism. Body transparency allows microscopic tracking of particles within the organism. We used unipolar polystyrene particles spiked with fluoranthene (FLA) and normalized the particle amount according to surface area. The translocation of polystyrene particles (50, 90, 128, 250, 305 nm) could be observed by confocal microscopy using fluorescent polystyrene particles. They were mainly found to accumulate in the intestinal tissue; the particles smaller than 100 nm occasionally appeared in the uterus as well and were excreted through the vulva. The bioavailability and detoxification activity of FLA was determined by gene expression profiles of cyp35C1 as an indicator of the metabolization of PAHs in *C. elegans*. FLA spiked particles were tested in comparison with pure growth medium (control) and FLA solution via liquid exposure (0,9mg/L). The results supported our hypothesis of an increased bioavailability of FLA via the particulate exposure route. A trend of increased gene expression with decreasing particle size was also suggested from our results. All samples caused a significant reduction of the reproductive success of *C. elegans*. In order to assess these nanoscale specific effects, this study investigates the ecotoxity of a nano-TiO_2 (P25, 21nm) in comparison to a bulk TiO_2 (NM100, 100 to 200nm). The results of this investigation stress the importance of the primary particle size for the ecotoxicity of TiO_2 materials. In contrast to bulk TiO_2, nano-TiO_2 significantly inhibits *C.elegans* reproduction and growth with a LOEC of 10mg/L and 30mg/L respectively, while mean particle sizes of nano- and bulk TiO_2 in the test system are similar and both materials are ingested by *C.elegans*. There is evidence for photocatalytic activity and phototoxic effects of nano-TiO_2 induced by exposure to simulated sunlight, while no phototoxic effects are evoked by the photocatalytic activity of bulk TiO_2.

130 Determination of carbon nanotube concentrations in terrestrial and aquatic organisms a using microwave-induced heating method

J.E. Cañas-Carrell, Texas Tech University / Department of Environmental Toxicology; S. Li, EPA / Environmental Toxicology; F.O. Atore, Texas Tech University / Chemical Engineering; A.M. Parra, Environmental Toxicology; F. Irin, Texas Tech University / Chemical Engineering; B. Shrestha, Texas Tech University / Department of Environmental Toxicology; M.A. Saed, Texas Tech University / Electrical and Computer Engineering; M.J. Green, Texas Tech University / Chemical Engineering

Carbon nanotubes are currently one of the most widely used nanomaterials. There are limited analytical methods to quantify carbon nanotubes. A novel technique utilizing microwave-induced heating was developed to determine carbon nanotube concentrations in biological samples. The new method has been used to determine multiwalled carbon nanotube (MWNT) and single walled carbon nanotube (SWNT) uptake and bioaccumulation in terrestrial and aquatic organisms. Carbon nanotubes were taken up into corn exposed to various concentrations of MWNTs in soil for 40 d. MWNT uptake into corn roots was less than 10 µg/kg in all MWNT treatments and uptake was not dose-dependent. Translocation of MWNTs to the above ground portion of the plant (leaves and stem) was very low. Carbon nanotubes were also taken up into earthworms, but did not bioaccumulate with a low bioaccumulation factor of 0.015 ± 0.004. Considering the lack of detection and quantification methods for carbon nanotubes in biological samples, this new method is not only novel but will be useful in the risk assessment of carbon nanotubes.

131 Exposure to Carbon Nanotube Contaminated Sediment Increases Morbidity in Juvenile Fathead Minnow (Pimephales promelas)

E.R. McReynolds, University of Georgia / Environmental Health Science; M.C. Black, University of Georgia / Dept. of Environmental Health Science

Multi-walled carbon nanotubes (MWCNTs) are produced on a large scale with the potential to contact the aquatic environment through product use or accidental releases. Sediment is predicted to be the ultimate sink for MWCNTs in aquatic environments, as MWCNTs are extremely hydrophobic. The objectives of this study were to determine the fate and toxic effects of MWCNTs in a realistic aquatic exposure. Juvenile fathead minnows (*Pimephales promelas*, 14-d old) were exposed to ^{14}C-labeled MWCNTs (0, 0.5, 5, and 50 mg/kg) added to aged sediment collected from an USDA reference site. Exposure vessels contained 500 g of sediment and 2 L of moderately-hard water. Fish in half of the replicates for each concentration were excluded from the sediment to determine the bioavailability of MWCNTs in the water column without sediment disturbance by fish. Toxicity endpoints were growth, morbidity (swim bladder deflation, gill infections, and GI tract abnormalities), and MWCNT accumulation. Water samples collected at 0, 1, 5, and 10 days confirmed the presence of MWCNTs in the water, with highest MWCNT concentrations detected when fish had access to sediments (5, 50 mg/kg; p< 0.0001), suggesting that fathead minnow behavior increased MWCNT transport from sediments to the water column. Minimal concentrations of MWCNTs were detected after filtering the water (0.45 µm filter), suggesting that most MWCNTs attached to larger sediment particles suspended in the water rather than dissolved particles. Morbidity was observed in fish at all exposure concentrations, but was most frequently detected at the 50 mg/kg exposures, including anal protrusion of the GI tract (6.5% incidence) and fungal growths on gills (3% incidence). Following a 24 h depuration, fish had no statistically significant accumulation of MWCNTs at any exposure concentration (p=0.515). Although fish are able to excrete the MWCNTs once placed in clean water, exposures to MWCNTs at all concentrations may have inhibited proper immune system functioning, increasing the risk for infections. Future research should measure immune system responses during chronic sediment exposures to evaluate the effect of MWCNTs on fish immune systems and minnow population health; traditional toxicity testing protocols should be adjusted to reflect the unique behavior of MWCNTs.

132 The effects of evapotranspiration rate and dimorphic growth phase on gold nanoparticle uptake in the aquatic macrophyte, Myriophyllum aquaticum

B. Glenn, Clemson University / Clemson Institute of Environmental Toxicology; S.J. Klaine, Clemson University / Institute of Environmental Toxicology

It has been observed that 4-nm gold nanoparticles are able to translocate from the roots to the shoots of free-floating *Azolla caroliniana* and fully submerged *Myriophyllum simulans.* It was also observed that *A. caroliniana* had the highest tissue gold concentrations, perhaps due to the presence of a transpiration stream. Here, *Myriophyllum aquaticum* was investigated as it undergoes a dimorphic growth phase. Initially, it remains fully submerged, then it becomes emergent before flower. In order to study the effect of transpiration rate, *M. aquaticum* in the emergent phase was placed under three evapotranspiration conditions, a high humidity chamber with an evapotranspiration rate of 0.16 + 0.09 ml H_2O/g/24h, normal growing conditions with an evapotranspiration rate of 3.93 + 0.64 ml H_2O/g/24h and a low humidity chamber with an evapotranspiration rate of 4.80 + 2.36 ml H_2O/g/24h. Exposure to nominal 200 µg/L, 4-nm gold nanoparticles

(AuNPs) took place over 16 days under each condition with 48h renewals. Roots and shoots were separated and results indicated that AuNPs concentrate in the root systems, moving in with bulk water flow. However, tissue concentrations in the shoots remained consistent in each treatment. This root filtering effect indicates that xylem transport of AuNPs is unlikely, and that passive diffusion from roots to shoots in the apoplastic region of the plant is the most likely route of travel. To determine the effect of only evapotranspiration not dependent on length of exposure, plant were grown from node cuttings through both growth phases over 30 days. Results indicate that upon emergence, the presence of an evapotranspiration stream does increase tissue concentration, suggesting the importance of evapotranspiration in bulk AuNP root uptake.

Deepwater Horizon/MC 252 Well Incident Update – Part B

133 Physical, chemical and toxicological characterization of micron size droplets created from MC-252 source oil

P.R. Gardinali, Florida International University / Department of Chemistry & Biochemistry and SERC; K. Sandoval, Florida International University / Department of Chemistry and Southeast Environmental Research Center (SERC); B. Echols, Florida International University / Ecotoxicolgy and Risk Assessment Laboratory, Southeast Environmental Research Center (SERC); A. Smith, Florida International University / Department of Biological Sciences and Southeast Environmental Research Center; G.M. Rand, Florida International University / Ecotoxicology & Risk Assessment; T. Nordtug, SINTEF Materials & Chemistry / Applied Environmental Biology and Chemistry; B. Hansen, SINTEF Materials and Chemistry / Applied Environmental Biology and Chemistry

Macondo oil released to the Gulf of Mexico produced heterogeneous mixtures composed of dissolved hydrocarbon constituents and undissolved, phase-separated oil residues, including oil droplets. The size distribution, density, and composition of these droplets depend on key parameters such as the physical and chemical properties of the oil, the energy of mixing at the release point, and the water temperature and pressure at the release location. Toxicity of oil is often described as a function of the hydrocarbons present in the dissolved fractions and a considerable data gap exists on procedures to properly assess the contribution of oil droplets, in equilibrium with an aqueous phase. The main objective of this study was to evaluate a conceptual model to determine such contribution by generating droplet-specific EC50s using two well-characterized reference species (*Americamysis bahia* and *Menidia beryllina*). A turbulent-flow pressurized flow injection generator (PFI) developed by SINTEF and capable of producing oil-water dispersions (OWDs) of predictable and reproducible physical and chemical compositions was used to generate exposure media with similar droplet sizes but increasing oil concentrations. Water-soluble fractions (WSFs) of the OWDs were prepared and tested simultaneously by filtering the OWDs to determine the differential toxicity of the oil/water mixtures in the presence and absence of oil droplets. A simple, conceptual model representing the parameterized effect of droplets on aquatic toxicity was used to contrast the OWD and WSF results. Preliminary results suggest that droplets may have an effect on survivorship, but at Total PAH concentrations greater than the vast majority of the samples collected from the water column during the DWH response in 2010.

134 The effect of sediment bioturbators on the hydrocarbon distribution in hydrocarbon-dosed laboratory mesocosms

P.L. Klerks, A. Oguma, E. Blankson, C. Curtis, University of Louisiana at Lafayette / Department of Biology; M. Goodwin, University of Louisiana at Lafayette / School of Geosciences; F. Louka, University of Louisiana at Lafayette / Department of Chemistry

While it is well established that oil spills can affect organisms inhabiting marine sediments, it is not well appreciated that these organisms can in turn have an effect on the fate and distribution of the petroleum hydrocarbons. Such an effect can be either direct or indirect. Burrowing, feeding and other activities of benthic animals can move sediment, sediment pore water and water overlying the sediment, thereby directly moving dissolved or particle-bound hydrocarbons. With respect to an indirect effect, the bioturbators' activities change sediment characteristics such as redox potential, organic content, and particle size – which affect hydrocarbon binding characteristics as well as microbial degradation of the hydrocarbons. This project is using laboratory mesocosms (with bioturbators, marine sediment and water) to assess the influence of bioturbators on the distribution and biodegradation of petroleum hydrocarbons. Model bioturbators being used are ghost shrimp and razor clams; these differ in bioturbation characteristics including burrowing depth, and both are ecosystem engineers that are common in Gulf of Mexico intertidal to shallow subtidal zones. Variables being measured include hydrocarbon distribution between sediment and water column, hydrocarbon distribution within the sediment, and hydrocarbon loss from the mesocosms. Results will be presented for experiments being conducted with a single hydrocarbon (phenanthrene) and experiments in which the mesocosms are dosed with a surrogate Macondo Well oil.

135 The contribution of oil droplets to PAH bioaccumulation and toxicity in filter-feeding copepods following acute exposure to dispersed oil

B. Hansen, SINTEF Materials and Chemistry / Applied Environmental Biology and Chemistry; T. Nordtug, SINTEF Materials & Chemistry / Applied Environmental Biology and Chemistry; I.B. Overjordet, SINTEF Materials and Chemistry / Applied Environmental Biology and Chemistry; A.J. Olsen, I. Salaberria, Norwegian University of Science and Technology / Department of Biology; D. Altin, BioTrix

The toxicity of oil dispersions is believed to be largely attributed to the dissolved phase because of its bioavailability. However, for filter-feeding organisms, particulate oil droplets may contribute significantly to uptake and toxicity due to filtration of oil droplets. The objectives of this study were to investigate the potential contribution of small oil droplets to the acute toxicity and the uptake of oil components from mechanically dispersed oil (no chemical dispersants were used) in filter-feeding copepods. Parallel flow-through exposure chambers were set up with oil dispersions and filtered oil dispersions, corresponding to the equilibrium water soluble fraction (WSF) of the dispersion. In this way we were able directly compare the two treatments and thereby isolate the potential contribution of oil droplets to uptake of oil components and toxicity. The copepod *Calanus finmarchicus* was used as a model species for filter-feeding organisms, and acute toxicity and uptake of oil components (body burden) was determined following 96 h exposure (Nominal concentrations: 0 [ctrl], 0.6, 2 and 10 mg oil/L). Three oils with different properties (paraffinic, naphthenic and waxy oils) were used in separate but identical experiments to evaluate if observed effects (mortality and uptake) could be generalized for different oils. Concentration-dependent increases in body burden were observed for dispersions as well as WSFs. The contribution of oil droplets to body burden ranged between 20-60% dependent on the oil type and concentration. Particulate PAHs were significantly less bioavailable than dissolved PAHs. Reduction in food uptake (algae) was observed, possibly reducing oil filtrations by copepods as well. Reduction in survival was only observed for the highest dispersion concentration (T-PAH 50-100 μg/L), and was consistent between different oil types.

136 Exposure to sediment contaminated with Deepwater Horizon oil alters growth and survival of the southern flounder

R.J. Griffitt, University of Southern Mississippi / Coastal Sciences; N. Brown-Peterson, B. Bailey, University of Southern Mississippi / Gulf Coast Research Lab; M. Krasnec, I. Lipton, R. Takeshita, J. Lipton, J.M. Morris, Stratus Consulting Inc.

The 2010 Deepwater Horizon (DWH) explosion resulted in the largest oceanic oil spill in the history of the United States. In order to investigate the effects of this event on estuarine fauna, we exposed juvenile (>60 days post hatch) southern flounder (*Paralichthys lethostigma*) to sediment contaminated with oil from the DWH incident. Two separate experiments were performed. The initial experiment used field-collected sediments from several locations in Louisiana that contained a range of PAH concentrations. This experiment was performed for 30 days as a chronic flow-through exposure with field-collected sediment and uncontaminated water. The most contaminated sediment resulted in severe mortality, and there appeared to be a relationship between PAH concentration and fish size at the end of the test, suggesting an adverse effect of sediment-associated contaminants on flounder growth. A second experiment using clean sediment spiked with increasing concentrations of weathered oil skimmed from the water surface during the DWH spill. In the second experiment, juvenile flounder were placed into individual cages containing 75 grams of contaminated sediment and observed daily for 32 days. The exposures were performed as chronic flow-through experiments with uncontaminated water flowing through the test containers. The flounder were videotaped and measured twice weekly for growth. Increasing sediment PAH concentrations resulted

in a dose-dependent increase in mortality and a corresponding decrease in growth over the course of the experiment. After 32 days, the surviving flounder were sacrificed and dissected for further analysis. Histological and molecular analyses are ongoing.

137 Overview of Deepwater Horizon phototoxicity testing: Acute and trans-generational effects on early lifestage organisms

A.P. Roberts, University of North Texas; J.T. Oris, Miami University / Department of Biology; M.M. Alloy, University of North Texas / Department of Biological Sciences; L. Taylor; M. Grosell, University of Miami / Division of Marine Biology and Fisheries; A. Volety, Florida Gulf Coast University / Department of Marine and Ecological Sciences; J.A. Stoeckel, Auburn University; J.M. Morris, Stratus Consulting; J. Lipton, Stratus Consulting, Inc.

The 2010 Deepwater Horizon (DWH) oil spill resulted in a large release of polycyclic aromatic hydrocarbons (PAHs) into the Gulf of Mexico. PAHs exert a variety of effects on aquatic organisms including photoenhanced toxicity. Photoenhanced toxicity is a well-described phenomenon in which molecules of certain PAHs interact synergistically with ultraviolet radiation (UV). This interaction generates reactive oxygen species resulting in oxidative stress and acute toxicity. Translucent, early life stages of aquatic organisms that inhabit surface waters and cannot migrate away from high UV regimes are generally more susceptible than juvenile and adult stages. Here we present results of numerous tests using Gulf of Mexico species including early lifestages of blue crab (*Callinectes sapidus*), eastern oyster (*Crassostrea virginica*), sheepshead minnow (*Cyprinodon variegatus*), mahi-mahi (*Coryphaena hippurus*), and fiddler crabs (*Uca longisignalis*). Tests were conducted by exposing organisms to DWH oil in aqueous water accommodated fractions (WAF) or oiled sediments. Exposures were conducted in outdoor microcosm exposure systems equipped with plastics/neutral density filters to achieve a range of UV light conditions. Phototoxic effects were observed in both a UV and PAH concentration-dependent manner for all test species. Trans-generational phototoxicity was observed in fiddler crab larvae reared from PAH-exposed adults but otherwise not exposed to PAH post-hatch. These data demonstrate the importance of considering combined effects of non-chemical and chemical stressors in ecological risk assessment.

138 Native Species in Ecotoxicology: The Use of Non-Standard Organisms to Assess Impairment Following the Deepwater Horizon Oil Incident

B.S. Echols, Florida International University / SERC; A.J. Smith, Florida International University; B.C. Seda, Florida International University / graduate student; G.M. Rand, Florida International University / Ecotoxicology & Risk Assessment

Recommended test species are often used to assess the potential risk to an aquatic ecosystem, either prior to or in response to a perturbation. However, these species may be less sensitive than the native organisms inhabiting a system. Native species are not frequently used in laboratory toxicity studies due to a number of reasons, including limited availability, sensitivity to handling stress, a lack of established testing guidelines and insufficient background information on sensitivity and reproducibility of test results. As part of the Natural Resource Damage Assessment program in response to the Deepwater Horizon Oil incident, a toxicological testing program was developed and included the use of representative native species, including sensitive life stages of vertebrates and invertebrates. Studies were conducted with MC252 source and field-collected weathered oils, as well as a reference toxicant. Acute responses of sensitive life stages have been evaluated for fish species, including Red Drum, Spotted Sea Trout, Red Porgy, Florida Pompano and Cobia. Invertebrate species are also being evaluated. Results of fish tests suggest that the native fish species are generally more sensitive to both reference toxicants as well as water-accommodated fractions of weathered and unweathered oils, compared to the standard test organism, *Menidia beryllina*. Juvenile fish were also more sensitive to unweathered oil, than the weathered oil.

139 Impacts of Deepwater Horizon (DWH) oil on pelagic fish from the Gulf of Mexico (GOM) – An overview of relevant endpoints and effects

M. Grosell, University of Miami / Division of Marine Biology and Fisheries; E. Mager, University of Miami / Rosenstiel School of Marine and Atmospheric Science / Division of Marine Biology & Fisheries; A. Esbaugh, University of Texas Marine Science Institute / Rosenstiel School of Marine and Atmospheric Science, University of Texas – Austin, Marine Science Institute / Department of Marine Science; J. Stieglitz, University of Miami – RSMAS / Division of Marine Biology and Fisheries; R. Hoenig, RSMAS, University of Miami / Marine Affairs and Policy; C. Bodinier, RSMAS, University of Miami / Marine Biology and Fisheries; J. Incardona, NOAA Fisheries / Env. Conservation Division; N. Scholz, Ecotoxicology Program, North West Fisheries Science Center NOAA; D. Benetti, RSMAS / Marine Affairs and Policy

Captive brood stock allowed for exposure of early life stages of mahi (*Coryphaena hippurus*) and yellowfin tuna (*Thunnus albacares*) to water accommodated fractions (WAFs) of oil collected from the Gulf of Mexico and was achieved through development of novel test procedures ensuring high control performance. Tests, conducted under indoor conditions, revealed high sensitivity of embryos and larvae with 72-96 h LC50s as low as 5 µg/L ∑PAHs, depending on oil type and WAF preparation method. Interestingly, tests performed outdoors under natural light conditions indicated significantly photo-enhanced toxicity. From indoor tests, the mortality thresholds tended to coincide with thresholds for induction of a cardiotoxic syndrome associated with tricyclic PAH exposure although a clear causative link remains to be established. Swim performance in young juvenile mahi, as assessed by maximum sustained swim speed (Ucrit), was impacted by even short term (24 h) exposure to concentrations as low as 30 µg ∑PAHs /L. 48 hours of exposure to 1.2 µg ∑PAHs /L initiated shortly after fertilization resulted in significantly impaired swim performance even following 30 days of rearing in clean water. Basal metabolic rate, maximum metabolic rate, and aerobic scope of embryos/larvae or young juveniles were not impacted by exposure to DWH WAFs suggesting that reduced swim efficiency rather than reduced cardiac performance may account for the reduced swim performance.

Sustainability Ethics: An Experiential, Game-based Approach

140 Experiential Sustainability Ethics

T.P. Seager, Arizona State University / School of Sustainable Engineering & Built Environm

This session introduces participants to a game-based, experiential approach to understanding the Tragedy of the Commons problem in the context of inter-generational equity. Participating fully requires attendance during the entire session period, but the exercise will extend to on-line interaction over a period of several days. The exercise is deliberative, active, participatory, and typically surprising. Topics explored include moral luck and the obligations we have to others in an increasingly inter-connected world.

Mercury Characterization and Contaminated Site Remediation: Methods, Challenges, and Lessons Learned Part B

141 Mercury Dynamics and Bioaccumulation in the Berry's Creek Study Area, New Jersey

D. Glaser, B. Henry, N. Kelsall, Anchor QEA; J.L. Durda, Integral Consulting Inc.; D. Himmelheber, Geosyntec

Berry's Creek is a tidal tributary to the Hackensack River in northeastern New Jersey. Its 31 km^2 watershed is almost 90 percent developed land, except for small areas of open channel bordered by extensive *Phragmites* marshes. The creek has a long history of discharges from industrial facilities and municipal sewage treatment plants, landfills, and constraints on water flow from existing structures and tide gates. The site is currently the subject of a multi-media investigation by a group of private companies and public agencies under the purview of USEPA. Sampling began in 2009 and will continue at least through 2013. Primary contaminants of interest include PCBs, mercury and other metals. Total mercury concentrations in sediments of upper Berry's Creek are considerably higher than regional background levels and those found at most other "mercury sites". Yet, methylmercury concentrations in sediment, pore water, surface water, and biota (e.g., *Fundulus heteroclitus*) are lower than would be predicted based on data from other sites. The low bioavailability of mercury is likely a reflection of site-specific methylation dynamics rather than unique bioaccumulation processes. Pore water and sequential extraction data suggest that elevated sulfide levels bind mercury, reducing the pool of mercury available for methylation in many portions of the system. Substantial stands of *Phragmites* (10:1 emergent marsh to waterway) also contribute to sequestration of mercury, through abundant organic carbon and stable marsh sediment. Another key

process controlling mercury cycling is deposition and subsequent burial of mercury associated with particles as evidenced by patterns of mercury concentration with depth in marsh and waterway sediment. Methylmercury cncentrations are greater in marsh sediments than waterway sediments, but the significance of this source to surface water and biota remains unclear. Attributes of the Berry's Creek food web, such as its detrital nature, short length of the food chain, and large home range of some secondary consumers, serve to limit bioaccumulation in upper level receptors. These site-specific physical, chemical and biological factors influence mercury dynamics and bioaccumulation in Berry's Creek and will be considered during risk evaluation associated with and potential efficacy of remedial options at the site.

142 Use of Adsorptive Media Structures to Treat Mercury-Contaminated South River Water and Improve Physical Habitat

R.N. Brent, James Madison University / Department of Integrated Science and Technology; A. Cotting, S. Henke, James Madison University; K. Mumaw, J. Roller, B. Wagner, Ecosystem Services, LLC

A 130-mile stretch of the South River and South Fork Shenandoah River in Virginia is posted with a fish consumption advisory due to mercury contamination. Mercury in the river originated from a textile facility in Waynesboro, Virginia, that used and discharged mercuric sulfate from 1929-1950. While industrial discharges of mercury to the river ceased more than 60 years ago, legacy mercury in the floodplain and stream channel banks continues to feed mercury to the river system. Within the river, mercury from legacy sources is methylated and biomagnified through the aquatic food web to produce top predators (smallmouth bass) with 1-2 ppm methylmercury content. Remediation planning is underway for this river, and various options for reducing mercury uptake are being investigated. One such option is in situ treatment using habitat structures designed to house adsorptive media. Habitat structures, such as log sills or cross veins, are being infused with biochar to adsorb and treat dissolved mercury in the water column. These structures are being tested in stream-side mesocosms to determine their effectiveness in reducing mercury uptake in periphyton, the base of the South River food web. Initial studies have shown that mercury uptake in South River biota is highly correlated with water column mercury concentrations, indicating that passive water column treatment with adsorptive media may be effective at reducing uptake. Traditional applications of adsorptive media, such as in reactive caps or amendments, are problematic in the South River, because it is a high gradient, shallow, cobble-bottom stream. The use of habitat structures infused with adsorptive media may provide a mechanism for stabilizing adsorptive media within a high energy system and passively treating the water column. If implemented as part of a natural stream channel restoration design, these structures can provide the added benefits of: 1) improving ecological health and habitat conditions within the river, and 2) reducing bank shear stress and erosion, a major ongoing source of mercury to the South River.

143 The effect of in-situ treatment and capping amendments on mercury fate and behavior

P. Schierz, University of Texas at Austin / Department of Civil and Environmental Engineering, Duke University / Pratt School of Engineering, Duke University / Civil and Environmental Engineering, The University of Texas at Austin / Center for Research in Water Resources; P. Bireta, The University of Texas at Austin / Environmental and Water Resources Engineering; J.S. Grundy, L.E. Katz, The University of Texas at Austin / EWRE; D.D. Reible, The University of Texas-Austin, University of Texas / Environmental and Water Resources, The University of Texas at Austin / Dept. of Civil & Environmental Eng.

In this study, the ability of amendments to increase the effectiveness of conventional sand capping and in-situ treatment was evaluated. First, baseline sediment batch slurry and mesocosm experiments with unamended sediments were performed to evaluate net mercury release and methylmercury production and identify key controlling factors. Slurry and mesocosm experiments were also used to evaluated amendments including sorbents to reduce the amount of bioavailable mercury and chemical additives to control redox chemistry or interfere with the production of methyl mercury. Sorbents tested including activated carbon, biochars, organophilic clays while chemicals considered included MnO and FeS to help control redox conditions away from conditions that tend to maximize methyl mercury production. The performance of each of the tested materials in controlling mercury availability, redox conditions and methylation will be presented.

144 A Plan for Monitoring an Aquatic Field Based Pilot Study for the Effectiveness of Mercury Treatment with Biochar

C. Mancini, URS Corporation / Environmental Services; N.R. Grosso, DuPont Company; R.C. Landis, DuPont; J. Badner, URS Corporation

Treatment of soil and sediment with amendments such as biochar and activated carbon is a new technology being considered for the control of mercury and other bioaccumulative constituents in the environment. One of the more challenging aspects of studying potential remedial technologies in the field pilot stage is testing for significant improvements within the context of natural sources of variability. In addition to basic tenets of good experimental design (e.g., hypothesis testing), the design of the monitoring program must be robust enough to detect small but significant changes; this is particularly challenging when biota are part of the data set. In the South River Amendment Pilot Study, biochar is being assessed with respect to its utility in reducing the bioavailability of mercury to aquatic biota dwelling in a pond on the floodplain of the South River, Virginia. The data collected are used to test hypotheses that are directly related to understanding potential impacts of the carbon amendment on environmental media and benthic macroinvertebrate communities in the pond. In this case, the ability of biochar to decrease the bioavailability of mercury in pore water, surface water and sediments, is being tested. These exposure pathways are assessed by several lines of evidence, including biological tissue and benthic community analyses. The uptake of THg and MeHg by biological tissue is an important measure of mercury bioavailability in the pond environment. Consideration of the life histories and feeding habits of the test biota are critical to realistic assessment of the efficacy of the treatment. The targeted benthic organisms in the Amendment Pilot Study were selected based on the following three criteria: inclusion in complete exposure pathway associated with mercury relative abundance in the pond importance in the aquatic food web Preliminary tissue results support the importance of consideration of the food web and relevant exposure pathways in the selection of monitoring organisms.

145 Mercury in fish tissue in Mexico: towards a regional approach to monitoring persistent and bio-accumulating toxics

N. Daoust, CEC

In pursuit of a regional North American approach to monitoring persistent and bio-accumulating toxics, the Commission for Environmental Cooperation has recently summarized data on mercury (Hg) in fish tissue from Mexico. This information complements similar data on mercury concentrations in fish from the United States and Canada. The study is based on data sets originating from various sources and different laboratories: from samples of market fish, a literature search, samples of fish from the Lake Zapotlán watershed and other ecosystems in Mexico, and in muscle samples of thirteen species. Using a STELLA-based, one-compartment model to test the relationships of various data sets for human exposure to methylmercury (MeHg), simulation runs were made to evaluate the expected Hg levels in hair for different consumption profiles. Overall, the fish mercury content was found to be low, below the levels specified by the fish consumption advisories from Canada and the United States. However, three fish species presented Hg levels higher than the commercial fish guideline of 0.5 ppm. The results of this report, although a preliminary attempt at comparing data from the three countries, will give decision-makers baseline information and develop strategies to address risks posed by consuming mercury-contaminated fish in Mexico.

146 Residential mercury contamination and exposure in Huancavelica, Peru

N. Hagan, University of North Carolina at Chapel Hill / Environmental Sciences and Engineering; N. Robins, North Carolina State University; H. Hsu-Kim, Duke University / Department of Civil & Environmental Engineering; S. Halabi, Duke University / Department of Biostatistics and Bioinformatics; R.D. Espinoza Gonzales, Environmental Health Council; E. Ecos, E. Ecos, Departmental Hospital of Huancavelica; D. Richter, Duke University / Nicholas School of the Environment; J. Vandenberg, USEPA / National Center for Environmental Assessment

Between 1564 and 1810, nearly 17,000 metric tons of mercury (Hg) vapor were released from cinnabar refining in the small town of Huancavelica, Peru, much of which was locally deposited. Because of the historical release of Hg, a focused exposure characterization was conducted to inform mitigation and remediation strategies. Ambient soil Hg concentrations are among the highest levels in surface soil in the world, ranging up to 1200 μg/g. More

than 80% of the houses in Huancavelica are built using adobe bricks made using this contaminated soil. Potential health problems arise because the interior walls and floors are often unsealed, allowing Hg to be released to indoor air as particles and vapor. In the first study of adobe brick contamination anywhere in the world, we measured concentrations of total Hg in adobe bricks, dirt floors, and surface dust ranging up to 1070 µg/g, 926 µg/g, and 9.69 µg/surface wipe, respectively. Measurements of Hg in the hair of residents of adobe brick homes ranged up to 3610 µg/g, comparable to measurements in the hair of residents of towns with present-day gold refining using Hg. This study combines total and speciated Hg from residential samples, total Hg in hair, and data regarding factors influencing exposure from self-reported questionnaires (e.g., frequency of fish consumption, occupation) to characterize individual exposure to Hg. A comprehensive analysis of residential Hg contamination and exposure in Huancavelica will help guide the development and implementation of mitigation and remediation strategies in the community to reduce potential health risks from residential Hg exposure. Disclaimer: The information in this abstract has been funded in part by the US Environmental Protection Agency and in part by the EPA/UNC Toxicology Training Agreement CR-83515201-0, with the Curriculum in Toxicology, University of North Carolina at Chapel Hill. It has been subjected to review by the National Center for Environmental Assessment and approved for submission. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US Environmental Protection Agency.

147 Remediation Effectiveness Monitoring of a Former Hg-Cell Chloralkali Plant

R.R. Turner, RT Geosciences Inc.

Extensive remediation of soil and groundwater at a large chlor-alkali plant site in British Columbia was completed in 2003. Onsite and intertidal groundwater, as well as surface water and selected biota within the groundwater discharge zone, have subsequently been monitored intensively to demonstrate compliance with established criteria and to track recovery of these environmental media. In spite of extensive excavation, treatment and/or removal of contaminated soil, as well as a period of exhaustive extraction and treatment of contaminated groundwater, subsequent monitoring failed to demonstrate acceptable continued recovery three years after remediation was completed. The initial groundwater extraction program was implemented in July 2001 and halted in September 2002 after 50,000 m^3 of contaminated groundwater had been extracted and treated. At this time mercury concentrations in boundary wells had decreased below 1mg/L, an interior plume had virtually disappeared and most soil remediation was nearing completion. However, within a few months mercury in boundary wells had returned to former levels and by 2006 mercury in foreshore (intertidal) groundwater was exceeding established "trigger" levels. A new program of groundwater extraction and treatment was initiated in 2007 focused on maximizing mercury capture instead of complete plume capture. Monitoring results after 2006 demonstrated both effectiveness of the new groundwater extraction system as well as a "superimposed" longer term trend of decreasing mercury concentrations in intertidal groundwater, surface water and biota. Following subsequent decreases in mercury concentrations in the extraction wells in 2011 to below the trigger levels in boundary groundwater the new system was placed on standby. In 2013 concentrations in boundary wells have again increased to near the trigger levels, raising the possibility of yet another period of extraction and treatment. This work illustrates both the complexity of mercury transport in intertidal groundwater and the challenges of assuring permanent restoration of Hg-contaminated sites, few of which are monitored sufficiently following remediation to allow assessment of which remedies work in the long term.

148 Lessons learned from thirty years of mercury bioaccumulation monitoring in Oak Ridge, Tennessee

M. Peterson; T. Mathews, Oak Ridge National Laboratory / Environmental Sciences Division; G.R. Southworth, George Southworth; K. Roy, Oak Ridge National Laboratory / Environmental Sciences Division

Monitoring of fish for mercury bioaccumulation has been conducted downstream of industrial facilities in Oak Ridge, Tennessee for over thirty years. Mercury concentrations in fish have remained problematic, exceeding Tennessee fish consumption advisory limits, despite numerous actions to reduce mercury releases from the facilities. Initially, spatial trends in fish mercury concentrations in East Fork Poplar Creek exhibited a pattern consistent with a point source release and decreasing concentration with distance downstream. However, in more recent years the spatial pattern of fish bioaccumulation is more uniform throughout the stream, reflecting decreased concentrations in fish near the facilities and increased concentrations at downstream sites. Changes in aqueous methyl mercury exposure in the downstream part of the creek are thought to explain the increased fish concentrations, but the primary cause of higher methyl mercury levels is not known and is under investigation. Temporal patterns of mercury bioaccumulation have varied over decade-long time scales depending on sampling location. In upper East Fork Poplar Creek, fish concentrations initially decreased commensurate with decreased aqueous concentrations, but then fish levels have plateaued, and have remained unresponsive to further reductionsin aqueous mercury concentrations. In contrast, decreased aqueous mercury concentrations in White Oak Creek, another mercury contaminated stream in Oak Ridge, ultimately resulted in fish concentrations below advisory limits (< 0.3 µg/g), probably due to the comparably low levels attained in water (< 20 ng/L total mercury). During the course of the thirty year monitoring program, short-term studies have shed light on the role of various factors thought to affect bioaccumulation in the system, including flow and water quality impacts, species-specific differences and food chain effects, in-stream mercury sources vs. facility release, seasonal differences in mercury exposure, and fish size and movement. The unique decades-long record of mercury bioaccumulation trends in Oak Ridge, and the associated studies of potential causal factors affecting variability, may be a useful case study for other mercury contaminated aquatic sites.

Scientific Approaches to Support Restoring Beneficial Uses and Delisting Great Lakes Areas of Concern

149 Using riparian spiders as sentinels of ecosystem exposure and recovery at contaminated sediment sites

D.M. Walters, USGS / Fort Collins Science Center; M.A. Mills, USEPA, ORD / Remediation and Pollution Control Division, NRMRL

Identifying important pathways of wildlife exposure and developing reliable biological indicators of these exposures are important for managing risks associated with contaminated sediments. Aquatic insects are significant movers of contaminants between aquatic and terrestrial habitats. They accumulate compounds in their tissues as larvae and then move these contaminants into riparian zones when they leave the water to reproduce. Contaminants are then transferred to riparian species that feed on these insects. Riparian spiders are among their key predators, and they are, in turn, eaten by predators such as birds. Thus, spiders can play a critical role in regulating contaminant flux between aquatic and terrestrial food webs. We have been using spiders as ecological indicators of contaminant exposure at at Great Lakes Areas of Concern (AOCs) and other contaminated sediment sites over the past 6 years. Here, we provide an ecological overview of contaminant flux in riparian zones and the research that went into developing spiders as ecological indicators of contaminated sediment. We also provide case studies highlighting the application of riparian spiders for (1) improved site characterization and monitoring, (2) source identification, (3) removal of beneficial use impairments, and (4) assessment of remedy effectiveness for a diversity of aquatic habitat types (backwater, river, harbor, and lake) across various AOCs.

150 Intersection of Beneficial Use Impairments: Reducing Bioaccumulation of Mercury and Restoration of Aquatic Habitat Using Navigation Dredged Material

P. Horner, US Army Corps of Engineers; J.P. Kreitinger, US Army Corps of Engineers, US Army Engineer Research and Development Center / CEERD-EP-E; P. Gidley, US Army Engineer Research and Development Center; B. Suedel, US Army Corps of Engineers / CEERD-EP-R, US Army Engineer Research and Development Center / Environmental Laboratory; A. Friona, US Army Engineer Research and Development Center

Beneficial use impairments (BUIs) for the St. Louis River Area of Concern in Duluth, MN/Superior, WI include restriction of fish consumption due to mercury residues, degradation of fish and wildlife populations and loss of habitat due to shoreline alterations and contaminated sediments, and degradation of benthos due to physical disturbances and proximity to known pollutants. State restrictions on open water placement and a lack of capacity for management of dredged material using the Erie Pier confined disposal facility (CDF) have created a challenge for maintaining the Harbor's

navigation channels. A pilot project has been initiated to assess the feasibility of using dredged material for capping mercury impacted sediments, restoring shallow water habitat and creating a living shoreline. As part of this project, a study is being conducted to evaluate the potential for changes in mercury flux and the change in mercury exposure to biota that may result following use of dredged material for future habitat restoration projects required for delisting BUIs. Available estimates of methyl mercury flux and sediment partitioning coefficients (Kd values) developed for St Louis River Estuary sediments and other sites, will be reviewed and discussed as they relate to a conceptual site model and quantitative estimates of mercury flux developed using the USACE RECOVERY model. Conservative estimates of mercury flux and potential exposure of biota following placement of dredged material will be presented including the relative risk compared to pre-placement conditions. In addition, the relative impact of mercury flux from habitat restoration projects will be compared to the larger scale mercury flux associated with atmospheric deposition and contributions from the St Louis River watershed. An overview of the current project, risk concerns, model results and considerations for future monitoring programs will be presented.

151 Removing the fish consumption BUI at the Ashtabula Ohio Area of Concern

A.P. Mucha, USEPA / Great Lakes National Program Office; T. Conlin, A.J. Klei, Ohio Environmental Protection Agency; D. Green, USEPA

The Ashtabula AOC lies in northeast Ohio and empties into Lake Erie. Removing beneficial use impairments (BUIs) and delisting Areas of Concern (AOCs) are a high priority for USEPA and a significant component of the Great Lakes Restoration Initiative. To date, significant remediation and habitat improvement has occurred at the Ashtabula AOC. Following these actions, data efforts are underway and in place to begin removing beneficial use impairments. One of the first of the six remaining BUIs planned to be removed soon is the restrictions on fish and wildlife consumption BUI. The main reason for this BUI being in place was PCB contamination in fish tissue residues. Fish tissue residues have been monitored by the State over time, and during the sediment remediation there was a do not eat advisory issued to address anticipated increases in fish tissue concentrations due to resuspension of contaminated sediments. Since then that advisory has been lifted and extensive multi-species sampling of ambient fish has occurred. In 2011, a great deal of data was collected by multiple agencies for multiple purposes, and included collection of fish in the lower Ashtabula River and Harbor. Utilizing these data, and based on comparisons to concentrations in fish collected in Lake Erie and on analysis of trends over time, this BUI is now able to begin the removal process. This presentation will describe the delisting criteria for this BUI, discuss the relationship to fish advisories and present the data that support removal of the fish consumption BUI at Ashtabula.

152 Engineering Assistance and evaluation of technologies for pilot scale remediation at the former Muskegon Zephyr Refinery Site

D. Gent, ERDC; A. Martin, US Army Corps of Engineers / Environmental Research and Development Center; P. Horner, US Army Corps of Engineers; A. Friona, US Army Engineer Research and Development Center; M. Kuhn, US Army Corps of Engineers

The Michigan Department of Environmental Quality Remediation Division (MDEQ/RD) has worked toward cleanup of extensive petroleum contamination from the former Zephyr Refinery, Muskegon, Michigan within the Muskegon Lake Area of Concern. The site was used as a refinery and bulk storage facility beginning in the 1930s through the early 1990s. Numerous spills have been documented over the years, including two major gasoline spills totaling over 320,000 gallons in 1984. A groundwater pump and treat system has been in operation for more than a decade with the objective of limiting the migration of petroleum contaminants to surrounding surface water bodies. The contaminant mass has remained stable for years and shows no sign of natural attenuation. The pump-and-treat biological containment treatment system treats both fuel oil and gasoline contaminated groundwater. A series of recovery wells are used to pump contaminated groundwater to an aboveground bioreactor system with a liquid-phase carbon polishing stage that treats the contaminated groundwater for discharge to a National Pollutant Discharge Elimination System (NPDES) permitted outfall and to subsurface injection trenches within the contaminant plume.

153 Beyond SWAC – Integrating Assessment Methodologies for baseline sampling of contaminated sediment sites

M. Loomis, USEPA GLNPO; A.P. Mucha, USEPA / Great Lakes National Program Office; B.R. Jones, USEPA GLNPO / Great Lakes National Program Office

The Great Lakes Legacy Act (GLLA) is managed under the Great Lakes National Program Office and is charged with cleaning up contaminated sediments in Great Lakes Areas of Concern. The goal of this project is to assess the effectiveness of the proposed remedial action for the Buffalo River GLLA project. The question being answered is how can system-wide "snapshot" baseline conditions be appropriately sampled in a complex site that has multiple contaminants of concern (COC)? This presentation describes how an integrated sampling approach was implemented at the Buffalo River Area of Concern to assess pre-remediation baseline conditions. The approach is unique in that it provides for a holistic assessment of the environmental system beyond just surface-weighted area concentration analyses. Multiple assessment methods were employed along biological, chemical and physical lines of evidence to account for different chemical processes and movement(s) within the environment. Specifically, while PAHs are the main driver for the site cleanup there is also heavy metal and PCB contamination which impact different biotic components of the system. If a straight SWAC assessment was used, areas of biological relevance would be missed – from bioaccumlative effects in fish to benthic toxicity impacts. Additionally, physical improvement of the habitat can also improve biotic components of the system. Our approach followed three main steps: (1) develop and refine a conceptual site model (CSM); (2) choose assessment methodologies along the three lines of evidence, ensuring that the resulting assessment data will match the project area's Remedial Objectives; (3) Execute sampling with eventual post-remediation sampling. Outcomes of the case study revealed several lessons learned. These include: (1) sediment sampling – need to match assessment method with remedial action, validation of composite sampling through coefficient of variation analysis; (2) ability to correlate sediment contamination to biotic indicators (fish tissue, benthic toxicity) including what methods and parameters to use; (3) how to sample fish efficiently – choosing target species and appropriate chemical analyses.

154 Understanding rivermouth processes as a key to remediating beneficial use impairments (BUIs)

J. Schaeffer, USGS Great Lakes Science Center

Areas of Concern (AOCs) are often based on legacy contaminants, but there is growing recognition that lower river habitats provided valuable ecosystem services whose restoration would benefit coastal communities where AOCs are found often. We studied 5 Great Lakes rivermouths to understand how their hydrodynamics function, and how flows support habitat and nutrient processing. We found that Great Lakes rivermouths are not simply the terminus of freshwater pipelines with diffuse flows, but rather function more like coastal estuaries. While not tidal, we found complex flows, mixing zones, and frequent backflushing via seiches. These support both mixing and nutrient processing, but processes are likely constrained in all dimensions by infrastructure. This has consequences for a variety of benefical use impairments (BUIs) ranging from contaminants to wetland loss. Managers can gain insight into remediation strategies by understanding rivermouth dynamics because what maintains (BUIs) are system alternations that deviate from traditional processes. While challenging to restore, this would not only help achieve delisting, but restore ecosystem services that might revitalize Great Lakes Coastal communities.

155 Evaluating sources of PCBs in aquatic sediments using multiple metrics and a weight of evidence approach: Manistique River AOC case study

M.A. Mills, USEPA, ORD / Remediation and Pollution Control Division, NRMRL; D.M. Walters, USGS / Fort Collins Science Center; A.P. Mucha, USEPA / Great Lakes National Program Office; S. Swart, Michigan DEQ / Office of the Great Lakes; E. Johnson, NOAA/NCCOS/CCM; K.L. Kimbrough, NOAA / NCCOS Mussel Watch Program; J.M. Lazorchak, USEPA / National Exposure Research Laboratory

Sources of contaminants, such as polychorinated biphenyls (PCBs), to sediments can be difficult to identify and address before initiating remediation. Identification is necessary to allow source control strategies to be implemented to ensure remedy effectiveness of sediment-associated contamination. Potential sources may include outfalls, contaminated groundwater plumes, erosion of contaminated soils, hot spots in sediments, etc. Due to the

complicated hydrology, often episodic nature of sources, unknown points of discharge, etc., multiple lines of evidence may be required to reach a weight of evidence decision on the source location and contribution to the sediments. This presentation will focus on an on-going source investigation at the Manistique River Area of Concern (AOC) in Manistique, MI. EPA and its federal and state partners have designed and are executing a source investigation using biological, chemical, and physical lines of evidence. These include techniques such as: macroinvertebrate and mussel body burden analyses, riparian spiders tissue analyses, passive samplers, sediment and water chemistry, and groundwater flux measurements. The results of these studies will be used to determine if there is a current source of PCB contamination in the watershed and its contribution to downstream sediment.

156 Comparative Evaluation of Sorbents for Sediment Remediation

W. Azhar, University of Texas at Austin / Department of Civil, Architectural and Environmental Engineering; D.D. Reible, The University of Texas-Austin, University of Texas / Environmental and Water Resources, The University of Texas at Austin / Dept. of Civil & Environmental Eng.; P.J. Dunlap, Black & Veatch; X. Lu, The University of Texas-Austin / Civil, Architectural and Environmental Engineering; E.F. Barth, USEPA; S. Chattopadhyay, TetraTech

With the advent of active capping of contaminated sediments as an attractive remediation technology, the need to evaluate the sorption capacity of different commercially available sorbent materials has become of increasing interest. Adsorptive removal of contaminants is not just an option for use in a sediment cap but also as an in-situ treatment to be applied directly to the top layers of sediment. The ability of a sorbent to adsorb contaminants onto its surface making them less bioavailable serves as an effective remediation technique for treating contaminated sediment sites where toxic chemicals are often bioaccumulative and pose human health hazards. The adsorptive potential of different commercially available sorbent materials for the adsorption of metals as well as organic contaminants was evaluated at The University of Texas at Austin. The sorption capacity of each sorbent was evaluated by performing laboratory isotherm experiments using laboratory prepared water as well as different site waters. Several sorbent materials were chosen based on commercial availability and expected sorption capacity of metals and organic contaminants. Sorbent materials used for the isotherm experiments included clays CETCO Organoclay™ PM-199 and MRM, alumina pillared montmorillonite, dimethyldioctadecylammonium modified montmorillonite (DMDODA-MMT) and Kaolinite, and carbon based sorbents Calgon granular activated carbon, iron-palladium coated granular activated carbon and sulfur impregnated activated carbon. These materials were tested for the sorption of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), mercury and copper. Sorption capacity of activated carbon for organic contaminants proved to be higher than organophilic clays but the former is subject to fouling due to presence of natural organic matter (NOM). Numerical modeling was done to evaluate the predicted performance of granular activated carbon and Organoclay™ PM-199 under simulated field flows and conditions. Models compared the efficiency of both sorbent types under field conditions for use as an amended cap or as in-situ treatment. Addition of granular activated carbon showed substantial improvements in sediment quality as measured by porewater concentration and flux to the overlying water. The addition of Organoclay™ resulted in less dramatic improvements but is expected to perform better in the presence of NAPL.

Endocrine-disrupting Chemicals and Pharmaceuticals in the Environment: Part B

157 UV Spectrophotometry of Copper-derivatized Alendronate

P.J. Pagnozzi, Civil and Environmental Engineering; M. Barnes, Rensselaer Polytechnic Institute; M. Nyman, Rensselaer Polytechnic Institute / Dept. of Civil and Environmental Engineering

The bisphosphonates are widely used in clinical medicine for treatment of osteoporosis and other bone disorders. Chemically, they are substituted structural analogs of pyrophosphate and work by binding to and deactivating osteoclasts. Because of poor bioavailability (< 2% absorption) and long biologic half-life, they have a significant potential for environmental accumulation. Bisphosphonates do not absorb UV light. However, a recent study describes a method of derivatizing bisphosphonates in powder form with copper sulfate and nitric acid yielding products that absorbs UV light at 233 nm. Our group has replicated those findings using alendronate as a preliminary investigation. Expanding upon that work a stock solution of 0.0103 M alendronate was prepared. Two methods of analysis were employed. In the first 25 ml of the stock solution was mixed with 13 ml of 1.5 M $CuSO_4$ and 12 ml of 1.63 M HNO_3 resulting in a 0.005 M solution. Following this dilutions of alendronate were made of 0.004 M, 0.003 M, 0.002 M and 0.001 M and UV absorbance at 233 nm was measured. In the second, 10 ml samples of the initial stock solution (0.01 M) and dilutions of 0.008 M, 0.006 M, 0.004 M and 0.002 M were prepared. Each dilution was then mixed with 5 ml of 1.5 M $CuSO_4$ and 5 ml of 1.63 M HNO_3 (same final concentrations as method 1) and UV absorbance at 233 nm was measured. Both methods demonstrated a linear concentration-absorbance relationship; however, there is significant variation in the slope of these graphs. The equation for the first method was y=209.04x -0.05 which is similar to the equation established in the initial report (y=303x -0.3). The equation for the second method was y=53x+0.8. This variability indicates formation of two different reaction products as a result of variation in the sequence of adding reagents. Despite the variation in the slopes of the lines either method yields reproducible results, findings which support the validity of this method. This work provides a platform both for further investigation into the reactions mechanisms and for further use of this method as an analytical tool. The next step in the former will be to identify the different products and elucidate the mechanism leading to formation of different products. Once these questions are resolved further assessment of stability of alendronate under environmental conditions and alendronate soil isotherm studies can be undertaken utilizing this method.

158 Analysis of Maternal Urine, Amniotic Fluid and Cord Blood to Explore Fetal Exposure to Endocrine Disrupting Compounds and Adverse Health Outcomes

L. Geer, SUNY Downstate / Environmental Health Sciences; B. Pycke, Arizona State University; R. Halden, Arizona State University / Biodesign Institute

Recent evidence suggests that certain bioactive and emerging contaminants (such as endocrine-disrupting chemicals (EDCs) can adversely affect fetal development and birth outcomes, as well as result in disease later in life. EDCs have been implicated in the increased incidence of a wide range of adverse health outcomes, including infertility, genital tract abnormalities, breast cancer, obesity, and early onset of sexual maturation, observed in American and European populations over the last 50 years. The World Health Organization (WHO) recently released a report on EDCs suggesting a potential role of EDCs in the rise of preterm birth worldwide. We employed liquid chromatography/tandem mass spectrometry (LC-MS/MS) to measure levels of the environmental contaminants triclosan (TCS), triclocarban (TCC) and bisphenol-A (BPA) in both urine specimens from pregnant women and in cord blood serum from a convenience sample in a predominantly Caribbean-born, black immigrant population in Brooklyn, New York. For TCS, for example, 17 of 131 samples (13%) were above the limit of detection (LOD), with a geometric mean of 2,910 ug/L (95% CI: 810-10,460 or 1,550 ug/g (95% CI: 420-5,750), when adjusted for creatinine levels. Analyses of amniotic fluid samples from a second cohort of this population are ongoing. An expanded list of target analytes in amniotic fluid includes bisphenol A, tetrabromobisphenol A, fipronil, methylparaben, ethylparaben, propylparaben, butylparaben, benzylparaben, triclosan, triclocarban, and human metabolites of the latter. Data analysis and an epidemiological exploration of potential associations between fetal EDC exposure and adverse birth outcomes are in progress and will be reported on.

159 Fate and Transport of Trenbolone Acetate Metabolites in Rangeland Runoff

G.D. Jones, E.P. Kolodziej, University of Nevada-Reno / Civil and Environmental Engineering

US beef cattle are implanted with over 4500 kg/yr of the synthetic anabolic growth promoter trenbolone acetate (TBA). At least 8% (380 kg/yr) of the implant dose is excreted in manure as hormonally active steroid metabolites, which are potent endocrine disruptors that can reduce aquatic vertebrate fecundity at low concentrations (~10 ng/L). The endocrine disruption potential is greatest downstream of agricultural lands, where runoff concentrations of 350 ng/L have been reported. Therefore, characterizing the environmental risk of TBA metabolite transport is critical to minimize exposure to nontarget organisms. Our objectives were to identify dominant fate and transport mechanisms and strategies to minimize steroid transport in grazing rangelands. We evaluated leaching rates and overland and subsurface

transport in both irrigation and rainfall scenarios using microcosms and plot scale systems. Using GC/MS/MS analysis, we evaluated steroid attenuation by quantifying concentration decreases. The data indicate that leaching and subsequent transport was greatest in rainfall events and subsurface flows, respectively. The steroid mass leached during light to moderate intensity rainfall events was twice that of leaching during irrigation and was up to ~50x greater during high intensity rainfall events. This was a result of two factors: 1) leaching rates are a function of manure/water interfacial area, which was greater in rainfall events and 2) rain dislodged more solids, which likely increased steroid transport. Surface attenuation on vegetated buffer strips (3, 4, and 5m) ranged from 60-90%. Attenuation was steady thru time and did not vary with increased strip length. Attenuation thru a 3m subsurface runoff plot ranged from 36-67% and decreased with time. The hydraulic retention time on surface and subsurface runoff plots was ~3 and 10 min, respectively. This rapid attenuation is likely a result of organic carbon (C) partitioning instead of transformation. We hypothesize that higher C content in the A horizon of surface plots explains the increased attenuation when compared to C contents of the AB horizon in subsurface runoff plots. Future experiments will characterize specific removal mechanisms in both systems. The results of this study are important because they identify when the risk of steroid transport is greatest and indicate that sorption is likely the dominant mechanism for attenuating TBA metabolites.

160 Occurrence and concentrations of Endocrine-disrupting chemicals in Wastewater Matrices

M.A. Mills, USEPA, ORD / Remediation and Pollution Control Division, NRMRL; K. Dasu, USEPA / National Risk Management Research Laboratory / Department of Agronomy, Crop, Soil and Environmental Sciences; L. Zintek, USEPA / Department of Chemistry; W. Brashear, PTS; K. Tadele, USEPA / Student Contractor to National Risk Management Research Laboratory; B. Crone, Student services, USEPA

Contaminants of Emerging Concern (CECs) enter the environment by a number of sources. These contaminants are detected globally in wastewater treatment plant (WWTP) discharges such as effluents and biosolids and play an important role in the transport of these contaminants into the environment. As many of the CECs are shown to have endocrine disrupting properties and other harmful effects, there is a growing concern about the potential risks associated with the release of treated effluents and land-application of biosolids to the aquatic ecosystems and humans. Once land-applied, the CECs present in biosolids can enter the surface and ground waters or carry over to the plants grown on such soils and this increases the potential exposure of humans and aquatic ecosystems to these compounds. The current work encompasses an interdisciplinary effort to monitor and quantify CECs in effluents and biosolids from different WWTPs. Several classes of contaminants monitored include steroid hormones, alkylphenol ethoxylates, alkylphenols, perfluorinated chemicals, pharmaceuticals and personal care products along with metals and microbial pathogens. The current presentation focuses on the chemical analysis and concentration trends of endocrine disrupting chemicals. Grab samples of effluents and biosolids are collected from 9 WWTPs across the United States in quarterly sampling events. The effluent and biosolid samples are extracted separately for different classes CECs and are analyzed on UPLC/MS/MS. Evaluation of fluctuations of the levels of these chemicals in each plant was done in these quarterly sampling events during a period of one year. Based on the concentrations and compositions of CECs, seasonal effects, differences in the treatment processes, additives and other factors will be evaluated. The data will aid in understanding the fate and transport of CECs in wastewater discharges and the results will provide information for the risk management of these chemicals in the environment. The data on the occurrence and concentrations of different ECs and their precursors or metabolites in wastewater matrices will be discussed in detail.

161 What's in your water? Chemical and microbial contaminants of emerging concern in source water and treated drinking water of the US

S.T. Glassmeyer, USEPA / Office of Research and Development; E.T. Furlong, US Geological Survey / National Water Quality Laboratory; D. Kolpin, US Geological Survey; A. Batt, W.H. Benson, USEPA; S. Boone, USEPA / Office of Chemical Safety and Pollution Prevention; O. Conerly, M. Donohue, M. Kostich, H. Mash, S. Pfaller, K. Schenck, J. Simmons, E. Varughese, S. Vesper, E. Villegas, V. Wilson, USEPA

The term "contaminants of emerging concern" (CECs) encompasses a wide array of unregulated chemicals and microorganisms that require more data on frequency of environmental occurrence, concentration and/or effects before the risks from exposure, if any, can be ascertained. To determine which CECs exist in sources of drinking water and persist through drinking water treatment, a joint USEPA / USGS study examined paired source and treated drinking waters from 25 drinking water treatment plants (DWTPs) from across the United States. All water samples were analyzed for over 250 CECs, including pharmaceuticals, perfluorinated chemicals, hormones, wastewater indicators, trace elements, bacteria, protozoa and viruses. Sampled DWTPs utilized a mix of surface and ground water sources, and used a variety of disinfectants (e.g., chlorine, chloramine, and advanced treatments such as ozone and UV). Of the 250+ analytes, 165 were detected at least once in the source water (median of 60), and 134 were detected at least once in the treated water (median of 47). An examination of the source water data suggests that CEC frequency may be associated with season of sample collection, watershed land use, waterbody type, and total organic carbon. When the source water data is compared to their corresponding treated drinking water samples, the qualitative efficacy of various treatment practices can be examined. In addition to the occurrence data, margin of exposure risk assessments are being conducted to potentially determine the implication of the exposure to groups of CECS to both human and ecological health. *Disclaimer: Although this work was reviewed and approved by the USEPA, it may not necessarily reflect official Agency policy.*

162 Monitoring Bisphenol A in Thermal Paper with Yeast-Based Bioreporter Assay

J. Rajasärkkä, University of Helsinki / Department of Food and Environmental Sciences; M. Virta, University of Helsinki

Bisphenol A (BPA) is a high production volume chemical that is one of the most frequently detected xenoestrogens in environmental samples. Plastic products and epoxy resins are regarded as the major source of BPA, but in recent years another BPA source, thermal paper, has gained attention. Whereas in plastic and resins BPA is mainly in polymerized form, in thermal paper BPA is used in its free monomeric form. From paper, BPA can absorb through skin upon contact. Frequency of BPA-containing thermal paper usage and bioavailability of BPA was assessed in a follow-up study conducted in years 2011 and 2013. In total 70 paper samples from grocery stores and other public sources were collected mainly in Finland. High-throughput yeast-based BPA-specific bioreporter (BPA-R) assay was used to detect and quantify BPA in non-treated and extracted paper samples. Of the samples 46% were tested BPA positive, with slightly less positives in year 2013 (42%) compared to 2011 (52%). Amount of BPA ranged from 2800 to over 10,000 $\mu g\ g^{-1}$. BPA in thermal paper was readily bioavailable to the yeast bioreporter: non-treated paper samples (ø 3.2 mm) induced a maximal signal during the 2.5 h incubation time. The extremely high amount of BPA restricted the quantitative measurement of non-treated paper samples. The dynamic working range (detection limit to EC_{85}) of the BPA-R bioreporter for BPA was 50 to 1,500 $\mu g\ L^{-1}$ in direct non-treated paper sample analyses, and 23 to 760 $\mu g\ L^{-1}$ in extract analyses (total concentrations). In addition, some thermal paper samples exhibited toxicity on the yeast assay. Several low-activity samples were detected (42% and 18% in years 2011 and 2013, respectively), and amount of BPA equivalents in these samples was 10-100 $\mu g\ g^{-1}$. It is possible that in these thermal paper samples another BPA derivative, such as BPS or BPF, is used. Both BPS and BPF were able to induce the BPA-R bioreporter but at about 2 and 3 orders of magnitude higher concentrations, respectively. As a conclusion, amount and bioavailability of free BPA in thermal paper is very high. Thus, thermal paper continues being a possible BPA exposure source for humans, and via paper waste disposal also for the environment. Waste channels of BPA-containing thermal paper should be monitored in order to reveal emission levels to the environment. The yeast-based BPA-R bioreporter is a cost-efficient monitoring tool for bioavailable BPA, and a good addition to the battery of BPA analysis methods.

163 Representative Pharmaceuticals in Small South Carolina Streams: A Catchment Based Approach to Identify Potential Impacts and Sources

A.J. Jones, Clemson University / Institute of Environmental Toxicology; E. Carraway, Clemson University / Department of Environmental Engineering and Earth Sciences

Anthropogenic activities have long been studied as potential sources for contamination of water in nearby streams, however the majority of the research conducted has been focused on larger water bodies in urbanized areas. Little is known about the impacts these activities have on small, rural streams. Research in small, rural watersheds may have the benefit of lower background contamination and lower thresholds of effect than larger urbanized watersheds. In 2006, Clemson University and the South Carolina Department of Natural Resources embarked on a multi-year study to investigate approximately 200 small streams throughout the state of South Carolina. This study assessed chemical pollution in the water column, biomarkers of effect, and fish and invertebrate populations and community structure. This presentation will focus on the occurrence and distribution of organic pollutants in small streams of South Carolina and relationships with land cover. Organic pollutants are characterized using liquid chromatography – tandem mass spectrometry after concentration through an Oasis HLB extraction cartridge. Analytes of interest are estrone, 17β-estradiol, 17α-ethynylestradiol, caffeine, triclosan, diphenhydramine, naproxen, and ibuprofen. Results indicate that organic pollutants are widespread in small streams, however they are not often related to land cover or point sources. Often, the presence of contaminants in small streams can be attributed to other physical factors – such as soil porosity in the case of 17α-ethynylestradiol. This research indicates that many pharmaceuticals are present in the environment despite the absence of known point sources.

164 Predicting the Fate of Emerging Contaminants in Different Wastewater Treatment Configurations – Varying the Importance of Different Removal Pathways

F. Cloutier, L. Clouzot, Universite Laval / Département de génie civil et de génie des eaux; P.A. Vanrolleghem, ModelEAU – Université Laval / Département de génie civil et de génie des eaux

The influent of wastewater treatment plants (WWTP) contains various contaminants of emerging concern (CEC) originating from pharmaceuticals and personal care products, other household chemicals, etc. Several studies have shown that these compounds can negatively affect living organisms in the environment. WWTP were built to remove organic matter and nitrogen and therefore were not designed to treat other specific pollutants. Nevertheless, some WWTP are able to achieve high removal efficiencies for the non-recalcitrant CEC. Their removal from wastewaters depends on the treatment configurations, the operating conditions and the physicochemical properties of the pollutants. Their main removal processes are biodegradation, sorption, volatilization, and photolysis. Modeling and simulation were carried out in the software tool WEST® (mikebydhi.com) to represent the dynamics and fate of pollution in different wastewater unit processes. Previous studies have shown that it is essential to model the fate of traditional pollutants (COD and nitrogen) as it determines for instance sludge production and aeration intensity, which then determine removal by sorption and volatilization, respectively. The models were implemented by extending state-of-the-art WWTP models with fate submodels for CEC. As examples, the removal efficiencies of 17α-ethinylestradiol (EE2), trichloroethylene (TCE), and bis(2-ethylhexyl) phthalate (DEHP) were studied under dry and wet weather conditions in six WWTP configurations: conventional activated sludge (CAS), nitrifying activated sludge (NAS), biological nutrient removal (BNR), CAS + sand filtration, chemically enhanced primary clarification + ozonation, and aerated lagoons. The input to the model was a 40 days dynamic influent file including dry and wet weather periods so as to subject the WWTP to typical disturbances. TCE, DEHP and EE2, chemicals characterized by quite different fate-relevant properties, were added to the influent file to allow for the comparison between the different treatment trains. Required physicochemical properties of the compounds were found in the literature. Results show that TCE (mainly through volatilization) and DEHP (mainly through sorption and biodegradation) are easily removed by all treatment trains studied. EE2 is mostly removed with wasted sludge, but some biodegradation occurred in NAS and BNR systems. The addition of an ozonation unit as tertiary treatment significantly increases the removal of EE2.

Can You Believe Your Model? Tools and Techniques to Aid in the Prediction of Environmental Fate and Transport

165 How much is the difference? Updating and improving multimedia fate model SimpleBox with newly gained insights

D. van de Meent, RIVM / Institute of Wetland and Water Research; A. Hollander, J. Meesters, Radboud University Nijmegen; T. Traas, D. Sijm, RIVM

A new version of the multimedia mass balance model SimpleBox has been released. In SimpleBox 4, new insights in substance specific transport and transformation mechanisms, acquired since the previous release of SimpleBox 3.1 (2004), have been incorporated. Water compartments were redesigned, allowing better simulation of global fate of water borne substances, whereas simulation of fate in plants was simplified. Hollander and Hessels' formulations for chemical-specific penetration depths in soil have been introduced to better simulate fate in soil of semi-volatile substances. Jolliet and Hauschild's formulation for simulating intermitted rain episodes was implemented to better predict fate of highly water soluble chemicals. Franco and Trapp's routines for partitioning of ionizing substances was implemented to allow modeling of acids and bases. Photodegradation in water was accounted for more properly. Most importantly, typical colloidal processes, such as homo- and heteroaggregation were implemented to make it possible to model fate of nanomaterials. Obvious aim of the model adjustments was to produce better (more realistic and more reliable) predictions of exposure concentrations of chemical substances, and to expand the model's capability to simulate behavior of chemicals of more complex structures than the traditional hydrophobic organic compounds. In order to check the performance of the new model, we have systematically compared model outputs of the "old" model (SimpleBox 3.1) with the outputs obtained from the "new" model (SimpleBox 4), analyzing the effects of the various individual model adjustments on target- and non-target chemicals. The results provide answers to general questions of the kind "does it matter"? To what extent have the model adjustments influenced the predicted environmental exposure concentrations?

166 Estimating Environmental Concentrations of Pesticides in Ecological Risk

J.P. Sullivan, Ardea Consulting

The US Environmental Protection Agency has developed a number of models for estimating the concentration of pesticides in various environmental compartments (vegetation, surface water, fish tissues, etc.). These models range in complexity from highly complex (e.g., Pesticide Root Zone Model [PRZM]) to simple (e.g., Terrestrial Residue EXposure [T-REX]). Those aspects of the models that estimate the environmental concentration are discussed and those aspects of various models that also estimate risk (i.e., calculate a Risk Quotient) will be briefly considered. Ease of use for each model is presented and identify underlying assumptions, model limitations, or difficulties in interpreting the model outputs.

167 Modeling the Environmental Fate and Bioaccumulation Potential of Selected Halogenated Organic Flame Retardants

B.P. de Jourdan, Department of Environmental Biology / School of Environmental Sciences; D.C. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division

The criteria for classifying a compound as a persistent organic pollutant (POP) include measures of long range transport potential (LRTP), persistence (P) bioaccumulation (B), and toxicity (T). A wide range of software tools have been developed for predicting physicochemical properties, environmental fate and toxicity. In this study we used a variety of techniques to assess these properties of flame retardants (FRs) substituted with bromine (BFRs) or chlorine (CFRs) atoms, or include a functional group containing phosphorous (PFRs). These halogenated and organophosphorous FRs have been identified as replacement compounds for the recently phased out and banned polybrominated diphenyl ethers (PBDEs), however very little is known about the PBT properties of these compounds. Using the USEPA's EPISuite program, OECD's The Tool, The EQuilibrium Criterion (EQC) model, and the US FDA's Endocrine Disruptor Knowledge Base (EDKB) we generated LRTP, and PBT data for selected BFRs (n = 55), CFRs (n = 18) and PFRs (n = 23). The P and LRTP as described by The Tool, indicate that most of these FRs are persistent, and are capable of travelling over 2000 km. The EQC model showed that for the majority of FRs the preference is to partition into the soil and sediment matrices, with partitioning into the air being predicted to be very limited. The results from EPISuite show that biotransformation of the FRs varies considerably, with many FRs showing a resistance to biotransformation. Incorporation of the biotransformation estimates into bioaccumulation models revealed that many of these FRs have bioaccumulation factors (BAFs) below the international criteria for identifying a substance as bioaccumulative. As well, the estimated BAFs indicated that trophic dilution was likely to occur for many of the FRs. These FRs tend to be outside the domain of standard acute toxicity models, so endocrine disruption was estimated using the EDKB database, which revealed some of these FRs to be estrogenic. The results of this modeling exercise demonstrate that these FRs have P and B characteristics and are capable of undergoing long range transport; however toxicity estimates were more uncertain. The modeled BCFs and BAFs were compared to measured values obtained from a 2006-2010 sampling program that examined a suite of non-legacy halogenated organic compounds (HOCs) in water and biota samples from Lake Ontario, Lake Erie, and Lake Opeongo.

168 Multimedia mass-balance models for chemicals in the environment: reliable tools or bold oversimplifications?

M. Scheringer, ETH Zürich / Institute for Chemical and Bioengineering; K. Hungerbuhler, ETH Zürich

Over the last 30 years, multimedia mass-balance models for chemicals in the environment have been used widely in science and decision making. After the introduction of the basic concept of mass-balance models in the early 1980s, many new and highly diverse models of this type have been developed. They cover different spatial scales from local to regional and global; different types of ecosystems (Arctic, temperate, tropical); and different types of chemicals, from non-polar organic chemicals to ionizing substances, metals, and even engineered nanoparticles as a recent development. Also cascades of parent compounds and their environmentally relevant transformation products have been described in terms of these models. In many cases, the concentrations of chemicals in the environment as calculated by these models show surprisingly good agreement with measured concentrations. What is the general feature of these models that makes them capable of reproducing levels of so many diffferent types of chemicals in a range of different ecosystems and environmental media? We investigate the uncertainties associated with the set-up (model equations, process descriptions) of multimedia mass-balance models and with the model inputs (emission rates and chemical property data). On this basis, we discuss for several case studies to what extent we can "believe" each of these components and derive a small set of truly decisive components that determine the success of failure of a multimedia mass-balance model in a certain application. Engineered nanoparticles are highlighted as a case where it is not just parameter uncertainty or lack of data that make environmental fate modeling a challenge, but where completely new process descriptions need to be developed in order to make the concept of mass-balance modeling applicable.

169 Empirically Modeling PCB Origins in the Sediments of a US Gulf Coast Bay

J. Atmadja, The Louis Berger Group, Inc.; E.A. Garvey, The Louis Berger Group, Inc. / Site Assessment and Remediation; X. Wang, The Louis Berger Group, Inc.

Development of a conceptual site model (CSM) from available data is an essential part of understanding PCB sources and transport. In this gulf coast bay and its associated river, PCBs levels in fish remain elevated despite the passage of time, resulting in state fish consumption advisories. PCB levels in sediments of the river and bay are relatively low with typical values less than 20 ug/kg. Low detection level PCB congener measurements via EPA method 1668A were used in a forensic analysis of PCB-contaminated sediments in this river, bay and associated marshlands to develop a conceptual model of PCB source areas and their relative magnitude. Despite the low absolute levels, the forensic analysis was able to discern the existence of at least 2 PCB sources, a minor one originating in the upper reaches of the river, and a second, larger one originating roughly 10 km upstream of the river's confluence with the bay, coincident with a chemical plant along the river. In this study, we used a statistical tool called principal component analysis, along with other lines of evidence to understand the spatial distribution of PCB concentrations and patterns throughout the river and bay and formulate our CSM. Based on the linear portion of the system (the river) we were also able to estimate the relative magnitude of the 2 sources. The interpretation

of PCB data within the river and the bay suggests that it is likely that the plant site continues to be an environmentally significant source of PCBs to the river and the bay. Our examination showed that there is a statistically significant increase in PCB concentration and TOC-normalized PCB concentration downstream of Km point 10 (KP10), coincident with the plant site location. Accompanying this increase in PCB concentration is a change in the PCB pattern to lower molecular weight PCBs, such that all locations with elevated PCB concentrations below KP10 are characterized by the pattern originating at KP10. The PCB pattern of the upstream river, while being generally higher in molecular weight is also characterized by the presence of PCB-11, a common paint pigment contaminant. We were able to make use of PCB pattern, PCB concentration and the level of PCB-11 in identifying the existence of a major downstream source downstream of KP10. Concentrations and PCB patterns in the bay are consistent with a scenario involving dilution of the main PCB source originating below KP 10 with cleaner solids originating outside the bay.

170 Comparison of USEPA Surface Water Models: Pros, Cons and What Users Need to Know

D. Bonnar

The US Environmental Protection Agency (USEPA) uses a number of simulation models to predict pesticide concentrations in surface waters. These models (PRZM, EXAMS, AgDisp) are complex, process-based simulation codes that combine chemical and physical data, application method details, and descriptions of the environmental settings into which pesticides are released. While their complexity allows for sophisticated, scenario-specific analysis, this may render them difficult for some users without programming experience. Graphical User Interfaces (GUIs) are used to overcome this complexity and to provide standardized regulatory "scenarios". Two of these GUIs, EXPRESS and PE5, are explored and the ease of use, advantages, and limitations of each are discussed. A brief introduction to the new USEPA Surface Water Calculator (SWC) will also be given.

171 Reducing a Numerical Simulation Model to a Simplified Lumped Parameter Model Through Reverse Engineering: An Illustration Based on the Hudson River

J.W. Kern, Kern Statistical Services, Inc.; J. Field, National Oceanic and Atmospheric Administration; L.B. Rosman, NOAA/OR&R/ARD

Remediation decisions at large contaminated sediment sites with bioaccumulative contaminants often rely on complex mechanistic models for comparing remedial options. In the near past more simplified Tier-I models based on lumped parameters and less spatially resolved model inputs were used for similar purposes. With increasing availability of higher speed computing, the trend has been toward more complexity with the promise that higher complexity brings greater predictive power. Conversely, much effort in statistical analysis revolves around identification of the simplest models that capture the essential elements of a particular system—the principle of parsimony or Occam's razor. This presentation illustrates this interplay through an example from the Hudson River PCBs Superfund Site. At the Hudson River PCBs Superfund Site EPA's 2002 Record of Decision called for dredging and natural recovery of contaminated sediments in the UHR, based on results of fate and transport models predicting future levels of PCBs in UHR sediment, water, and fish. The models, considered state of the art at that time, were linked to Lower Hudson River (LHR) models to predict PCBs in fish tissue in the LHR. Subsequent to these modeling efforts, sediment cores were collected in (2002-2005) and surface PCB concentrations were found to greatly exceed contemporaneous sediment bed concentrations used in the models. To understand the implications of these newer sediment data for remedy selection, we reverse engineered a lumped-parameter model capturing the behavior of the numerical model. Our greatly-simplified lumped-parameter model reproduced numerical model outputs (PCB concentration in water and fish tissue) from model inputs accurately—nearly 1-1 and precisely (R^2=0.95). This simplified model was then used to evaluate a range of new scenarios including some not anticipated at the time the original models were developed. This lumped parameter model greatly simplified the evaluation process as virtually no runtime is required and hence many more scenarios could be evaluated. This experience raises questions about the merits of long expensive model development processes when similar results may be available from much less demanding and costly models. This approach also provides an avenue for more rigorous uncertainty analyses which are impossible with more complex models due to prohibitively long run-times.

172 A Rigorous Dual Continuum Model for Solute Transport in Karst

J. Harris, R. Painter, Tennessee State University / Civil & Environmental Engineering; T.D. Byl, US Geological Survey / Department of Interior; L. Sharpe, Tennessee State University / Massie Chair of Excellence

The advection dispersion equation (ADE) applied to pipe flow often successfully models solute transport along major karst features. This approach has had success when flow within these reaches is predominately conduit flow. However, the slower solute transport associated with flow in the diffuse continuum may explain the tendency of tracer response concentration data to have a long upper tail compared to the diminishing tail predicted by the ADE. The hydraulic response of karst to a rain event also reflects the dual continuum for karst flow. The high permeability of the conduit network allows for a subsequent quick response at the spring to a rain event. A slow recession to pre-rain event spring flows is often related to water being released from storage in the fractured media or epikarst. It is in this context that the case is made for a dual continuum transport model for karst. In existing double continuum models the matrix and the conduit network are each represented by a continuum and the exchange of water between the two continua is governed by lumped exchange parameters. This paper presents a dual continuum model for karst, which is based on the finite element solution of the rigorous model in terms of the Navier-Stokes and continuity equations describing conduit flow and a modified Darcy equation describing the diffuse phase flow, and finally, the transient ADE describing solute concentration. In the model, the two adjacent continua share a common boundary. Due to the extreme differences in the flow regimes near the common boundary, the boundary conditions are characterized by very steep gradients. Furthermore, the hydraulic characteristics are independent of the concentration of the contaminant. Logically conservation of mass and momentum require that the gradients be continuous and in this model the continuity at the common interface was achieved computationally using smooth Heaviside-unit functions in a finite element model.

Molecular, Genetic, Multi-Generational and Evolutionary Ecotoxicology: Part A

173 Temporal dynamics of metal accumulation and impacts on biochemical oxidative stress biomarkers and stress-responsive gene expression in yellow perch

M.A. Defo, Université du Québec / INRS-ETE; P. Couture, INRS / Centre Eau Terre Environnement; P.G. Campbell, Universite du Quebec, INRS / INRS-ETE; L. Bernatchez, Universite Laval / Department of Biology

In a previous study, the transcriptional response of one thousand genes to metal contamination was investigated using a cDNA microarray in yellow perch (*Perca flavescens*) exposed in the laboratory to cadmium at environmentally relevant concentrations for 6 weeks. The microarray, supported by posttranscriptional validation, revealed that multiple metabolic pathways including oxidative stress and retinoid and carotenoid metabolism were affected. The transcriptional responses to metal contamination revealed distinct patterns of regulation. For example an increase of catalase(*cat*) and retinol dehydrogenase 2 (*rdh-2*) expression levels were detected at the end of the exposure, whereas a decrease in the microsomal glutathione S-transferase 1 (m*gst1*) level was reported. These data suggest that the transcription level of oxidative stress-related genes can be a good indicator of metal exposure. In order to validate the lab response in the field and to determine the temporal dynamics of metal accumulation and biomarker responses, young of the year (age 1+) yellow perch were caged for 1 and 4 weeks in either a reference (Opasatica) or a metal-contaminated (Dufault) lake. The mesh size of the cages allowed free exchange with the surrounding lake water and zooplankton, the main food source of this life stage. Kidney Cd and Cu significantly increased after 4 weeks of exposure in the contaminated lake, but no variations in kidney Zn or Ni were observed. At the biochemical level, yellow perch transplanted into the metal-contaminated lake had significantly lower concentrations of liver dehydroretinol and dehydroretinyl esters than did fish from the reference lake, indicating that Cd exposure impairs vitamin A (antioxidant) levels in wild fish. We also documented a significant decrease in the percentage of hepatic free dehydroretinol with increasing renal Cd concentrations, suggesting that the enzymes and the binding proteins

involved in retinoid homeostasis are inhibited by the presence of Cd. The relationships among metal accumulation, biochemical responses of biomarkers of oxidative stress and the transcription levels of stress-responsive genes will be discussed. The combination of molecular and physiological tools and combining laboratory and field studies clearly provides clues to the mechanisms of metal toxicity in wild fish.

174 Detoxification and genetic recovery capacities of Danio rerio after an experimental metal contamination (Cd and Zn)

A. Arini, School of Public Health; P. Gourves, P. Gonzalez, M. Baudrimont, University of Bordeaux 1

Cadmium (Cd), a non-essential metal, is widely distributed in the environment as a result of natural and anthropogenic activities. Because of its low rate of excretion from the body, Cd strongly accumulates in aquatic organisms and can generate severe damages at cellular and individual levels. This study aimed to evaluate the kinetics of metal decontamination in zebrafish (*Danio rerio*), and the reversibility potential of sequestration protein synthesis (metallothionein) and genetic damages after a metal contamination. First, fish were exposed during 15 days to Cd and/or Zn, at environmentally relevant concentrations. Then, fish were maintained in metal-free water for 75 additional days. Throughout the depuration period, genes involved in fight against metal stress and in recovery processes were studied. Bioaccumulation and metallothionein (MT) concentrations were also measured. The main results demonstrated the high Cd accumulation capacity of zebrafish, with concentrations reaching 3716.4 ± 578.6 µg Cd/kg FW in gills after 15 days of contamination. The 75-day decontamination failed to completely eliminate Cd whereas Zn, poorly accumulated, was quickly depurated. The partial Cd depuration in the gills likely resulted from a metal transfer to the liver. MT response was clearly correlated to the Zn contamination, while genetic responses were more pronounced in case of Cd contamination. Cd induced over-expressions of genes involved against oxidative stress (*sod*, *sodmt*), and involved in detoxification mechanisms (*mt1*, *mt2*), mitochondrial mechanisms (*cox1*) and DNA repair (*rad51* and *gadd45*). Zn binary contamination with Cd was demonstrated to provide protective effects on Cd-induced toxicity in *Danio rerio*. Results highlighted that the genetic response was metal- and tissue-dependent. The brain and the muscles showed very few genetic responses, probably due to the low bioaccumulations measured in these tissues and suggesting fast adaptive responses in the brain to limit metallic damages. Conversely, genes expressed in gills and in liver of fish exposed to Cd were strongly affected. However, after 14 to 30 days of depuration, genes were no longer over expressed in response to Cd contamination in gills and liver of fish exposed to Cd and Cd/Zn conditions, suggesting an adaptation of fish to the residual Cd contamination.

175 Regulation of Cytochrome P450 Catalyzed Steroid Biotransformation in Coral-consuming Fish from Hawaii

A. Maldonado, University of California, Riverside / Environmental Science; D. Schlenk, University of California-Riverside / Department of Environmental Sciences; G. Ostrander, Florida State University; M. Slattery, University of Mississippi / Department of Pharmacology; S. Ankisetty, University of Mississippi

Cytochrome P450 monooxygenase (CYP) is the primary enzyme system for detoxification of xenobiotics including dietary chemicals and pollutants. Little is known about the biotransformation and detoxification of allelochemicals derived from dietary products in marine organisms. Certain species of butterflyfish of the genus *Chaetodon* have been shown to feed on several species of chemically-defended corals including the soft corals *Sinularia* spp. 5-episinuleptolide (5ESL) is an allelochemical found at high concentrations in *Sinularia maxima*. This study examined the effects of 5ESL on the expression and catalytic activities of CYP3A and CYP2 in the *Sinularia*-consuming butterflyfish species *Chaetodon unimaculatus*. Fish were gavaged at ecologically-relevant concentrations of 1.0 and 3.0 mg/kg of 5ESL. Testosterone hydroxylase (TOH) 16 β was 16 times higher in fish treated with 5ESL. Testosterone hydroxylase (TOH) 16 β in control animals was 6 times higher than other hard coral feeding species. These results indicate an induction of CYP2 (16-α TOH) and CYP3A (6 β, 16 β TOH) catalytic activities in detoxification of 5ESL in *C. unimaculatus*, which preferentially feed on this coral species in Guam.

176 Molecular and physiological impacts of hypersaline acclimation on the sublethal toxicity of chlorpyrifos to salmonid olfaction

L.A. Maryoung, University of California Riverside / Department of Environmental Sciences; R. Lavado, University of California Riverside / Department of Environmental Sciences; G. Hardiman, R. Sasik, N. Agarwal, University of California, San Diego / Department of Medicine; K.B. Tierney, University of Alberta / Biological Sciences; D. Schlenk, University of California-Riverside / Department of Environmental Sciences

Acclimation to hypersaline environments enhanced the acute toxicity of certain organophosphate pesticides to euryhaline fish species; however sublethal effects have been far less studied. The present study focuses on the sublethal toxicity of chlorpyrifos to salmonid olfaction after hypersaline acclimation with the goal of linking molecular and physiological alterations. To determine molecular effects of combined exposure, coho salmon were acclimated to three different salinities (8ppth, 16ppth, 32ppth) for one week and mRNA was collected from the olfactory rosettes. Micrroarray hybridization was used to determine differences in gene expression for the different salinity treatments. Potential target genes involved in signal transduction, which have been shown to be impacted by chlorpyrifos in zebrafish, were identified and evaluated in coho salmon after saltwater acclimation. These genes included chloride interacellular channel 4, guanylate cyclase activator 1A (retina), guanine nucleotide binding protein (zgc:101761), calcium/calmodulin-dependent protein kinase II delta, and adrenergic alpha 2C receptor. To assess physiological effects, electroolfactograms (EOGs) were conducted on juvenile rainbow trout acclimated to freshwater and hypersalinity (16ppth) with co-exposure to environmentally relevant concentrations of chlorpyrifos (0.5 µg/L and 5.0 µg/L). Exposure to chlorpyrifos following acclimation to hypersalinity significantly decreased the response to L-serine and decreased the response to taurocholic acid additively. Gene expression of the five target genes was examined in olfactory tissue from rainbow trout used in the EOG study. Exposure to hypersalinity and chlorpyrifos up-regulated all five target genes. The combined results from the molecular and physiological sections show that sublethal exposure to chlorpyrifos after hypersaline acclimation impacts salmonid olfaction, which may result from diminished signal transduction (T32 ES018827 and NIEHS P30ES07033).

177 Assessment of the expression levels of genes involved in metal detoxification in killifish inhabiting a closed copper mine Superfund site

D. Jung, Dartmouth Medical School / Physiology, Geisel School of Medicine at Dartmouth / Microbiology and Immunology, Physiology; A. Cravero, R. Lehman, E. Cutting, Mount Desert Island Biological Laboratory; B. Jackson, Dartmouth College / Earth Sciences and Chemistry; B. Stanton, Geisel School of Medicine at Dartmouth / Microbiology and Immunology, Physiology

The Callahan Mine Superfund site in Brooksville, ME is a closed open-pit copper mine and is currently flooded with seawater. The sediments at this site are contaminated with extremely high levels of metals such as cadmium, lead, copper, and zinc. Consequently, killifish (*Fundulus heteroclitus*) inhabiting this Superfund site are constantly exposed to these metals. A survey has shown that killifish collected from the site have significantly higher metal concentrations in tissues compared to fish collected from a nearby reference site. Therefore, we hypothesized that killifish from the Superfund site have elevated expression of genes involved in antioxidant defense induced by metals, and metallothionein (MT), a protein involved in metal detoxification. To test this hypothesis, we collected fish from three different locations within the Superfund site, as well as from a reference site nearby. Gills and livers were isolated for gene expression analysis from twelve individuals at each location. Expression levels of five oxidative stress response genes (nuclear factor erythroid-related factor-2 (Nrf2), glutathione-S-transferase-alpha (GSTα), glutamate cysteine ligase catalytic subunit (GCLc), Mn-superoxide dismutase (SOD2), and catalase) and MT were measured by Q-RT-PCR. In addition, tissues were analyzed for metal content. Tissue samples collected from the Superfund site had significantly high levels of cadmium and copper. Surprisingly, MT mRNA levels were not different in the gills and livers of fish collected from the Superfund site compared to the reference site. In addition, none of the oxidative stress response genes showed significant differences among the collection sites. Therefore, constant exposure to extremely high levels of metals does not seem to affect the expression of genes involved in metal detoxification pathways. Examination of protein expression of these genes will provide more information on whether killifish at the Callahan Mine Superfund site have adapted to high levels of metals in the environment.

178 DNA Damage in Cichlid Fish and Environmental Forensic Analysis of the Origin of PAHs at a Guatemalan Oil Field

C.W. Theodorakis, Southern Illinois University Edwardsville / Department of Biological Sciences and Environmental Sciences Program, Environmental Sciences, Southern Illinois University / Environmental Sciences; J.W. Bickham, Battelle Memorial Institute; K.C. Donnely, Texas A&M University; T. McDonald, Texas A&M University / School of Rural Public Health; P. Wilink, Shedd Aquarium

This study focused on several wetlands in Laguna del Tigre National Park (Guatemala) as part of Conservation International's Rapid Assessment Program. Sediment and water samples were collected from a laguna near Xan field, Guatemala's largest oil facility, and 3 other sites for determination of levels of polycyclic aromatic hydrocarbons (PAHs). Cichlid fish (*Thorichthys meeki* and *Vieja synspila*) were collected for determination of DNA strand breakage (by gel electrophoresis) and chromosomal breakage (flow cytometry). PAHs in water and sediment were extracted with methylene chloride and analyzed by GC/MS. Environmental forensic analysis was also carried out using three metrics to draw inferences about the origin (pyrogenic, petrogenic, diagenic) of the PAHs in the sediment. These metrics included a) concentration (mg/kg) of PAHs with 4 or more rings, b) a "pyrogenic index" defined by as ∑(all other 3-6 ring PAHs)/∑(alkylated [napthalenes, phenanthrenes, dibenzothiophenes, fluorenes, and chrysenes]), and C) ratio of chrysene/phenanthrene in sediments. Multivariate analyses were also used to analyze all three of these variates together. For *T. meeki* from Xan field, chromosomal breakage and strand breakage was greater than in at least two of the three reference sites. For *V. synspila*, chromosomal breakage and strand breakage were greater in Xan than one of the two reference sites. Patterns of aqueous PAH concentrations, suggests that fish are affected by anthropogenic contaminants. PAHs were elevated at some reference sites, but environmental forensic analysis suggested a pyrogenic or diagenic origin. The environmental forensic analysis also suggested that PAHs near the oil well injection site were primarily of petrogenic origin, and the ratio of petrogenic:pyrogenic PAHs decreased with distance from the oil field. The data are consistent with the hypothesis that oil field brines injected into the ground water caused genotoxicity in fish at Xan field, and it is also possible that pyrogenic PAHs influence levels of DNA damage in reference sites. These analyses represent one of the first efforts to examine genotoxicity in native Mesoamerican cichlids.

179 Genome Sequencing of Hyalella azteca: a model for evolutionary toxicology and ecological exposure

H.C. Poynton, Molecular Indicators Research, University of Massachusetts-Boston / School for the Environment; B.J. Blalock, University of Massachusetts-Boston / School for the Environment; J.M. Lazorchak, USEPA / National Exposure Research Laboratory; D.P. Weston, University of California / Department of Integrative Biology; G. Wellborn, University of Oklahoma; M.J. Lydy, Southern Illinois University Carbondale / Fisheries and Illinois Aquaculture Center and Department of Zoology, Southern Illinois University-Carbondale / Department of Zoology

Hyalella azteca is a freshwater epibenthic crustacean of interest to both ecotoxicology and evolutionary biology. It is the primary invertebrate crustacean used in the US for freshwater sediment toxicity testing and has been the subject of recent gene expression studies in ecotoxicogenomics. *H. azteca* is a species complex, which due to its low morphological divergence was originally characterized as a single cosmopolitan species indigenous in lakes, streams and ponds from Latin America to Canada. However evolution of different ecotypes of *Hyalella* has become of recent interest because isolated populations have become genetically distinct over the past 11 million years. Because of its broad interest to complementary fields of ecology *H. azteca* was recently nominated by the i5K Arthropod Sequencing Initiative and is currently undergoing whole genome sequencing. This effort will greatly facilitate the development of genomic tools for environmental assessments as well as broaden its role in evolutionary studies. This presentation will discuss the advantages of *H. azteca* as a model of environmental genomics and discuss recent research using transcriptomics to provide insights into the exposure of emerging contaminants as well as the evolution of pesticide resistance across distinct species groups of *Hyalella*.

180 Complications with the use of the Hyalella azteca species complex in toxicity tests

J. Leung, University of Waterloo; J. Witt, University of Waterloo; W.P. Norwood, Environment Canada / Aquatic Contaminants Research Division; D. Dixon, University of Waterloo

Hyalella azteca, an amphipod crustacean, is frequently used in freshwater toxicity tests involving metals, organics and sediments. Numerous organizations have collected and established cultures of *Hyalella azteca* originating from localities across North America. However, *Hyalella azteca* is actually a large cryptic species complex whose members satisfy both the biological and the phylogenetic species concepts. The potential use of different species in this complex may explain some of the variability among toxicity test results obtained from different laboratories. In this study, two cryptic species were delineated using DNA barcoding. These lineages of *H. azteca* were exposed to two-week-long single metal toxicity tests using copper or nickel. Differences were determined for survival between lineages exposed to copper or nickel. In addition, differences between species were observed in juvenile survival as well as production, despite having been cultured in the same laboratory conditions and water. The results of this study indicate that genetically characterized cultures of *H. azteca* should be identified in toxicity tests.

Evaluation of Veterinary Pharmaceuticals in the Environment

181 Overview of the FDA Environmental Risk Assessment Process for Veterinary Pharmaceuticals

K.M. Beckhorn, US Food & Drug Administration / Center for Veterinary Medicine; W.S. Hunter, US Food and Drug Administration / Center for Veterinary Medicine; E.M. Silberhorn, US Food & Drug Administration / Center for Veterinary Medicine; H. Zahner, US FDA / Center for Veterinary Medicine

The Food and Drug Administration (FDA), Center for Veterinary Medicine (CVM), regulates animal drugs, food additives, animal devices, and medicated feeds. Most actions taken by the agency are governed by the Federal Food, Drug and Cosmetic Act (FFDCA). However, following the National Environmental Policy Act (NEPA), CVM also evaluates the environmental impact of agency actions. Agency actions include requests to conduct investigational research on new animal drugs and the approval of animal drugs for commercial use. NEPA provides tools, such as categorical exclusions and environmental assessments (EA), for evaluating the environmental impact of agency actions. An EA is a document that summarizes the results of a science-based risk analysis and is used to determine whether significant impacts to the quality of the human environment are expected as a result of an action. CVM has published guidances for conducting an environmental impact assessment that were developed in cooperation with regulatory agencies in the European Union, Australia, and Japan. The Phase II Guidance for Industry (GFI #166) recommends a tiered approach to testing that includes an evaluation of the fate, transport, and effects of veterinary drugs in the environment with tests conducted according to OECD guidelines. It describes recommendations for evaluating risk of veterinary drugs used in aquaculture, pasture animals, and intensively-reared (e.g., feedlot) animals. The risk analysis involves comparing the predicted environmental concentration (PEC) to the predicted no effect concentration (PNEC). If a potential environmental risk is identified, additional testing, analyses, or risk mitigation measures may be necessary. After an animal drug is approved, the EA is published on the CVM website and with a Finding of No Significant Impact (FONSI). A brief overview of recent EAs will be presented.

182 Application of higher tier methods for assessing the environmental risks of veterinary parasiticides

A. Boxall, University of York / Environment Department

Veterinary parasiticides tend to exhibit high potency to non-target organisms such as dung organisms and aquatic invertebrates. Consequently an environmental risk cannot usually be excluded for these products using standard environmental risk assessment approaches such as those recommended by VICH. Non-standard higher tier assessment approaches are therefore often needed that better reflect risks in the real environment. However, limited guidance is currently available on how these assessments should be performed and on how the results from higher tier studies should be interpreted. Using the anthelmintic, ivermectin, as a case study, this presentation will illustrate how a range of higher tier testing approaches can be used to assess whether a parasiticide poses a risk to the environment or

not and to identify which environmental compartments are most at risk. The presentation will draw on data from a variety of higher tier studies that have been performed on ivermectin, including: 1) long-term non-standard laboratory studies on aquatic invertebrates; 2) aquatic and terrestrial mesocosm studies; 3) plot-scale fate studies into the persistence and mobility of parasiticides in pasture systems and the farmyard environment; 4) ecological and chemical monitoring of pasture systems; and 5) the use of exposure models and population modelling approaches.

183 Estimation of Veterinary Drug Concentrations In Canadian Soils: Piled Higher and Deeper

G. Rattray, Health Canada / Environmental Impact Initiative Division; A.M. Belknap, S. Kullik, G. Stringer, Health Canada

The *Canadian Environmental Protection Act* (CEPA) 1999 requires that all substances in Canadian commerce be evaluated for their potential risks to the Canadian environment and human health. A regulatory framework has been developed specifically for active pharmaceutical ingredients (APIs) in products regulated by the *Food and Drugs Act* (F&DA) to assess their potential environmental effects. Designed to harmonize with the drug approval process stipulated by the *Food and Drugs Act* (F&DA) and its regulations, the framework assesses the potential impacts of APIs in human pharmaceuticals and veterinary drugs on exposed aquatic and terrestrial organisms in soils, sediments and surface waters. In particular, APIs in veterinary drugs used in food animals primarily enter ecosystems through the application of livestock waste to agricultural land and a means to predict environmental concentrations (PECs) of APIs as part of a directed testing approach is required. Variability in agricultural practices and the complexity of calculating a screening-level predicted environmental concentration of veterinary APIs in soil (PECsoil) can be a challenge for regulatory authorities. A science-based and transparent methodology to calculate PECsoils that incorporates default values representative of typical Canadian confined animal husbandry and agricultural practices has been developed and will be used to identify new medicinal ingredients that may require terrestrial ecotoxicity and fate testing. A comparison to approaches used in the United States and the European Union for generating PECsoil values with a subset of currently used veterinary drugs will be presented and, where possible, compared to measured environmental concentrations. Finally this talk will explore how this model might be utilized to better direct the testing requirements for veterinary APIs within the proposed regulatory framework.

184 Predicting environmental exposure of aquaculture medicines

J.P. Staveley, Exponent, Inc.; R. Endris, Merck Animal Health; G. Scheef, MSD Animal Health Innovation GmbH

Potential risks to the environment from the use of veterinary pharmaceuticals are required to be evaluated in a number of jurisdictions including the European Union, the United States, Japan and Australia. Harmonized guidance (VICH, 2000; VICH, 2004) as well as more recent guidance from the European Medicines Agency (EMEA, 2008) provides a process for conducting these environmental assessments which essentially involves comparing the Predicted Environmental Concentration (PEC) to the Predicted No-Effect Concentration (PNEC) for key receptors. However, available guidance is much more developed for drugs used on terrestrial animals than for aquatic animals. In particular, guidance is limited on approaches for derivation of the PEC for aquaculture drugs. In this presentation, case studies are used to illustrate approaches for a variety of aquaculture systems. In the environmental assessment of the use of Aquaflor® (florfenicol) for freshwater-reared finfish, PECs were derived for pond, flow-through raceway, and recirculating aquaculture systems. In the environmental assessment for the use of SLICE® (emamectin benzoate) for saltwater salmonids, approaches were developed to derive PECs for net pen aquaculture systems. PECs for water, soil, and sediment matrices were derived, as relevant for the particular exposure scenarios. The assumptions and calculations used in these case studies to derive the initial PECs for Tier A (acute) and Tier B (chronic) exposures, as applicable, are discussed. In most instances, a typical case as well as a worst-case scenario was used. The PECs were then compared to the PNECs to determine the risk quotients, and refined if necessary using additional information. The use of monitoring data as well modeling to estimate environmental exposures is also discussed.

185 An Environmental Impact Assessment (EIA) of Derquantel (DER) a New Parasiticide for Treatment of Drug Resistant Gastrointestinal Nematodes of Sheep

W.J. Smolenski, Pfizer Animal Health / Metabolism & Safety; R. Greening, Zoetis Animal Health; J. Mackie, Regulatory Compliance Ltd

DER is one of the active components of Startect® Oral Drench, and is a member of a new class of parasiticides (spiroindoles) for treatment of drug-resistant gastrointestinal nematodes of sheep. DER is a nicotinic cholinergic receptor antagonist in both nematodes and mammals. Startect is a combination product which also contains abamectin. This presentation will focus on the EIA for DER only. EIAs for Startect® have been approved in major sheep-producing countries (Australia, New Zealand, South Africa and UK). DER is extensively metabolized in sheep with 0.6% and 5.8% of the dose excreted as DER in urine and feces, respectively. All metabolites were < 10% of the original dose, therefore the EIA was performed on the parent molecule. The initial predicted environmental concentration in soil (PEC_{SOIL}) was estimated to be 9.6 and 12.8 µg/kg soil for lambs and ewes, respectively. Although these values were less than the VICH Phase II threshold, parasiticides automatically require a Phase II EIA. In soil, DER was shown to be immobile and degraded quickly in each of the soils used. A number of terrestrial ecotoxicological studies were conducted with DER to potentially identify organisms at risk. These included earthworms, dung flies and dung beetles. Toxicity studies were also conducted for aquatic organisms; *Daphnia*, algae and fish. Toxicity endpoints and safety assessment factors were combined to determine predicted no-effect concentrations (PNEC). The risk to terrestrial and aquatic organisms was assessed by comparing PNEC to PECs in soil, manure and water. It was established that DER did not present a significant risk to terrestrial and aquatic organisms. From this it was determined that no environmental risk from the use of DER in sheep was identified. The combined effects of DER and abamectin on dung organisms were also evaluated, and it was established that the combination product, Startect® (DER and abamectin), posed no greater environmental risk than that from abamectin alone.

186 Novel Transformations of Trenbolone Acetate Metabolites Suggest Incomplete Environmental Risk Assessment for Trenbolone

E.P. Kolodziej, University of Nevada-Reno / Civil and Environmental Engineering; D.M. Cwiertny, University of Iowa / Department of Civil and Environmental Engineering; S. Qu, UI Civil and Environmental Engineering Department

In general, the existing regulatory and risk assessment paradigm for veterinary pharmaceuticals and other potential environmental contaminants is relatively simplistic as it equates observations of contaminant degradation with significant reduction in associated ecological risk. However, it is becoming clear that there exist a number of environmental contaminants whose behaviors in the environment confound this assessment paradigm and whose environmental risk cannot be accurately assessed by laboratory studies demonstrating degradation or attenuation of compound concentrations in model environmental systems. For example, trenbolone acetate (TBA) is an androgenic growth promoting steroid used widely in animal agriculture in the United States, with the vast majority of US beef cattle receiving TBA implants. Despite their significant economic value (~ $1 billion annually), TBA metabolites can be potent endocrine disrupting compounds for sensitive species of aquatic organisms, capable of endocrine disruption at low ng/L concentrations. TBA metabolites are often considered rather reactive and prone to degradation, and risk assessment studies specifically point to their rapid degradation as evidence for limited ecological risks. However, we have recently demonstrated a most unexpected observation for TBA metabolite fate in environmental systems: namely that product to parent reversion is possible for TBA metabolites. Also, a variety of structural analogs and stereoisomers can arise from environmental transformation processes of TBA metabolites, yielding a range of uncharacterized steroid structures potentially capable of receptor interactions. None of these possibilities are accounted for in current risk assessment approaches for trenbolone or any other veterinary pharmaceutical. Concurrent analytical approaches also suggest that novel, uncharacterized steroids derived from TBA metabolites are present in agricultural runoff. These observations confound most all current environmental risk assessment and contaminant fate models, and therefore improving our approach to environmental risk assessment needs to specifically account for these possibilities. The implications of this data suggest that improved environmental risk assessment should include a more complete characterization of transformation products and identification of possible non-target receptor interactions as part of exposure assessment process.

187 The effects antimicrobial compounds in grass shrimp Palaemonetes pugio and the development of antibiotic resistant bacteria

M.E. DeLorenzo, NOAA / NOS/NCCOS / CCEHBR; M. Kelly, College of Charleston / Grice Marine Laboratory; K.W. Chung, National Ocean Service / NOS / NCCOS / CCEHBR; P.B. Key, J. Moore, M.H. Fulton, NOAA / NOS / NCCOS / CCEHBR

Antimicrobial compounds may enter coastal ecosystems through wastewater, agricultural runoff, and aquaculture effluents. The environmental risks posed by antimicrobials to the marine ecosystem are not well understood, and few studies have addressed effects on marine crustaceans. This study characterized effects of antimicrobial compounds common to human and veterinary medicine, aquaculture, and consumer personal care products (erythromycin, sulfamethoxazole, oxytetracycline, and triclosan) in the grass shrimp *Palaemonetes pugio*. Effects on grass shrimp survival were assessed after 96h exposures and samples were preserved for sublethal biomarker analysis. Effects of antimicrobial exposure on bacterial density (*E. coli* and *Vibrio* spp.) in the surviving grass shrimp was determined by spread plating tissue homogenates and counting bacterial colonies after incubation. The development of multiple antibiotic resistance in the grass shrimp bacterial community was then determined by measuring growth of bacterial isolates in 12 different antibiotics. The antimicrobial exposures were not found to affect grass shrimp survival, while sublethal biomarker effects were observed. Antimicrobial exposures were found to decrease grass shrimp bacterial densities compared to controls. There were also significant changes in the overall percentage of antibiotic resistant isolates in shrimp bacteria, and changes in bacterial resistance to specific antibiotics. This research provides data on the risks of antibiotics in the coastal environment to environmental and human health.

188 Aquatic fate and effects of erythromycin in laboratory studies

J.R. Coats, Iowa State University / Department of Entomology

Antibiotics in surface water and sediment systems are of concern due to the potential adverse effects as a result of their environmental fate. The fate of 14C-erythromycin was investigated in microcosms containing pond water and submerged pond sediment. The bioavailability of 14C-erythromycin was also evaluated in the microcosms, with and without the addition of pond sediment using C8 Empore extraction disks and the aquatic oligochaete *Lumbriculus variegatus*, for comparison. The comparisons of relative uptake indicated a steady-state bioconcentration factor of 2.2 for *L. variegatus* compared to 18 for the disks in the aquatic system, which were reached between Day 3 and Day 7. The disk "biocontration factor" was 8.2 times the worm bioconcentration factor, without normalization to lipid and carbon content. Daphnids showed the highest erythromycin BCF at Day 3, followed by a decrease in tissue contentration of the compound through Day 14. Toxicity testing was also conducted for erythromycin and two other veterinary antibiotics, tylosin and sulfamethazine. Erythromycin was slightly toxic to the aquatic invertebrates tested. The median effects concentrations (LD/EC50s) were also used to calculate toxic units as a way to characterize chemical interactions in mixtures of two or three of the antibioitcs. Using this approach we found that a less-than-additive (antagonistic) effect was indicated by the acute and chronic tests for survival for all species exposed to every combination of antibiotics. Reproduction studies in *D. magna* the summed toxicity units resulted in a total TU of less than 1. All combinations were either additive or antagonistic.

Harmful Algal Bloom Toxins in Inland Waters: Environmental Contaminants of Emerging Concern

189 Risk assessment of Microcystis aeruginosa from the San Francisco Estuary on threadfin shad, Dorosoma petenense

S. Acuña, Aquatic Health Program, School of Veterinary Medicine; D.A. Deng, Oceanic Institute / Aquatic Feeds and Nutrition Department; D. Baxa, University of California, Davis / Aquatic Health Program, School of Veterinary Medicine; P. Lehman, California Department of Water Resources / Environmental Services Office; S. Teh, University of California-Davis

Blooms of toxin-producing *Microcystis aeroginosa* have been detected in the San Francisco Estuary (SFE) since 1999. The bloom represents a threat to wildlife, the ecosystem, and the millions of people of California that depend on the SFE. The cyanobacterium produces the hepatoxin microcystin (MC), including MC-LR, LA, and RR forms. Concurrently, in the SFE significant declines in pelagic fish recognized as the Pelagic Organism Decline (POD) have been recognized by state and federal agencies since 2000. The presence of the toxic algal bloom, *M. aeruginosa* has been suggested as a link to the POD. As one of the POD species, threadfin shad (TFS), *Dorosoma petenense*, were collected from the SFE to ascertain a link between the presence of *M. aeruginosa* and biomarkers of exposure. TFS were collected in four sites: 1) two sites were low *M. aeruginosa*, Sherman Island (SI) and Stockton (STK) and 2) two sites with high *M. aeruginosa*, Brannon Island (BI) and Mildred Island (MI). The samples were examined morphologically, biochemically, and histologically. For TFS from BI and MI, *M. aeruginosa* was localised in the gut cavity. MCs were localized in the tissues of the gut and liver, and lesions increased in prevalence and severity compared to SI and STK. Liver damage characteristic of MC exposure was detected in the TFS from BI and MI with the presence of vacuolar degeneration, necrosis and sinusoidal congestion. The TFS from BI and MI exhibited significant reductions in condition and hepatosomatic indices suggesting the health of TFS from these sites were negatively impacted compared with SI and STK. The results indicate that sites with high *M. aeruginosa* exhibited toxic effects characteristic of MC exposure suggesting that the bloom may significantly impair the healt of threadinfin shad in the SFE.

190 Does Risk Assessment of Harmful Algal Blooms need to consider combined and interactive effects with pesticides (and vice versa)?

J. Asselman, Ghent University / Laboratory of Environmental Toxicology; M. Pfrender, Notre Dame University; J. Lopez, Notre Dame University; J. Shaw, Indiana University; W. Waegeman, University of Ghent; B. De Baets, Ghent University; K. De Schamphelaere, Ghent University / Environmental Toxicology and Aquatic Ecology

The widespread occurrence of toxic cyanobacterial blooms, associated with the production of a wide range of toxins, is an increasingly important concern in aquatic ecosystems. Furthermore, the frequency of occurrence and intensity of these blooms is projected to increase under climate change conditions. In addition, aquatic ecosystems may also be polluted with plant protection products, due to their widespread use in agriculture. Thus, in agricultural areas *Daphnia* may be confronted with a mixture of PPPs and cyanobacterial blooms. Such mixtures can result in combined adverse effects which may either be additive, antagonistic (less than additive effects) or synergistic (more than additive effects). The aim of our study was therefore to investigate the toxicity of binary mixtures of carbaryl and four species of cyanobacteria to gain more insight into the occurrence of interactive effects on *Daphnia pulex* at both life history level and molecular level. Here, we exposed *Daphnia* for 21 days to *Aphanizomenon* sp., *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa, Oscillatoria* sp and carbaryl and all binary combinations of carbaryl and each individual cyanobacterium. The estimated EC50 (median effect concentration) for carbaryl was comparable between the experiments, ranging from 2.28 to 5.94 µg/L. EC50 for cyanobacteria ranged from 13.45% of the total diet ratio for *Microcystis* to 66.69% of the diet ratio for *Oscillatoria*. In combination with carbaryl, the response of *Daphnia* to three out of the four cyanobacteria demonstrated antagonistic deviation patterns ($p < 0.05$). Exposure to combinations of carbaryl and *Cylindrospermopsis* did not result in statistically significant deviations. Next, we exposed four day old *Daphnia* for ten days to one carbaryl treatment, one cyanobacteria treatment and one binary mixture combination of carbaryl and the respective cyanobacteria combination. RNA was extracted for gene expression analysis with cDNA microarrays. Hence, we can identify pathways and gene families involved in the underlying mechanisms of both the individual and combined effects of carbaryl and four selected cyanobacteria. Such mechanistic information may enhance our knowledge of mixture toxicity and its potential occurrence in the environment. In addition, it can also be used as supportive information in risk assessment to identify those combinations that are of particular concern for the aquatic ecosystem and the environment in general.

191 Influence of pH on Prymnesium parvum bloom development and toxicity in inland waters

W.C. Scott, Baylor University / Department of Environmental Science; K.N. Prosser, Baylor University; T.W. Valenti, Syngenta Crop Protection LLC / National Research Council / Environmental Safety; B.W. Brooks, Baylor University / Department of Environmental Science

Since first identified in Texas inland waters in 1985, *Prymnesium parvum* has produced devastating harmful algal blooms (HABs) responsible for significant damage to water resources. Traditionally, *P. parvum* HABs

primarily occurred in coastal systems, but are increasingly observed in inland waters and have now been documented in 19 States. Increased frequency of droughts coupled with human population growth and thus a growing need for water sequestration over the past decade in Texas has led to inflow reductions to reservoirs, which appears correlated with increases in salinity and pH variability of impoundments. Our research group has examined abiotic factors, including salinity, light intensity, inflows, temperature and nutrient enrichment, and biotic factors governing *P. parvum* bloom initiation, termination and ambient toxicity. Here we present findings from laboratory, field and in situ mesocosm studies examining the influence of site-specific pH on *P. parvum* toxicity and bloom development. We further examined the aquatic toxicity of fatty acid amides, recently reported by NOAA scientists as causative toxins produced by *P. parvum*, using *Daphnia magna* survival and reproduction and *Pimephales promelas* survival and growth. Such observations appear particularly relevant to regions affected by climatic changes, instream flow alterations and salinization of inland water from natural or antropogenic (e.g., natural resource extraction) activities.

192 Microcystin-LR in Minnesota: Human Health-Based Drinking Water Guidance Values

J. Dady, H. Goeden, C. Greene, MN Dept. of Health

In recent years, the Minnesota Department of Health (MDH) has responded to citizen concerns regarding algal blooms in various eutrophic lakes. Concerns regarding drinking water contamination from eutrophic lakes are two-fold: (1) potential drinking water contamination for municipalities that derive source water from eutrophic surface waters and (2) potential for migration into groundwater which could contaminate near-shore wells. Microcystin-LR (MC-LR) was nominated to MDH's Drinking Water Contaminant of Emerging Concern Program (CEC), a program funded by Minnesota's Clean Water Fund established under a constitutional amendment passed by Minnesota voters in 2008. MC-LR ranked as a high priority chemical for review and derivation of guidance based on toxicity and exposure parameters. Using MDH's risk assessment methods promulgated in 2009, which includes use of age-adjusted drinking water intake levels, MDH developed and evaluated values for short-term, subchronic, and chronic exposure durations. MDH recommends health-based drinking water guidance of 0.04 µg/L for MC-LR, a value that is protective of all three exposure durations. Three emerging concerns were identified: (1) the ELISA screening test method commonly used does not have congener specificity or sufficient sensitivity relative to the new HBV; (2) the predominant health endpoint is liver toxicity; however, a new study raised some concern about potential male reproductive toxicity; and (3) MC-LR is presumed to be the most common and/or toxic congener; however, recent mechanistic receptor uptake studies have shown much greater hepatocyte toxicity *in vitro* for MC-LF and MC-LW. Congener-specific analytical methods are available with sufficient sensitivity; however, to resolve emerging concerns regarding reproductive toxicity and comparative congener toxicity, further research is needed.

193 Harmful Algal Blooms in Wisconsin: Results of a Statewide Public Health Surveillance Program

D.A. Perkins, C. Hedman, University of Wisconsin – Madison / Wisconsin State Laboratory of Hygiene; G. LaLiberte, Wisconsin Department of Natural Resources; E. Wollenburg, M. Werner, Wisconsin Department of Health Services / Wisconsin Division of Public Health

The presence of freshwater harmful algal blooms (HABs) and the toxins they may produce are of increasing concern in recreational and drinking waters due to their potential public health risk. The Wisconsin Department of Health Services, the Wisconsin Department of Natural Resources and the Wisconsin State Laboratory of Hygiene conducted a multi-year surveillance study to measure the public health burden of HABs and to better characterize environmental conditions during these blooms. Between 2009 and 2012, the Wisconsin Department of Health Services received 133 complaints of human and animal illness associated with exposure to HABs. The most common HAB-related illnesses reported were gastrointestinal distress, dermal rash, respiratory irritation and cold-like symptoms. Cyanotoxins were detected in over 51% (22 of 43) of the environmental samples analyzed, with microcystin-LR, -LA and -RR the most common toxins in Wisconsin surface waters. Air monitoring along lake shorelines also indicated elevated levels of ammonia and hydrogen sulfide, which may contribute to respiratory symptoms reported by lakeshore residents. In addition to collecting health and water quality data, this surveillance program also expanded outreach to veterinarians, local lakes associations and the general public in order to: 1. increase reporting of HAB-related illnesses and 2. increase awareness of the potential hazard associated with algal blooms. The information gathered in this surveillance study will enhance our understanding of the public health burden of HABs and facilitate the development of prevention measures aimed at reducing exposures to harmful algal blooms.

194 Application of passive sampling for assessment of microcystin contamination and removal efficiency in drinking water treatment plants

P. Babica, L. Jasa, J. Sadilek, Institute of Botany, ASCR / Department of experimental phycology and ecotoxicology; J. Kohoutek, RECETOX – Research Centre for Toxic Compounds in the Environment, Masaryk University; L. Strakova, Institute of Botany, ASCR / Department of experimental phycology and ecotoxicology; B. Marsalek, Institute of Botany, ASCR / Department of experimental phycology and ecotoxicology

Occurrence of toxic cyanobacteria in drinking water reservoirs may represent serious human health hazard, hence effective tools for assessment and management of health risks related to cyanobacteria contamination of drinking water supplies are required. Passive sampling represents interesting alternative to traditional grab sampling techniques, since it allows low-cost but sensitive time-integrated evaluation of contamination, even at ultratrace concentration levels. This perspective approach could be effectively utilized also for purposes of scientific and regulatory monitoring of cyanotoxins. In our study, we used an optimized and calibrated time-integrative passive sampler (POCIS-based) for monitoring of cyanotoxins microcystins (MCs) in three selected drinking water reservoirs and adjacent treatment plants in the Czech Republic. Alongside traditional grab sampling, passive samplers were exposed in reservoirs and different steps of water treatment and replaced in two-week intervals during Jun-Oct 2011 or 2012. Deployed passive samplers well-sequestered MCs and the amounts of accummulated MCs reflected both seasonal dynamic and spatial variations of MC concentrations determined by grab sampling. For the employed experimental setup, minimal detectable time-weighted average (TWA) concentrations of MCs were at pg/L level. MCs were found using this sensitive method in all samples of final treated drinking water, with TWA concentrations ranging from 0.003 to 0.02 µg/L. Such concentrations are orders of magnitude below WHO guideline value for MC-LR in drinking water (1 µg/L), thus most likely did not present any human health risks. Passive sampling provided excellent sensitivity without a need of time consuming and laborious sample processing and showed good correlation with data obtained by grab sampling. Passive sampling was demonstrated to be a valuable tool for studies of MC environmental occurrence and contamination, for drinking water quality control, evaluation of MC removal by drinking water treatment technologies, and human health risk assessment. Supported by the SoMoPro project no. 2SGA2858 (funded from the EC within the 7th Framework Programme FP/2007-2013 under Grant Agreement No. 229603 and co-financed by the South Moravian Region), by project funded by Thomas Bata Foundation granted to Babica in 2012, and by CETOCOEN project from the European Regional Development Fund (Z.1.05/2.1.00/01.0001).

195 Development and application of sonication on the removal of harmful cyanobacteria

S. Baik, C. Park, KIST Europe; J. Kim, KIST Europe / Institute of Environmental Sciences; Y. Jung; J. Choi, Korea Institute of Science and Technology; S. Lee, Korea Institute of Science and Technology / Water Research Center; S. Kim, KIST Europe / Chemical Management Lab

Blue-green algae, known as cyanobacteria, are of increasing concern due to their potential impact on drinking and recreational waters as well as toxicological effects on aquatic life and human. Lab-scale sonication with 20 kHz of its frequency was conducted to investigate on the removal of cyanobacteria. *Microcystis aeruginosa, Microcystis* sp., *Anabaena flos-aquae, Anabaena* sp. were target algal strains for their removal efficiencies by sonication. Diverse ultrasonic densities, from 0.15 to 1.5 W/ml, were applied and it was shown that exposure times had inverse relationship with ultrasonic densities regarding reduction rate of target algal strains. Microcystin and anatoxin-a are major toxins released from target algal strains, and application of sonication initially led to release those toxins as increasing in levels. However, as increasing exposure time to sonication at high densities, levels of microcystin and anatoxin-a were reduced significantly. Both batch and continuous stirred-tank reactors were tested as simulators for lakes and

rivers, respectively. All toxins from target algal strains were quantitatively and qualitatively analyzed by HPLC-MS. This work was supported by the KIST Institutional Program (Project No. 2E24280)

196 Seasonal Analysis of Microcystin degradation by Biological Treatment Facility at a Water Purification Plant in Japan

K. Shimizu, Toyo University; M. Utsumi, H. Akasako, J. Li, University of Tsukuba; H. Maseda, The University of Tokushima; N. Sugiura, University of Tsukuba

In recent years, various drinking water reservoirs suffer from the pressure of cyanotoxins, such as microcystin (MC). Moreover, it has been predicted that the global warming around the world are likely to increase both occurrence and intensity of toxic cyanobacterial blooms. Toxic cyanobacterial blooms consisting of belonging to the genera *Microcystis*, *Anabaena*, *Planktothrix*, and *Nostoc* have occurred in lakes and reservoirs. Those cyanobacteria produce some kinds of microcystins (MCs) which are potent hepatotoxic compounds with cyclic haptapeptide, resulting in the death of many kinds of wild and domestic animals and human, such as serious affair in Caruaru, Brazil in 1996. MCs inhibit protein serine/threonine phosphatases 1 and 2A leading to tumor promoting activity. As a result of these concern about toxic effect by MCs, the World Health Organization regulate a guideline value of 1.0 mg/L for MC-LR, most known MC. MCs are chemically stable in water body and have been documented to be recalcitrant to conventional water treatment processes. Biodegradation is one of the effective methods to remove dissolved organic matter and MCs. Previous studies have shown that biofilm attached plastic carrier wihin biological treatment facility (BTF) degrade MCs due to MC-degrading bacteria on the biofilm, possessing *mlrA* gene. However, *in situ* analysis of the relationship between MCs concentration and population dynamics of MC-degrading bacteria in BTF is still unclear. The purpose of the present study was to reveal the population dynamics of MC-degrading bacteria using qPCR analysis targeted for *mlrA* gene on the biofilm attached plastic carrier within BTF at a water purification plant in Japan. The field sampling at the water purification plant has been performed monthly from August 2010 to October 2011. The biofilm attached to honeycomb tube carrier were sampled at three points in BTF. Water samples were also collected from the same points. HPLC analysis indicated that MCs were removed effectively through BTF treatment process. QPCR assays revealed that MC-degrading bacteria existed in the all three points biofilms throughout the period, and the maximum value of *mlrA* gene copy number was quantified on September 2010 when MCs were completely removed after BTF treatment. We revealed *mlrA* gene copy number is closely related to MCs degradation. Therefore, *mlrA* gene copy number is monitoring parameter for MCs degradation by BTF.

Amphibian and Reptile Ecotoxicology: Progress and Challenges in Understanding Chemical Effects, Exposure, and Risk

197 Comparison of Single Concentration Interaction experiments to Concentration Curves using isobole diagrams for Determining the Toxicity of Mixtures

J.R. Rayburn, Jacksonville State University / Department of Biology

Determining the toxicity of mixtures has been of interest for toxicity testing due to the difficulty of interpreting results and predicting effects of mixtures. Two methods are used, first single concentrations testing effects at single concentrations and measuring the increase or decreases of effects such as mortality or malformation. These methods often assume a response addition for effects. The second involves generation of a response curve for chemicals alone and in binary mixtures to determine differences in LC50 values. Many of these assessments admit to limitations with respect to predictions of mixture interactions that include antagonism and synergism. The purpose of this presentation is to compare single point estimates of interactions with that of isobole diagrams that indicate antagonism, synergism and response addition. This presentation explores what we can learn from these experiments for predicting developmental toxicity. Data from frog and shrimp embryo assays that assess the developmental toxicity of these chemicals and mixtures of the chemicals will be used. The data used are from the Frog Embryo Teratogenesis assay-Xenopus and the Shrimp Embryo Teratogenesis Assay-Pugio. Both assays expose embryos at early stages to free living larvae all collected from a single laboraoty0. These chemical interactions will include pharmaceuticals (Caffeine and Pseudoephedrine), environmental complex mixtures (oil and oil dispersants), and chemicals of concern in food chemistry (Acrylamide, Furan, L-cysteine). The Caffeine and Pseudoephedrine demonstrate concentration addition using the frog embryo assay. The oil and oil dispersants demonstrate synergism using the shrimp embryo assay. L-cysteine shows antagonisms with acrylamide and response addition with furan using the frog embryo assay. These results lead us to a real world setting of interpretation of results and a better understanding of when it is best to choose a single concentration comparison or isobole diagram comparisons.

198 Developmental Toxicity in Xenopus leavis embryos Exposed to Pairwise Mixtures of Acrylamide, Furan, and L-Cysteine

R. Williams, Jacksonville State Universy; J.R. Rayburn, Jacksonville State University / Department of Biology

Acrylamide and furan are of interest in toxicological studies due to their presences in common foods. L-cysteine has been shown to inhibit toxicity in acrylamide due to it being a glutathione precursor. Acrylamide is produced in starchy foods as a Maillard Reaction product, and industrially for producing polyacrylamides. Furan has been found in foods as the result of degradation of polyunsaturated fatty acids and ascorbic acid derivatives. L-cysteine is found in many protein rich foods, and is produced in most animals as a semi-essential amino acid. This study determines the degree of protective effects of L-cysteine on acrylamide and furan toxicity as well as investigating interactions between acrylamide and furan, by looking at changes to the 96hr-LC50 (mortality), 96hr-EC50 (malformation), and Teratogenic Index (TI), using the Frog Embryo Teratogenesis Assay – Xenopus (FETAX) protocol for individual and pairwise mixtures of the chemicals. Toxic Unit analysis will be used to analyze for synergistic, antagonistic, or additive responses for each chemical pair. The toxic unit ratios of 0:1, 1:1, 1:3, 19:1, and 1:0 acrylamide to L-cysteine were chosen, with 19:1 chosen as the upper limit due to being the equimolar concentrations. For furan to the paired compound (acrylamide or L-cysteine) 0:1, 1:1, and 1:0 toxic unit ratios were used for both acrylamide and L-cysteine. Xenopus eggs, at the small cell blastula stage, were exposed to controls, toxicants, and mixtures with20 embryos per dish in 8mL of test solution. At the end of the 24, 48, and 72hr intervals, the solutions were renewed with freshly made solutions. At the end of 96hr, mortality, malformation and embryo lengths were recorded. The 96hr-LC50 and 96hr-EC50 were calculated using Systat 13, probit analysis, and Teratogenic Index (TI=96hr-LC50/96hr-EC50) was then determined. Lowest observable effects for mortality, malformation, and length were determined using ANOVA and Bonferroni's multiple comparison test. This series of tests demonstrated that L-cysteine inhibits the toxicity of acrylamide at high ratios of toxicant to inhibitor, 19 Acrylamide: 1 L-cysteine, while furan had additive effects with both compounds. This work demonstrates the effectiveness of using FETAX protocols in conjunction with Toxic Unit Analysis for determining the toxicological interactions of compounds in mixtures.

199 Challenges associated with directly applying laboratory ecotoxicology studies to natural settings: an example with two amphibian species

S.L. Lance, University of Georgia / Savannah River Ecology Laboratory; R.W. Flynn, Savannah River Ecology Laboratory; D. Soteropoulos, D. Scott, University of Georgia / Savannah River Ecology Laboratory

Copper (Cu) is a widespread contaminant that can be toxic at concentrations just above the normal physiological range. Copper causes lethal and sub-lethal effects in amphibians, which are experiencing global declines. An artificial wetland system (H-02) was constructed on the Savannah River Site to treat process and storm water discharge that has elevated levels of Cu and zinc. Constructed wetlands can provide new habitat for local wildlife, but if poor water quality limits recruitment, these wetlands could become population sinks. The H-02 system includes a retention pond and two treatment cells. Copper levels in the retention pond and the influent end of the treatment cells range from 30 to 60 µg/L, while in the effluent ends it is below 10 µg/L. We conducted field experiments to evaluate the ability of southern leopard frogs and southern toads to develop and metamorphose along the concentration gradient of Cu. We constructed floating enclosures with screened tops and bottoms and reared tadpoles individually. For leopard frogs we reared tadpoles in six locations: both ends of the retention pond, influent ends of both treatment cells, and the effluent ends of both treatment cells. We used 6 clutches of eggs and reared 12 tadpoles per clutch per location. For southern toads we used one location within the retention pond, resulting in a 5 location x 6 clutch x 12 replicate factorial. Survival

and size at metamorphosis for leopard frogs was significantly lower in the retention pond. In addition, the time to metamorphosis was significantly longer in the retention pond and influent ends. These results agree with laboratory studies in which southern leopard frogs experienced reduced survival and increased larval periods at levels of Cu >100 µg/L. Southern toad larvae, on the other hand, survived to metamorphosis equally well at all locations, and had the shortest larval period and largest size at metamorphosis at the influent end locations. We conducted physiological tests on the toads that metamorphosed, and those from the influent end displayed better endurance in hopping trials. These results are in direct contrast to laboratory studies in which southern toads had 100% mortality at Cu concentrations above 15 µg/L. We will discuss our findings and their implications for amphibian ecotoxicology studies.

200 In vitro multi-endpoint assessment of common marine contaminant toxicology in sea turtles using C. caretta primary skin fibroblast cell cultures

S.J. Webb, Texas Tech University / The Institute of Environmental and Human Health; B.M. Higgins, NOAA/NMFS; M.J. Hooper, USGS Columbia Environmental Research Center; C.A. Godard, Texas Tech University / The Institute of Environmental and Human Health

The seven species of sea turtle are currently listed as either threatened or endangered under the United States Endangered Species Act or the Convention of International Trade of Endangered Species. A potential contributing factor in the decline of sea turtle populations is the impact of chemical contaminants on sea turtle health. Sea turtles are in contact with a variety of chemical contaminants in the marine environment, such as components of crude oil and oil-related contaminants, polychlorinated biphenyls (PCBs), perfluorinated compounds (PFCs), and heavy metals. When working with endangered species, methods that are minimally invasive and non-lethal are essential. In this study, an in vitro approach employed primary skin fibroblast cell cultures derived from loggerhead sea turtles to assess toxicity potential of marine environmental contaminants. Cultured cells were exposed to one of several common marine contaminants, including benzo(a) pyrene (B(a)P) (0.01 µM, 0.1 µM,, 1.0 µM, 10 µM, 100 µM), polychlorinated biphenyl (PCB) 77 (0.01 µM, 0.1 µM,, 1.0 µM, 10 µM), and PCB 126 (0.01 µM, 0.1 µM, 1.0 µM, 10 µM) at four time points: 24 hr, 48 hr, 72 hr, and 96 hr. These environmentally relevant dosing concentrations were determined based upon concentrations of chemicals reported in seawater, prey items, and sea turtle blood. Cell health and toxicity assays included 1) cell death assays using tetrazolium dye reduction (MTT) and lactate dehydrogenase (LDH) leakage from cells, 2) sublethal toxic effects on cell cycling, and 3) enzymatic activity and expression levels of cytochrome P450 with the biomarker alkyloxyresorufin-O-dealkylase (AROD) and real-time quantitative PCR (qPCR). Preliminary MTT and LDH data showed no significant cell death following exposure to either B(a)P or PCBs. Induction of cytochrome P450 1A5 was confirmed via qPCR following B(a)P exposure at 72 hours but not detected following 72 hours of exposure to PCB77. Cell cycle analysis and AROD assays are being optimized for these cell cultures. Demonstrating quantitative contaminant-tissue interactions provides relevance to contaminant concentrations measured in the tissues of these endangered species.

201 Quantifying amphibian pesticide body burdens for active ingredients versus formulations through dermal exposure

R. Van Meter, Environmental Protection Agency; D. Glinski; T. Purucker, USEPA

Widespread pesticide applications throughout agricultural landscapes pose a risk to post-metamorphic amphibians leaving or moving between breeding ponds in terrestrial habitats. Recent studies indicate that the inactive ingredients in pesticide formulations may be equally or more toxic to amphibians than the active ingredient itself. While pesticide active ingredients must be listed on the labeled formulation product by law, disclosure of inactive ingredients is not required. In an effort to indentify the total accumulation of chemicals associated with real world pesticide applications, our study was designed to compare pesticide body burdens (ppm) for 6 active ingredients and associated pesticide formulations through dermal exposure in terrestrial phase amphibians. In spring 2013, 5 southern leopard frog (*Rana sphenocephala*) egg masses, 5 Fowler's toad (*Bufo fowleri*) egg masses and 3 mating pairs of gray treefrogs (*Hyla versicolor*) were collected from ephemeral pools in University of Georgia's Whitehall Forest. Amphibians were reared through 30 days post-metamorphosis at the US Environmental Protection Agency (USEPA) in Athens, GA. All experiments were conducted in 10-gallon glass aquariums lined with Plott series soil in the laboratory at the USEPA. The pesticide active ingredients and associated formulations tested were atrazine and Hi Yield, imidacloprid and Dominion, triadimefon and Triadimefon 125EC, fipronil and Taurus SC, pendimethalin and Prowl, and glyphosate and Roundup. Amphibians were exposed to test compounds indirectly through contact with pesticide contaminated soil for 8 hours. Pesticide body burdens were analyzed using LC-MS after whole body tissue extractions using the QuEChERS method with slight modifications. Preliminary results indicate that formulated pesticide products lead to higher amphibian body burdens as compared to active ingredient only. Given that formulations are sprayed in agricultural fields as opposed to single pesticide active ingredients, this may contribute to increased total chemical exposures and bioaccumulation by amphibians moving throughout contaminated terrestrial habitats. To improve conservation efforts of amphibians worldwide, future research efforts exploring dermal exposure and accumulation of pesticides and their associated carrier compounds by this group of non-target organisms in terrestrial settings is imperative.

202 Developing Toxicity Testing Designs for Herps Applicable for Risk Assessment: developing soil levels for terrestrial salamanders

M.S. Johnson, US Army Ctr for Health Promotion & Preventive Med., US Army, Public Health Command / Health Effects Research Program, US Army Institute of Public Health / Health Effects Research Program / Toxicology Portfolio

Ecological risk assessments for substances in soil have largely ignored reptiles and amphibians founded on the logic that few data exist for this purpose. Although limited toxicity data for reptile and amphibian species do exist in the literature, the data are largely not collected for this purpose and are often inappropriate for extrapolation in the derivation of toxicity reference values (TRVs) or for media-specific values (e.g., soil screening values) for risk assessment purposes. Several study designs will be presented that are intended to provide data for TRV or media-specific risk assessments. Examples include an objective analysis of benefits and short comings for each, providing the basis for guidance intended to serve as a foundation from which to develop data that could be more applicable for the purpose of making decisions at multiple hazardous waste sites.

203 The Influence of Industry: How Conflicts of Interest Compromise Pesticide Regulation

M.D. Boone, Miami University / Department of Biology

Decisions for pesticide registration need to be based on the best available data to benefit regulators, the public, and natural world. However, current assessments for registering or reregistration pesticides can eliminate much to most of the research in the published, peer-reviewed literature allowing regulatory decisions to be based on only a few studies that are often conducted or directly funded by the pesticide manufacturer who is unambiguously impacted by the conclusions reached in these studies, thereby representing a clear case of conflict of interest. Furthermore, although scientific advisory panels are consulted by regulatory agencies, there is no clear requirement to heed the panel's advice. Our objectives here are to 1) highlight how conflicts of interest can compromise regulation; 2) evaluate the effectiveness of current assessment process of pesticides in the US, particularly regarding herpetofauna; 3) examine why studies are included or excluded in regulatory decision-making; and 4) offer recommendations for improving the process. We all have an interest in ensuring that regulatory decisions are based on all available research with sound experimental designs lacking conflicts of interest. Although current practices fall short in the US and other countries, a regulatory process that removes conflicts of interest is both plausible and essential to protect and manage human and environmental health.

Tennessee Valley Authority Kingston Fly Ash Recovery Project: Part A

204 Regulatory Framework and Overview of the TVA Kingston Fly Ash Recovery Project; Roane County, TN

C. Zeller, USEPA – Region 4 / Superfund Division

On May 11, 2009, the Tennessee Valley Authority (TVA) entered into an Administrative Order on Consent (AOC) with the Region 4 Office of the US Environmental Protection Agency (EPA), under the regulatory authority

of the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA), to address the approximate 5.4 million cubic yards (CYs) of coal ash released to the environment on December 22, 2008. The TVA Kingston Ash Recovery Project was divided into 3 distinct phases using Time Critical and Non Time Critical Removal Action authority under CERCLA. Phase 1 was a Time Critical Removal Action to mitigate potential upstream flooding and downstream transport of ash. Phase 1 consisted of hydraulic dredging, mechanical excavation, ash dewatering, rapid materials handling, and off-site rail transportation and disposal of 3.5 million CYs of ash. Approximately 4.0 million tons of ash recovered from the river was safely transported via 414 trains to the approved Arrowhead Landfill in Perry County, Alabama. Phase 1 activities were completed in December 2010. Phase 2 is a Non-Time Critical Removal Action that involves mechanical excavation of approximately 2.8 million CYs of ash in the north and middle Swan Pond Embayments of Watts Bar Reservoir. Recovered ash is consolidated on-site in a 250 acre disposal area that is being re-engineered with a subsurface perimeter stabilization wall to withstand liquefaction forces caused by the design seismic events. When ash consolidation and wall construction is completed, the cell will be capped and covered. Phase 2 construction will be completed in late 2014/early 2015. Phase 3 involves a comprehensive human health and ecological risk assessment of the estimated 500,000 CYs of residual ash that was not removed during Phase 1 or was transported downstream during storm events. The Phase 3 Baseline Ecological Risk Assessment (BERA) evaluated around 20 measurement endpoints for coal ash related impacts. In addition, extensive geochemistry studies, sediment/pore water bioassays, benthic macro invertebrate assessments, 2 dimensional sediment-ash fate/transport modeling, and groundwater modeling (MODFLOW) were conducted. The Phase 3 Action Memorandum was issued on November 7, 2012 and selected Monitored Natural Recovery as the preferred remedy. This presentation will provide an overview of the TVA Kingston project and introduce the other more specific technical sessions of this Symposium.

205 Using ordination and clustering techniques to assess multi-metric and multi-year fish health response following the Kingston fly ash spill

M. Bevelhimer, Environmental Sciences Division; M. Adams, Oak Ridge National Laboratory (retired) / Environmental Sciences Division; A.M. Fortner, M.S. Greeley, C. Brandt, Oak Ridge National Laboratory

Multivariate statistical procedures, such as cluster analysis and canonical discriminant analysis, are useful techniques for combining multiple variables into a single analysis for the purpose of investigating integrated or holistic responses. We used these two procedures to evaluate the collective temporal and spatial response of a suite of fish health bioindicators (i.e., blood chemistry parameters and various energetic and condition indices) to exposure to fly ash from TVA's Kingston Fossil Plant. Bluegill sunfish, largemouth bass, redear sunfish, and channel catfish were collected from the Clinch and Emory river systems from 2009 to 2012 in spring and fall. Sampling sites included three sites in close proximity to the spill (0 to 9 km downstream) and from three upstream reference sites (7 to 9.5 km from spill). Each speciesXseason combination was analyzed separately. The hierarchical clustering results suggest that year to year variation is greater than variation among sites or site types (spill versus reference) within any one year. Dendograms (i.e., tree diagrams) constructed from the clustering analysis revealed no consistent differences between spill sites and reference sites, but did identify some siteXyear combinations that stood by themselves, separate from the other groupings. Of the nine outliers identified by the dendograms, eight were from the two spill sites downstream from the ash spill, and seven of the nine outliers were from 2009 and 2010. The hierarchical clustering results suggest that any changes in the health status of fish at the spill sites are small and that any effect has decreased or recovered since the first two years after the spill. The canonical discriminant analysis results also suggest that year to year variation is greater than variation among sites or between site types. The principle findings suggest that the three spill sites are very similar and not that different from the reference sites. Based on the distances between the group centroids, the greatest difference between the reference and spill sites seemed to have occurred in 2010 for most of the cases analyzed. This further suggests that the ash spill may have caused some health response in fish to the spill and that those differences appear to be diminishing.

206 Effects of the Kingston Ash Release on Fish Reproduction and Larval Development

M.S. Greeley, S.M. Adams, L.R. Elmore, Oak Ridge National Laboratory; T. Mathews, Oak Ridge National Laboratory / Environmental Sciences Division; M.K. McCracken, Oak Ridge National Laboratory; M. Peterson; A.M. Fortner, Oak Ridge National Laboratory; T.F. Baker; R.M. Sherrard, Tennessee Valley Authority / Kingston Ash Recovery Project

In December 2008, a dike containing a mixture of fly ash and bottom ash failed at the Tennessee Valley Authority (TVA) Kingston Fossil Plant in East Tennessee resulting in the largest release of coal ash in US history into the adjacent Emory River and nearby Clinch River. A byproduct of coal-burning power plants, coal ash is enriched in various metals, including selenium that in sufficient quantities is considered to pose a significant risk of reproductive failure in exposed fish populations. In support of ecological risk assessment efforts associated with Kingston ash release, a series of studies were initiated as early as the spring of 2009 to examine the potential effects of the ash release on fish reproduction and fish larval development in affected reaches of the Emory and Clinch Rivers. These studies focused on: (1) assessing metal bioaccumulation in the ovaries of adult fish at ash-impacted sites which could lead to the maternal transfer of developmental toxicants such as selenium to the offspring; (2) examining the relative frequencies of developmental abnormalities in larval fish from ash-exposed sites and non-exposed reference sites; (3) assessing fecundity and other indicators of reproductive competence in adult fish sampled from ash-exposed sites and reference sites; (4) measuring the toxicity to fish embryos and larvae of river bottom sediment containing Kingston coal ash in short-term laboratory tests; (5) examining the effects of long-term laboratory exposures to coal ash on fish survival and reproductive competence; and (6) evaluating the success of reproduction and early larval development in fish exposed in situ to ash for over two years through in vitro spawning trials. Selenium, although clearly elevated in ovaries along with other metals following the Kingston ash release, has remained below current and proposed aquatic life criteria based on whole-body concentrations. Similarly, the results of these diverse field and laboratory studies and related fish community assessments by TVA suggest that the effects of the ash release on fish reproduction and fish populations in the Emory and Clinch Rivers have been negligible to date. The relevance of these results to the Kingston ash release situation in particular and the broader issue of the environmental risks of coal combustion wastes in general will be summarized and discussed.

207 Relationship between selenium body burdens and fillet concentrations in fish exposed to coal ash at the Tennessee Valley Authority Kingston spill site

T. Mathews, Oak Ridge National Laboratory / Environmental Sciences Division; A.M. Fortner, T. Jett, Oak Ridge National Laboratory; M. Peterson; N. Carriker, TVA

In December 2008, 4.1 million m^3 of coal ash were released into the Emory and Clinch Rivers by the Tennessee Valley Authority (TVA) Kingston Fossil Plant. Coal ash contains several contaminants, including the bioaccumulative metalloid selenium (Se). The US Environmental Protection Agency's (EPA) draft water quality criterion for Se is a whole body fish tissue Se concentration (7.91 mg/g, dry wt.) but many biological monitoring programs, including the one established at the Kingston coal ash spill, measure contaminant concentrations in fillets because of concerns about human health risks. We examined the relationship between the Se content in fillets and body burdens in fish collected around the Kingston spill site to determine whether Se exposure due to the spilled ash poses chronic ecological risks. The highest mean whole body Se concentration observed in this study (4.11 mg/g dry wt) was approximately half the draft criterion. Using the relationship between whole body and fillet Se concentrations established in this study, we calculated the fillet concentration corresponding to the whole body criterion to be 15.3 mg/g (dry wt). To date, all fillet concentrations from the Kingston spill site have been below this concentration. While further monitoring is needed to assess longer-term trends, our results suggest that exposure to Se has not led to exceedances of water quality guidelines at the Kingston spill site.

208 Evaluation of Human Health Risk from Potential Exposures to Fly Ash at the TVA Kingston Fly Ash Recovery Project

M.E. Stack, Jacobs Engineering / Federal Operations; S.J. Young, ARCADIS / Environmental; N. Carriker, TVA; D.S. Jones, ARCADIS

The release of fly ash at the Tennessee Valley Authority (TVA) Kingston Fossil Plant (KIF) on December 22, 2008 discharged approximately 5.4 million cubic yards of coal ash slurry into the adjacent terrestrial and aquatic systems. The initial response focused on public protection and stabilization of the released ash, but rapidly evolved to include comprehensive monitoring of ambient media and ecological receptors. The objective of the human health risk assessment for the TVA Kingston Fly Ash Recovery Project was to develop quantitative and qualitative estimates of potential cancer risks and non-cancer hazards for human receptors potentially exposed to the ash. These estimates were developed to support remediation decision making for the River System consisting of the Emory, Clinch, and Tennessee Rivers. Potential receptors were residents adjacent to the river and recreational users. The risk analysis was based on analytical data collected from the Emory, Clinch, and Tennessee Rivers. Legacy constituents (PCBs and mercury) in fish filets were the only constituents of potential concern for human exposures.

209 Laboratory Toxicity Studies in Response to the TVA Kingston Fossil Plant Fly Ash Spill

R.M. Sherrard, Tennessee Valley Authority / Kingston Ash Recovery Project

TVA engaged in numerous aquatic and sediment toxicological investigations following the December, 2008 Kingston Fossil Plant spill of 5.4 million cubic yards of fly ash into the Emory River. In this presentation, we provide an overview of these studies and explain how the results were used by risk assessors in their evaluation of ecological effects resulting from the Kingston fly ash spill. Initially, these studies were aimed at assessing effects to biota in short-term (48-hour to 10-day) laboratory exposures. The results from these earlier tests revealed few, if any, adverse effects in aquatic exposures (e.g., affected river water and dredge plume), and very limited adverse effects in sediment exposures with benthic species (particularly the amphipod *Hyalella azteca*). Once the early investigations had been completed, a laboratory study design was developed that focused on long-term exposures to sediments containing residual ash from the Emory and Clinch Rivers. These long-term sediment exposures included 28-day *H. azteca* survival and growth tests and *Chironomus dilutus* (a midge) partial life cycle (PLC) tests. Four strategic sample locations in the Emory River and Clinch River were chosen based on the results from 10-day screening tests included in this study design. In Clinch River sediment exposures, adverse effects on amphipod growth were observed from only two sample locations, and were not assessed as being attributable to chemical parameters associated with fly ash (ash content < 41% in samples). Minor effects (PLC survival and growth) were observed for midges at one sample location. No other adverse effects were observed in these long-term exposures to Clinch River sediment samples. Laboratory exposures to Emory River sediment samples (ash content >45%) resulted in more pronounced effects to test species. In two of the sediment samples, amphipod survival was adversely affected; growth effects were observed in all four sample exposures. With midges, 20-d growth and PLC survival and emergence were adversely affected in exposures to all four Emory River samples. Once incorporated into the analyses by risk assessors, these findings contributed significantly to the selection of remedial actions and long-term monitoring goals.

210 Adult Mayflies as a Contaminant Source to Terrestrial Consumers Following a Coal Ash Spill at the Tennessee Valley Authority's Kingston Fossil Plant

J.G. Smith, Environmental Sciences Division; T.F. Baker,

Emerging aquatic insects are an important energy subsidy to terrestrial consumers living near water. However, in contaminated water bodies they also can contribute to the transfer of contaminants from aquatic to terrestrial ecosystems. Following a spill of ~5.4 million yd^3 of coal ash into the adjacent Emory River in 2008 after a dike failed at the Tennessee Valley Authority's (TVA) Kingston Fossil Plant in Kingston, TN, a study on the spatial and temporal trends in bioaccumulation of potential ash contaminants was initiated on the nymph and adult stages of the common and widespread burrowing mayfly *Hexagenia bilineata*. This study focuses on the nymph to adult transfer of arsenic (As) and selenium (Se), metalloids that were identified as key ash-related contaminants from the spill that posed potential risks to terrestrial wildlife. Nymph and adult stages of *Hexagenia* were collected annually from 2009-2012 at up to 11 sites upstream (reference) and downstream of the spill site, and then analyzed for As and Se. Highest mean concentrations of As (46.2 µg/g DW) and Se (9.67 µg/g DW) in nymphs were found in 2010 ~1.5 miles downstream of the spill site. The highest mean concentration of As found in adults downstream of the spill site was 0.43 µg/g DW, indicating that little As was being transferred to the terrestrial ecosystem by this species. In contrast to As, Se concentrations were generally ≥ 6% higher in the adults than in the nymphs. These results suggest that emerging aquatic insects have the potential to be an important source of some contaminants to terrestrial consumers that feed near the water's edge.

211 Does selenium from Kingston coal ash spill accumulate in biota?

R.R. Otter, Middle Tennessee State University / Biology; D. McKinney, Tennessee Wildlife Resources Agency; T. Mathews, Oak Ridge National Laboratory / Environmental Sciences Division; F.C. Bailey, Middle Tennessee State University / Department of Biology; B. Brown, S. Lainer, D. Hubbs, Tennessee Wildlife Resources Agency; B. Read, Tennessee Department of Health; A.M. Fortner, Oak Ridge National Laboratory; M. Hayden, W.A. Monroe, Middle Tennessee State University

Coal burning power plants were responsible for the production of 42% of the electricity in the US last year. As a by-product of this process millions of tons of residual fly ash were also produced. On December 22, 2008, an impoundment failure at the TVA's Kingston Fossil Plant resulted in the largest coal ash spill in US history releasing approximately one billion gallons of coal ash into the Emory River. We conducted a comprehensive sampling campaign at the Kingston spill site to understand selenium bioaccumulation in organisms at different trophic levels in aquatic food chains. Different species of fish were collected from various sites up- and downstream of the ash spill. Redear sunfish were found to accumulate higher concentrations of selenium than any other species in this study. Field-exposed mussels showed increased uptake of selenium both during and after dredging compared to reference sites, but also indicated a decrease in total exposure after cleanup was completed. Tetragnathid spiders collected from sites downstream of the ash spill had higher selenium concentrations than those collected from reference sites, and could be a vector for selenium transfer from aquatic to terrestrial ecosystems.

Sustainability and Resource Use

212 Impact models from water use: comparing to better harmonize

A. Boulay, CIRAIG – École Polytechnique de Montréal; C. Bulle, CIRAIG – Polytechnique Montreal / Chemical engineering department; S. Pfister, ETH Zürich; M. Motoshita, National Institute of Advanced Industrial Science and Technology; M. Margni, École Polytechnique de Montréal / Department of Mathematical and Industrial Engineering

In the past decade, several methods have emerged to characterize impacts of water use in LCA. Building on an extensive review of these methods covering the LCA scope by Kounina and colleagues (2012), this paper aims to (i) compare selected methods addressing water scarcity as a midpoint indicator and identify the key elements and modeling choices that explain the main difference between the indicators, (ii) quantifying the significance of the differences between methods, including the assessment of model uncertainty and (iii) discuss the main methodological choices and provide recommendations to guide method development and harmonization efforts. This paper presents preliminary results of the third deliverable of the WULCA project (Water Use in LCA) of the UNEP/SETAC Life Cycle Initiative. Results from water scarcity methods are first normalized using a reference flow of a world average water volume and then compared using the Spearman rank correlation coefficient and the Gini mean difference coefficient. The former expresses the consistency of model response for different flows and the latter expresses the mean difference between two model results for the same flows. Similar models have high rank correlation and low mean difference. These tests are also performed for specific modeling choices to assess their relative influence: source of data, source of water (surface water vs. groundwater), consideration of temporal variation, use of consumption or withdrawal based scarcity index, choice of regional resolution scale and of aggregation methods. Results show that the type and scale of regional resolution, the source of data used and the consideration of consumed water to assess scarcity have a significant effect on the results. Other choices like the use of CTA

instead of WTA-based indicator or include seasonal variation, have shown to be of importance in few specific regions of the world. Maps are provided to identify the regions where such choices are relevant. Questions now need to be answered as to which data source is the most representative, how should scarcity be defined when using consumption-to-availability ratios and what is the most relevant spatial resolution.

213 A Holistic Approach to Understanding Water Risk at the Basin Level

S. Quinn, The Procter & Gamble Co.; S.D. Dyer, The Procter & Gamble Company / Central Product Safety; G. Carr, The Procter & Gamble Company / Quantitative Sciences

Growing human populations and economies coupled with global climate change have caused increased pressures on natural resources, including water. A lack of water, too much water or increased regulatory focus on water systems can cause issues for communities, businesses and manufacturers alike. To avoid disruptions in daily life and production, it is more important than ever to thoroughly understand water risks at both the basin and local levels so that mitigation plans can be created and risks can be managed appropriately. A tiered risk assessment approach, using a combination of diverse indicators, can help organizations identify and prioritize sites with the highest risk, and provide a prognosis that can lead to a tailored mitigation plan based on the highest risk areas. A combination of tools and internal data have enabled us to create a methodology to holistically assess water risks at the river basin level and use the results to inform decision making at the local level. Case studies using P&G manufacturing sites scattered throughout the globe and with diverse water risks will be presented. Inherent within water risk tools is the weighting of various metrics to derive an overall water risk score. To date, these weights have been based on best professional judgment. We present an assessment of these risk metrics, focusing on their importance to the overall risk scores, and our attempts to build simpler models of overall risk based on the most informative component metrics.

214 Which water scarcity index should I use?

J.C. Bare, USEPA / MS-466

Currently there are several water scarcity indices available. In many cases this is necessary since different information is necessary for different applications (e.g., agriculture, timber, paper products). In other cases, the water scarcity indices are simply confusing since they utilize the same information, but come to different conclusions. What are some things that need to be considered when choosing a water scarcity index for your application, especially in North America?

215 Spatial LCA of resource use in agricultural production: A United States case study

A.D. Henderson, University of Michigan / Environmental Health Science, School of Public Health, University of Texas School of Public Health / Environmental and Occupational Health Sciences; A. Asselin, University of Michigan; M. Heller, University of Michigan / Center for Sustainable Systems; O. Jolliet, University of Michigan / School of Public Health

Agricultural production directly affects land and water, two resources tied to sustainability and receiving attention in LCA. A case study of major crop production in the United States (e.g., corn, soybeans) and milk production presents the opportunity to compare methodologies related to resource use and compare the influence of sub-national resolution on inventory and impact assessment, which is important in a heterogeneous area such as the US. This presentation illustrates a spatialized used for agriculture, focusing on land use and water (consumption, as well as eutrophication). Working at the state, watershed, regional, and national level, we developed an original matrix approach to integrate crop production and dairy farm emissions (including enteric and manure management). Impacts can be considered from a an emission perspective (e.g., pesticide emissions in one area to grow feed for use in another area) and a receiver perspective (e.g., the impact of those pesticides may occur in yet another area). In addition, we developed a new impact assessment methodology for freshwater eutrophication and integrated recently developed approaches for water and land use into the existing Impact 2002+ structure. At state resolution, for example, we identified crop inventory variations ranging from a factor of 800 for water use to a factor of 2 for land use (both for corn grain). Characterization factors, which add an additional degree of spatial variation (e.g., up to 3 orders of magnitude for freshwater eutrophication), contributed further to variations across the US For example, the state of Nebraska accounts for over half of the water stress impact of national corn production. A US-level assessment would not see these spatial variations. Given the interest in resource use, comparing land use and water impacts can be insightful. In this study, we present a first order analysis to compare biodiversity losses due to land use, eutrophication, and water use, using global estimated species density information to compare terrestrial and aquatic impacts. Uncertainty will be discussed, as will the influence of spatial resolution. Further research questions will also be highlighted. For example, ongoing work for agricultural sustainability considers the suitability of cropland for alternative uses.

216 The Incorporation of a Net Ecosystem Service Analysis (NESA) Approach in Ecological Risk Assessment

K. Rouse Campbell, ENVIRON International Corp / Senior Science Advisor; J.T. Ipock, ENVIRON International Corp; J. Nicolette, ENVIRON International Corp

Traditional approaches to ecological risk assessment, such as the US Environmental Protection Agency's framework, typically do not consider ecosystem services, which are the benefits people obtain from naturally functioning ecosystems. Ecosystem services include provisioning services, such as food and water; regulating services, such as regulation of floods and droughts; supporting services, such as soil formation and nutrient cycling; and cultural services, such as recreational, spiritual, and religious benefits. In order to make an ecological risk assessment a more holistic evaluation, recent work has considered the use of ecosystem services as endpoints in the risk assessment. Furthermore, in cases where a natural resource injury does occur, the assessment endpoints and subsequent compensatory restoration will be more closely aligned in scope and matter. In addition to selecting particular ecosystem services endpoints during the problem formulation phase of a risk assessment, ecosystem services should be considered during the risk management and feasibility study stage. Assessing ecosystem services during decision-making is inherently more complex compared to considering traditional assessment endpoints because ecosystem services provide environmental, social, and economic benefits. To accomplish the task of considering and comparing the various alternatives, net ecosystem service analysis (NESA) could be included in the risk management decision-making stage. The overarching premise of the approach is that human well-being is directly related to changes in ecosystems and associated services. NESA evaluates the net change in ecosystem services, and hence human well-being, and provides another risk management tool during remediation/restoration planning for comparing alternative suites of services, quantifying how ecosystem services change with the implementation of an action (e.g., land development, restoration, preservation, and remediation), and evaluating how the human health and ecological risk profiles would change given implementation of each remedial alternative. Ultimately, the incorporation of NESA and ecosystem services will provide more information for decision makers and stakeholders and, in some cases, may even reduce uncertainty in the risk assessment process by utilizing more encompassing endpoints.

217 National Differences in Pesticide Application Practices Substantially Influence Human and Ecological Impacts in LCA

J.K. Saxe, EcoSafety Sciences

Agricultural practices used in the production of raw materials is considered in the life cycle assessment (LCA) of a wide range of products. Potential impacts due to pesticide use in agriculture can dominate the ecological and human health impact categories in an LCA. The environmental fate of pesticides can differ dramatically among nations, due to natural differences (e.g., soil types), and more importantly, due to differences in agricultural practices and national regulatory limits on how, when, and where pesticide application can occur. For example, pesticide use regulations dictate practices to minimize runoff (e.g., mandatory buffer zones), spray drift (e.g., limits on equipment used for application), and leaching (e.g., restrictions on application to certain soil types). This can result in substantially different potential impacts between nations where pesticide use restrictions differ. USETox is the most widely used tool for life cycle impact assessment (LCIA) of chemicals when assessing human and ecological health endpoints. The USETox database includes fate, transport, and toxicity data for a large number of pesticides, allowing LCIA to be completed with relative ease for these compounds. However, the USETox methodology and database do not account for any risk mitigation practices undertaken when pesticides are used. Furthermore, the USETox database does not tap the substantial library

of environmental fate studies – often applicable only to certain soil types or water chemistries – available through the pesticide registration process. This study demonstrates how nation-specific risk mitigation practices and soil- or water-specific empirical data not accounted for in USETox can be implemented in the model, and how accounting for these practices and data results in order-of-magnitude differences in the USETox outcome.

218 An Assessment Tool for Sustainability Education for Collaboration

A.J. Berardy, Arizona State University / School of Sustainability; T.P. Seager, Arizona State University / School of Sustainable Engineering & Built Environm; E. Selinger, R. Uhl, Rochester Institute of Technology

The field of sustainability requires the integration of multiple disciplinary perspectives to address a problem in a holistic manner. Most researchers become more specialized as they gain experience and expertise, so they must collaborate with other specialized researchers working on the same issue. Unfortunately, epistemological differences and varying terminology create barriers and conflicts between team members. Over time, effective teams are able to gain familiarity with the other disciplines and carry on high level conversations regarding the problem they are working on. This ability to converse expertly about a disciplinary topic without actually being an expert in that discipline is known as interactional expertise. Sustainability students in particular would benefit from this type of expertise, as they are expected to become familiar with multiple disciplines in their research. However, this typically requires extensive linguistic socialization over a long period of time to acquire. Sustainability students cannot be expected to gain interactional expertise in every discipline they study, and still must choose a discipline to focus on through their education. Furthermore, sustainability education is not oriented towards developing interactional expertise in students. Instead of focusing on communicative competency, it aims to create novice practitioners of a discipline. A more effective instruction methodology must be developed, and a way to assess its effectiveness is necessary to ensure that it is more useful than traditional sustainability instruction. The assessment instrument developed for this purpose is known as the Test of Ubiquitous through Real or Interactional Expertise, or TURINEX. TURINEX works by allowing a judge from the target expertise to communicate with three respondents. The role of the judge is to determine what the expertise level of each respondent is from three categories – fully expert, partially expert (also known as intermediate interactional expert), or not expert. A recognized expert provides a positive control for the test while a non-expert provides a negative control. The third respondent is the test subject. Results from this test can be compared before and after training, and can also be compared to results for a student who received traditional sustainability education. This comparison should then show how effective the training was.

219 Misapplication of Generic Hazard Classification Schemes for Versatile Building Materials: Copper as an Example

J.S. Meyer, Arcadis US, Inc., ARCADIS; J.W. Gorsuch, Copper Development Association Inc.; R. Dwyer, International Copper Association / Health, Environment and Sustainable Development Program

Inappropriate application of generic chemical-of-concern hazard classification schemes unnecessarily restricts the use of many versatile substances. For example, copper is a versatile building material that is used in many durable forms (i.e., resist decay and have limited water solubility), in addition to some "dispersive" forms (i.e., as soluble salts). Despite the fact that most copper in building materials is not present as a soluble salt but instead as a metal alloy, copper has recently been misclassified as a chemical of concern (COC). This misclassification results from incorrectly assuming that the fate and bioavailability of copper in soluble salts represent the fate and bioavailability of copper in the wide variety of durable materials used in construction. Although copper released into environmental media has the potential to cause toxicity, hazard does not equate to risk because environmental factors (e.g., species exposed, timing of exposure, soil and water chemistry) modify potential toxicity. Unlike refined risk assessments, several generic hazard classification schemes focus on extreme scenarios in which this potential toxicity is highest (e.g., the persistent, bioaccumulative, toxic [PBT] approach). However, the PBT approach is not appropriate for copper. Additionally, human-health risks from exposure to exterior copper building materials and even to copper-alloy antimicrobial "touch" surfaces are negligible. Greater than 98% of the copper produced is incorporated into durable products; and that copper is fully recyclable at the end of its life, with no loss in usefulness. Less than 2% of copper is produced as powders and chemicals, much of which is used in dispersive products: animal feed additives to meet nutritional deficiencies, friction materials in brake pads, and registered pesticides for plant protection and antifouling purposes. Copper is an essential element; and when recycled, the carbon foot print is minimal. Copper is a sustainable resource precisely because of its stability and durability, which are positive attributes for its use as a "green building" material. Sufficient scientific evidence demonstrates negligible risk associated with using copper as a building material. Therefore, the Precautionary Principle does not need to be invoked; and like some other metals and organics, copper does not need to be listed as a COC, be targeted in material-substitution schemes, and/or be discouraged from use as a building material.

PAH in the Environment: Advances in Assessment of Occurrence, Sources, and Human and Ecological Risks: Part A

220 Evaluation for the after-effects of 'The Japan Tsunami and Earthquake Disaster of 2011' from a viewpoint of PAHs pollution in Tohoku coastal waters

K. Miyazaki, Graduate School of Science and Technology; Y. Goto, H. Izumida, H. Nakata, Kumamoto University / Graduate School of Science and Technology; D. Ueno, Saga University / Faculty of Agriculture; T. Miyawaki, Fukuoka Institute of Health and Environmental Sciences; T. Matsumura, IDEA Consultants, Inc.; M. Nakamura, Hiyoshi Corporation; K. Nakai, Tohoku University / School of Medicine

In March 11, 2011, the earthquake of moment magnitude 9.0 occurred at Off-Miyagi Prefecture, Eastern Japan, and over 8 m Tsunami attacked the Tohoku Pacific coasts (TPC). After the disaster, oil contamination was occurred in whole TPC, due to a spillage of fuels from the storage tanks, automobiles and vessels by Tsunami. It has reported that fuels contain high concentrations of xenobiotics such as polycyclic aromatic hydrocarbons (Par-PAHs) and alkylated PAHs (Alk-PAHs). However, there has been little information on the PAHs pollution in TPC after the disaster so far. In this study, we analyzed Par-PAHs and Alk-PAHs in bivalves (n=65) and sediments (*n*=28) from 36 stations from Tohoku coasts to understand the occurrence, distribution and temporal trend of PAHs, and to evaluate the after-effects of the disaster. PAHs were detected in all samples analyzed. ΣPar-PAHs concentrations in bivalves from TPC were 4-1,800 times higher than those from reference sites. Furthermore, some samples showed high composition of high molecular weight Par-PAHs such as benzo[*a*]pyrene (BaP). This may be due to different exposure sources of PAHs among sampling locations. The high concentrations of ΣAlk-PAHs were also found in bivalves from the TPC. In addition, bivalves from TPC occupied greater percentage of dibenzothiophenes, which contained in bunker fuels, than those from reference sites. This implies the occurrence of PAHs pollution by the spillage of bunker fuels, due to destruction and outflow of fuel tanks into TPC. The high concentrations of PAHs were also detected in sediments from semi-closed bay of TPC. The compositions of Alk-PAHs in some sediment were much similar to that of bunker A, suggesting a discharge of bunker A into the bay. To evaluate potential risk of human exposure to PAHs, hazard quotient (HQ) of BaP were calculated in bivalves. The HQ values was less than 0.01, indicating that human risks of BaP exposure by eating bivalves from TPC may be small. As for temporal trend, PAH concentrations in bivalves from TPC were decreased after the disaster. Especially, the concentrations of Alk-PAHs drastically decreased in bivalves, indicating the improvement of seawater quality of the TPC. However, high concentrations of PAHs were still in sediments, so that it is necessary to monitor PAHs in aquatic environment in TPC.

221 Measuring and Modeling the Atmospheric Transport of Polycyclic Aromatic Hydrocarbons in Alpine Valleys in Arthur's Pass National Park, New Zealand

K. Orr, K.J. Hageman, University of Otago / Department of Chemistry; P. Zawar-Reza, University of Canterbury / Department of Geography; P. Shahpoury Bahry, University of Otago / Department of Chemistry

Polycyclic aromatic hydrocarbons (PAHs) are important contaminants because of their toxicity and tendency to undergo atmospheric transport. However, studies that investigate PAH atmospheric transport distances and dispersion mechanisms are often hindered by the mixing of PAHs generated from multiple nearby sources. Our project was designed to measure and model the atmospheric transport of PAHs originating from a line source,

i.e. State Highway 73 (SH 73), into three remote alpine valleys extending into Arthur's Pass National Park on the South Island of New Zealand. The three valleys were relatively flat, broad braided-river valleys and extended to the west, east, and north of SH73, respectively. PAHs were quantified in samples of lichen (*Usnea* sp.), which was used as a natural passive sampler, collected at 1-kilometre intervals along each 10-km long transect. Additionally, regional-scale high-resolution atmospheric modeling was performed to determine wind speed and wind directions at hourly intervals at a 1-km spatial resolution for the duration of the sampling period. The trends in measured PAH concentrations varied between valleys, with the concentrations decreasing linearly in the west-extending valley, remaining relatively constant in the east-extending valley, and dropping sharply and then leveling off in the north-extending valley. These trends could be largely explained by differences in the frequencies of up-valley winds in the three valleys and highlight the role that meteorology and geomorphology have on contaminant transport in mountain regions. To further understand the mechanisms influencing PAH atmospheric transport, PAH dispersion was simulated with the regional-scale model to obtain ground level concentration in each valley and calculated congener-specific characteristic travel distances. Finally, diagnostic ratios of specific PAH congeners that are typically used for source apportionment were calculated from both measured and modeled data and the different transport behaviors of individual congeners was used to illustrate the limitations of this approach.

222 High Resolution PAH Analysis of Hydrocarbon Products

S.D. Emsbo-Mattingly, NewFields Environmental / Forensics Practices, LLC; E. Litman, NewFields Environmental Forensics Practice, LLC; J. Occhialini, Alpha Analytical; R. Siegener, Alpha Analytical Laboratories

Recent improvements in benchtop GC/MS systems demonstrate a wide diversity of PAH compounds in a range of hydrocarbons products. These results help contextualize convential environemtnal monitoring data focussed on the measurement of 16 USEPA Priority Pollutant PAHs in accordance with Method 8270 from the SW-846 method compendium. Risk assessors often double the number of PAH analytes when evaluating risk to human and ecological receptors. Environmental forensic investigators expand this list still further to include approximately 50 analytes when identifying hydrocarbon sources and background conditions. The evolution of PAH testing methods using a modified version of Method 8270 extended the PAH analyte list now includes several dozen additional heavy PAH compounds with trace level detection limits. This presentation focuses on the distribution of more than 100 PAH analytes in a variety of commonly encountered petroleum products, tar products, and urban dust using a high resolution PAH analysis. The benzo(*a*)pyrene equivalent (BaPeq) concentrations for each product is determined using relative potency factors (RPFs) for human health risk assessments promulgated by USEPA in 1993 (n=6) and proposed by USEPA's Office of Research and Development (ORD) in 2011 (*n*=26). A comparison of the PAH distributions and BaPeq concentrations among the hydrocarbon sources in this study provide a context for evaluating PAHs in the environmental under different release scenarios. In addition, the data from this study offer reasonable benchmarks for performing high resolution PAH analyses on a routine basis in commercial laboratories.

223 Integrating passive sampling with UV irradiation and bioassays: an approach for modeling PAH degradation and phototoxicity in bioavailable mixtures

M. Elie, Oregon State University / Environmental and Molecular Toxicology Department; N.D. Forsberg, Oregon State University / Environmental and Molecular Toxicology; S.E. Allan, Environmental and Molecular Toxicology, National Oceanic and Atmospheric Administration / Office of Response and Restoration; S.G. O'Connell, Oregon State University / Environmental and Molecular Toxicology; R.L. Tanguay, K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology

In order to accurately assess the risk of polycyclic aromatic hydrocarbons (PAHs) in the environment, analytical approaches are needed to identify the effects of biogeochemical processes, such as ultraviolet (UV) radiation, on bioavailable PAH fractions. While passive sampling devices (PSDs) are established at measuring the bioavailable (C_{free}) fraction of organic chemicals in water, their coupling with laboratory-based UV irradiation experiments may provide further insights on the photo-modification and phototoxicity of PAHs in environmentally relevant mixtures. The objectives of this study were to: 1) assess if real-world mixtures of bioavailable PAHs can be photodegraded; 2) demonstrate that OPAHs are formed from PSD mixtures after UVB exposure; and 3) that PSD-UV extracts may be used for *in vivo* zebrafish bioassays. Experiments were conducted with PSD extracts collected from Portland Harbor Superfund sites in Portland, OR and the Gulf of Mexico during and after the Deepwater Horizon oil spill. The analytical approach resulted in a 20 to 100% reduction in PAH levels and simultaneous formation of several OPAHs, including 9,10-anthraquinone, 7,12-benz[a] anthracenequinone and 4H-cyclopenta[def]phenanthrene-4-one . Trends of OPAH formation are discussed. PSD-UV extracts were incorporated into biological-based assays using embryonic zebrafish to characterize the photo-induced toxicity of PAH/OPAH mixtures. Each embryo was assessed for mortality, and 18 developmental and neurological endpoints. Bioactivity pre-UV exposure and post-UV exposure were different as well as site specific trends. The results of this study may provide a framework for assessing the risk of weathered or remediated PAHs in the environment.

224 RAD Seq of PAH-Adapted Atlantic Killifish, Fundulus heteroclitus, Subpopulations from the Elizabeth River, VA

J.S. Osterberg, Duke University / Nicholas School of the Environment; T.F. Schultz, K.M. Cammen, Duke University / Nicholas School of the Environment, Duke Marine Lab; B.W. Clark, R.T. Di Giulio, Duke University / Nicholas School of the Environment

The highly industrialized Elizabeth River (ER) flows through Norfolk, VA to the Chesapeake Bay and contains numerous fossil fuel depots, naval installations, and dry docks, as well as the creosote-contaminated Atlantic Woods Industries Superfund (AW) site. The AW site supports a well-studied subpopulation of Atlantic killifish, *Fundulus heteroclitus*, that has a down regulated AHR pathway, is recalcitrant to CYP1 induction, shows resistance to PAH-induced tumors, and is resistant to the fatal embryonic cardiac teratogenesis seen in reference fish treated with PAH laden sediment extract from the site. Toxicological studies of fish collected from neighboring sites within the ER show similar adaptations, although to different degrees not necessarily correlated to sediment PAH loads at each site. Despite much data on the adapted phenotypes, the precise mechanisms of the adaptations remain elusive. We performed restriction site associated DNA sequencing (RAD seq) to identify regions of the genome under selection and better understand the genotypes that underlie the variously adapted phenotypes of six ER subpopulations with comparison to our long time reference site of King's Creek (KC), VA as well as a reference site in Beaufort, NC. Illumina sequencing of 32 individuals each from the AW and KC sites (other site analyses are on-going) produced 87.1 million reads with approximately 1.4 million average reads per fish. We used Stacks (v0.99993) to map the reads to the newly sequenced *F. heteroclitus* genome and identified 97,818 loci and 45,964 single nucleotide polymorphisms (SNPs). We calculated smoothed F_{ST} across 150 kB sliding windows for ~20,000 SNPs (read depths ≥10, ≥50% genotypes in both populations, and minor allele frequencies ≥10%) and identified 17 regions showing smoothed F_{ST} values significantly ($p < 0.0001$) elevated above background. Candidate genes (211) under selection were defined as those within 300 kB of a significant region and include several genes involved in the regulation of the canonical AHR pathway, as expected, as well as several genes relating to mitochondrial function, cancer and DNA repair, and muscle function. Results of RAD seq of the other ER subpopulations as well as candidate genes under selection will be presented.

225 Hyporheic flows in rivers as a vector of oil and PAH contamination of fish spawning shoals

P.V. Hodson, Queen's University / School of Environmental Studies; A.M. da Silva, K.M. Mumford, Queen's University / Department of Civil Engineering; R.S. Brown, Queen's University / Department of Chemistry; A. Rutter, Queen's University / School of Environmental Studies

An expanding North American oil industry has generated an increasing frequency of oil spills to inland waterways. The potential impacts include chronic toxicity to fish embryos of polynuclear aromatic hydrocarbons (PAH) that can partition from oil droplets or stranded oil into the waters where fish spawn. Steep gradient rivers with permeable bed sediments often support large populations of salmonids because currents interacting with the bottom topography of streams generate flows into and out of bed sediments. These 'hyporheic' flows supply oxygen to fish embryos buried in sediments, and remove metabolic wastes, thereby ensuring high rates of survival and recruitment when the embryos hatch. However, spilled oil can be entrained

from surface films into the water column as suspended droplets due to high energy mixing of river water associated with rapids, 'jumps', and turbulence due to obstacles. Thus, hyporheic flows may transport oil droplets deep into sediments where they may be filtered or trapped as pockets of oil or as oil stranded on the surfaces of gravel. The trapped oil may act as a source of PAH partitioning to interstitial waters, creating a continuous exposure of fish embryos to PAH at concentrations up to the limits of their solubility. If sufficient oil is trapped, the slow removal of PAH by dissolution could create long-term contamination and impacts on production of fish in affected rivers. This paper will review the toxicity of PAH to fish embryos, laboratory models that have examined the release of PAH from oil-contaminated sediments by interstitial flows, and hydraulic experiments needed to assess the potential risks of sediment contamination by hyporheic flows.

226 Snowmelt-induced concentration pulses of polycyclic aromatic hydrocarbons in stream water and macroinvertebrates in the Southern Alps, New Zealand

P. Shahpoury Bahry, K.J. Hageman, University of Otago / Department of Chemistry; C.D. Matthaei, University of Otago / Department of Zoology; R.E. Alumbaugh, University of Otago / Department of Pharmacology

Semi-volatile organic contaminants, including polycyclic aromatic hydrocarbons (PAHs), can travel through the atmosphere to locations distant from where they originated. In cold regions, these contaminants may then be scavenged by dry or wet deposition processes and transferred to the ground or water bodies. If the scavenging process happens by snowfall in the winter, the contaminants accumulate in the snowpack and may remain there as long as the ambient temperature allows them. When the temperature increases and the snowpack begins to melt, the contaminants accumulated in the snowpack may be released to the environment and specifically to the surrounding streams. This process can create pulses in concentrations of contaminants in the water and adversely affect the biological communities living in the streams. The present study aimed to determine which environmental and meteorological conditions are most likely to produce contaminant pulses in alpine streams. Additionally, a new approach for measuring snowmelt-induced concentration pulses was assessed and macroinvertebrates were collected from streams both to measure biological uptake of contaminants and to determine if contaminants effected macroinvertebrate population and biological traits. The concentrations of polycyclic aromatic hydrocarbons (PAHs) were measured in stream water and macroinvertebrates at three stream sites located in Arthur's Pass National Park, New Zealand. Measurements were made at regular intervals over the course of six months, starting before snowfall and ending after snowmelt (July to December 2010). Concentrations in water were measured with silicon passive samplers, allowing us to determine time-weighted average concentrations. Macroinvertebrates were collected on four occasions using a Surber sampler. Fluorene, phenanthrene, fluoranthene, pyrene, and retene were detected in all samples; naphthalene was also detected in macroinvertebrates. Overall, the results indicated that the total concentrations of PAHs in stream water and macroinvertebrates increased at the onset of the main snowmelt period. However, the magnitude of the contaminant pulse was influenced by site characteristics, especially the extent of vegetation in the stream drainage systems.

227 Characterization of PAHs and other Contaminants in Combined Sewer Overflow to the Gowanus Canal

E.A. Garvey, The Louis Berger Group, Inc. / Site Assessment and Remediation; S. Gbondo-Tugbawa, C. Prabhu, The Louis Berger Group, Inc.; E. Mahoney, New York City Department of Environmental Protection

The Gowanus Canal in Brooklyn, NY has become a center of environmental interest as the City of New York, the USEPA and the National Grid Corporation evaluate the extensive contamination that lies within its banks. The Gowanus Canal itself is a small, urbanized, commercial waterway with extensively bulkheaded shorelines. Its use for commercial shipping extends back to the late 19th century, and parallels the growth and decline of industrial activities in Brooklyn. There are a number of industrial contaminant sources to the Canal, in particular, three manufactured gas plants located along the banks. CSO discharges also impact water quality in the Canal. Recently, the New York City Department of Environmental Protection (NYCDEP) conducted an extensive sampling effort in the Canal to understand the nature of contamination in CSO discharges. In particular, the City obtained both dissolved and suspended matter samples from each of the four major CSOs to the Canal on at least four separate occasions. These samples represent time-weighted averages of the overflow discharge during discrete rainfall events. The samples were analyzed for an extensive set of parameters including PAHs, metals, PCB congeners, and many supporting parameters like total suspended solids. This investigation represents some of the first measurements of split–phase CSO discharges in the US. Among the more important observations was the consistency of particulate-bound PAH concentrations across CSOs and rainfall events, despite different urban drainage areas. This presentation will focus on the nature of particulate PAH discharges from CSOs and compare the concentrations and patterns to those of surface sediments within the Canal. Concentrations of TPAH in CSO solids, which average 30 mg/kg, are substantially lower than surface sediment concentrations in the Upper Canal (mean of 270 mg/kg) and Middle Canal (mean of 920 mg/kg). The fingerprint of PAHs on the CSO solids are differ from those observed on sediments of the Canal, providing a basis to exclude CSOs as significant contributors to sediment PAH contamination. TPCB concentrations were also measured on CSO solids. Like PAHs, PCB levels were much lower than concentrations found elsewhere in the Canal. Overall, these data suggest that the CSOs cannot be the primary contributor of PCBs and PAH to the sediments of the Gowanus Canal. The data also provide an accurate picture of the low levels of these two compound classes in CSO discharges.

Innovative Environment: New Tools for Addressing Issues in Environmental Toxicology and Chemistry

228 Taking Stock Online: A Tool for Improving our Understanding of North American Environmental Quality

O. Cabrera-Rivera, D. Vallee, Commission for Environmental Cooperation

Pollutant Release and Transfer Registers (PRTR) provide information on the amounts of pollutants released from facilities to the environment on-site (air, water, land, and injected underground), as well as transferred off-site for recycling, treatment or disposal. PRTR data can serve as an effective tool in tracking and assessing the generation, release, and fate of industrial toxic contaminants over time, and their potential impact on human health and the environment. The Commission for Environmental Cooperation's (CEC) *Taking Stock* Online (www.cec.org/takingstock), compiles and disseminates the latest data from North America's three pollutant release and transfer registers (PRTRs). It features an integrated, multi-year database covering over 30,000 industrial facilities and 500 substances from about 100 major industrial sectors that report to the PRTRs of Canada, Mexico and the United States. The site features tools to assist in PRTR data analysis, such as customized searches, Toxicity Equivalency Potentials (TEP) information for selected pollutants, a graphics generator, file exports for data analysis and mapping, and a tool to explore data on pollutants transferred across national borders. These tools also allow users to explore, analyze, and compare information on pollution from industrial facilities across North America. Additionally, the *Taking Stock* Online tool integrates social media outlets such as Facebook and Twitter in an effort to make online queries and analyses easy to share amongst users. An overview of *Taking Stock* Online and an analysis of PRTR data with respect to geospatial information such as watersheds, rivers and lakes, and population centers using data from the CEC's *North American Environmental Atlas* will be presented.

229 Innovation on Foot: Partnering with the Hult International Business School to Gain True Insight into Issues Facing the Environment in Emerging Markets

P. Sun, The Procter & Gamble Co.; S. Quinn, The Procter & Gamble Co.; K. Lee, Procter & Gamble; T.W. Federle, The Procter & Gamble Company / Environmental Stewardship; D. Mcmillan, The Procter & Gamble / Global Product Stewardship

Recently, P&G partnered with the Hult International Business School to collect consumer product habits and practices data in several emerging countries, including: Nigeria, Kenya, India and Thailand. The project used the global network of Hult School students to coordinate on the ground data collection in the countries of interest. Country leaders identified in-country researchers, who observed, interviewed, photographed and recorded the daily habits of consumers and the overall environmental state in their respective country. The one-on-one interviews (generally between 300 to 600 interviews per country) and widespread availability of cell phone cameras allowed real time, first-hand information on the daily lives of each

population segment to be sent to P&G scientists. Key environmental information collected included: overall water supply, usage and disposal of water for local population, product disposal behaviors (e.g., packaging waste), waste infrastructure availability and several other endpoints. Additional data have been provided on water use and disposal practices as a function of economic segmentation and locale (e.g., urban, rural, different areas of the countries). The resulting data will be used to improve the understanding of environmental safety of consumer products and their ingredients in their countries. One example of this, the water usage data from 380 responses in rural Thailand showed that 75% of the population uses a combination of rain water and tap water, 19% uses tap water only, and 6% uses well water. This first-hand dataset dispels the assumption that the majority of the rural population uses river water as the main water supply in Thailand. This innovative project gave us insight into the real world environmental issues that exist in countries where easily accessible environmental information is extremely limited.

230 Science Video Journals to Increase Productivity in Research and Education

K.M. Henderson, JoVE (Journal of Visualized Experiments) / Editorial

Research in both the physical and biological sciences chronically suffers from the low productivity and reproducibility of experimental studies. This is due in part because the traditional text-based format of science journals cannot provide an adequate description of complex research procedures. This creates a critical "bottleneck" problem of knowledge transfer for research and education. Addressing this challenge, a new generation of science journals employs online video to provide a systematic visualized publication of experimental studies. This presentation will provide an overview of the growing field of video publication and discuss its technical challenges, implications for scholarly communication and acceptance in the academic and library community. Results and recently conducted case studies will be shown in support of video publications as a valid communication venue in scientific publishing.

231 ChemSpider as an Integration Hub for Interlinked Chemistry Data

A.J. Williams, Royal Society of Chemistry / eScience

The internet has provided access to unprecedented quantities of data. In the domain of chemistry specifically over the past decade the web has become populated with tens of millions of chemical structures and related properties, both experimental and predicted, together with tens of thousands of spectra and syntheses. The data have, to a large extent, remained disparate and disconnected. In recent years with the wave of Web 2.0 participation any chemist can contribute to both the sharing and validation of chemistry-related data whether it be via Wikipedia, the online encyclopedia, or one of the multiple public compound databases. Toxicologists commonly wish to source data, either for reference purposes, to support the development of models or, when experimental data are not available, predicted data will suffice. This presentation will offer a perspective of the type and quality of chemistry data available today, our experiences of building the ChemSpider public compound database to link together chemistry on the internet and our efforts to both encourage and enable even greater integration and connectivity for chemistry data for the community.

232 Passive Sampling and Online Systems Offer Citizen Scientists the Ability to Monitor Their Exposure in the Event of a Disaster

K.A. Hobbie, Oregon State University / Environmental Molecular and Toxicology; M.L. Barton, Oregon State University / Environmental Health Sciences Center; E.S. Peterson, K.M. Waters, Pacific Northwest National Laboratory; K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology

A well informed and trained group of citizen scientist offers researchers the power to quickly respond to environmental monitoring needs when resources are limited. This is especially the case when environmental disasters occur and baseline data is required to determine the magnitude of the event. Our lab has developed passive sampling technologies to monitor bioavailable organic contaminants in air, water and sediment, with easy to use deployment equipment to sample in a variety of conditions. This technology, coupled with a series of online training modules that include videos and training evaluation, certify citizen scientists to utilize passive sampling devices to monitor environmental exposures to various organic compounds. A series of form-based web applications running on Drupal, an open source web content management system, document the scientists training, provide an online ordering system to request passive sampling devices and equipment, and enable tracking of sample custody. This public web system is integrated through RESTful web services to various internal data management systems, which coordinate PSD stocks, sample indexing and capture sample results. This seamless interface between data systems enables a high throughput analysis of monitoring samples that syndicate information to peripheral data systems for environmental risk forecasting in near real time. Passive sampling technology and online training of citizen scientists with access to sampling equipment enable rapid response to environmental events while maintaining chain of custody and QC documentation, resulting in high quality and traceable results to inform stakeholders and the public when the outcome of an event is unknown.

233 Open-source collaboration and a "big data" approach to household spectrometry

J. Warren, S. Dosemagen, Public Laboratory for Open Technology and Science

Online communities, such as DIYDrones, PublicLab.org and SafeCast, are tackling ever more complicated technical projects through open source sharing and collaboration, more recently even hardware designs. "Big data" projects which approach data collection, analysis, and publication as a database-scale problem, such as OpenStreetMap, have demonstrated the remarkably high quality of aggregated data. The Public Lab's project at SpectralWorkbench.org includes both open source hardware and software for low-cost, amateur VIS-NIR spectral analysis, with a focus on contaminated water and soil. By developing a web-based data collection tool (the spectrometer plugs in over USB and connects directly to a web browser) and encouraging open source publication of data online, the Public Lab community is creating an open spectral library for the exchange of data as well as a suite of online tools and APIs for manipulating, interpreting, and comparing spectra. This talk will introduce the concepts of open source collaboration and data analysis through use of one of Public Lab's tools, the DIY spectrometer. We will discuss how, over the last year, the Public Lab tool development and engagement process has supported and helped grow a community of developers, scientists and environmental advocates interested in open, lowcost spectrometry.

234 CELLALL: Ubiquitous chemical sensing using mobile platforms

S. Dennis; D. Deininger, Synkera Technologies Inc; J. Li, NASA

With the advent of personal computing devices come a wide variety of value added technologies and applications that can be used support our health, safety and security. Over the past 10 years, industry has placed an increasing emphasis on the integration of sensors into personal devices for market differentiation. Over a several year period CellAll program worked with health officials, first responders, and industry representatives to examine the efficacy of integrated miniature chemical sensors into platforms such as personalized MP3 players and cellular phones. With successful laboratory proof of concepts, the program produced two prototypes that were evaluated in variety operational settings. These prototypes and evaluations demonstrate a clear future for ubiquitous sensing applications. Technologies developed under the CellAll program are now in niche markets while continuing to make their way into the broader marketplace.

235 Truly field portable instruments for measuring environmental toxins

D. Adkins, P. Lewis, Defiant Technologies

The traditional way to measure contaminants of concern in the environment has been to collect samples from multiple locations at multiple times, send the samples to a commercial laboratory for analysis, and wait days or weeks for the results. Lost in this approach is the ability to perform an on-the-spot assessment of the environment and quickly identify the presence and extent of a potential problem. With the advent of micro electro mechanical systems (MEMS) technologies, it is now possible to perform a gas chromatographic analysis in the field for site characterization and real time decision making. This presentation will discuss the fundamental approach to measuring environmental toxins in water, soil and air, and introduce some of the new technologies that can be applied to making these measurements. These new technologies include micro preconcentrators that collect samples from the environment and provide sharp injections for analysis, micro-gas chromatographic columns that can separate more than a dozen compounds for chemical identification, and small photo-ionization detectors that can sense compounds at levels below one part per billion. We will discuss the

sample preparation required for different mediums, and the procedures that are involved in analyzing many of the commonly encountered toxins. A comparison will be made between laboratory results and results obtained with portable equipment. While this presentation will focus on measuring volatile organic compounds using gas chromatography, it will also provide a brief discussion of techniques and equipment that is used to identify other chemical toxins.

Occurrence, Fate, and Behavior of Per- and Polyfluoroalkyl Substances in the Global Environment

236 Transport Potential of Perfluoroalkyl Substances (PFASs) at AFFF-impacted Sites: 1-Dimensional Column Studies

J.L. Guelfo, Colorado School of Mines / Hydrologic Science & Engineering; J.E. McCray, C.P. Higgins, Colorado School of Mines / Civil & Environmental Engineering

Recent implementation of soil and drinking water regulatory guidance values for two perfluoralkyl substances (PFASs), perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) by the US Environmental Protection Agency, reflects growing concerns regarding the presence of these persistent and bioaccumulative chemicals in the environment. Because of their unique properties, PFAS have a wide variety of uses including food packaging products, stain repellants, nonstick coatings, and aqueous film-forming foams (AFFF). AFFF is used to extinguish hydrocarbon fuel fires by fire departments, the hydrocarbon industry, and the military. PFAS in groundwater have been measured in the ng/L – µg/L range at fire training facilities where AFFF was repeatedly used in training exercises. These sites may also be impacted by co-contaminants, including non-aqueous phase liquids (NAPLs). The present work investigates the transport and reaction processes for 10 PFAS in the subsurface by evaluating 1-dimensional (1-D) column transport through soil and aquifer material in systems in the presence and absence of NAPL. Preliminary column results suggest that, consistent with batch systems, PFAS transport will be chain-length dependent with the exception of the smallest perfluorocarboxylates (PFCAs), which may be impacted by steric effects and/or ion exchange. Measured retardation factors were smaller than expected based on equilibrium sorption. Coupled with early breakthrough, this suggested that rate-limited sorption impacts transport of these compounds. Flow interruption experiments confirmed rate-limited sorption was occurring in soils with higher (1.7%) organic carbon content. Breakthrough curves will be used to determine rate constants, and these constants will be incorporated into a two-site kinetic nonequilibrium sorption equation to model transport. Column PFAS transport will be measured in 1-D systems with residual saturation of trichloroethylene (TCE) to determine how the presence of NAPL impacts PFAS transport in an advective scenario. These results will be compared to impacts of NAPL in batch equilibrium systems wherein NAPL increased sorption of small PFASs such as PFBA and caused concentration dependent impacts on larger PFASs such as PFOS. Column experiments are expected to have important implications for evaluating factors influencing PFAS transport at the field level.

237 Implications of In Situ Chemical Oxidation on PFAAs Fate and Transport in Porous Media

E.R. McKenzie, Colorado School of Mines / Civil and Environmental Engineering; L. Kudryk, Colorado School of Mines; J.L. Guelfo, Colorado School of Mines / Hydrologic Science & Engineering; R.L. Siegrist, Colorado School of Mines / Civil and Environmental Engineering; C.P. Higgins, Colorado School of Mines / Civil & Environmental Engineering

Perfluoroalkyl acids (PFAAs) are constituents in aqueous film-forming foam (AFFF) used to extinguish fires, and their use at protection training areas has been observed to result in elevated PFAA groundwater concentrations. Co-contaminants, such as chlorinated solvents and fuel hydrocarbons, are also commonly present and *in situ* chemical oxidation (ISCO) has commonly been employed to remediate such contaminants; however, it is currently unknown how ISCO remediation would impact PFAA fate and transport. In this research, the effect of chemical oxidation on the behavior of ten PFAAs in a porous media environment was investigated. Three oxidants were tested: permanganate, activated persulfate, and catalyzed hydrogen peroxide. Both batch equilibrium studies and 1-D column studies were employed. ISCO may produce effects on PFAA fate and transport due to one, or a combination, of the following: destruction of organic matter, destruction of a non-aqueous phase liquid (NAPL; i.e., the co-contaminant), formation of a new solid phase (e.g., MnO_2 precipitate), and changes in pH and other water quality parameters. In preliminary batch ISCO experiments, persulfate generally increased long-chain PFAA partitioning coefficient values (K_d) for both PFCAs and PFSAs compared to either permanganate or no-oxidant systems; this could be partially or wholly due to pH suppression (final pH 2.5–3). Permanganate was observed to have less of an effect on PFAA partitioning coefficient values. Inclusion of TCE had less of an effect on post-oxidant-exposure PFAA behavior, compared to oxidant type, indicating that presence of (and destruction of) the NAPL phase is likely not the most important factor affecting PFAA fate. On-going research efforts aim to elucidate the roles of pH, NAPL, and pH-NAPL interaction on PFAA behavior. Preliminary column experiments demonstrated that a permanganate oxidation produce effluent concentrations that exceed influent concentrations (i.e., $C/C_o>1$) for some PFAAs. Permanganate was observed to increase the exportation of organic matter, which likely contributed to the sharper observed post-oxidation sorption and desorption breakthrough curves. For columns studies conducted with high average linear velocities (600 cm/d), fitted retardation factors were less than predicted (i.e., less attenuation was observed), indicating that PFAA-soil interactions may not have reached equilibrium.

238 Release of poly and perfluoroalkyl substances (PFASs) from municipal solid waste under model landfill reactor conditions

B. Allred, Chemistry Department; J.A. Field, Oregon State University / Department of Environ. & Molecular Tox.; J. Lang, M. Barlaz, North Carolina State University / Department of Civil, Construction, and Environmental Engineering

Landfills represent the dominant disposal alternative for municipal solid waste (MSW) in many parts of the world including the US The leachate generated from MSW landfills is a highly complex matrix containing a myriad of compounds of environmental concern, including poly and perfluoroalkyl substances (PFASs). PFASs are of particular concern because they are generally not degraded in the conventional biological wastewater treatment processes used to treat landfill leachate. Laboratory bioreactors filled with shredded MSW were operated with circulating pH-neutralized leachate under carefully controlled methanogenic conditions to mimic accelerated decomposition in a landfill. Biologically-active bioreactors and control reactors treated with microbial inhibitors were evaluated to differentiate PFAS release due to abiotic leaching and biologically-mediated MSW decomposition. Microcosms also were constructed to focus on selected fractions of solid waste including paper, textiles, and carpet. Streptomycin and Dowicil QK-20 (2,2-dibromo-3-nitrilopropionamide) were used to inhibit microbial growth in the control (abiotic) reactors and microcosms. Anaerobic decomposition in the reactors was characterized by methane production rate, leachate pH, and chemical oxygen demand (COD). The PFAS concentrations and composition of leachate were determined for the bioreactors and microcosms. Initial efforts focused on verifying that the bioreactors gave low background PFAS concentrations. Reactors containing no solid waste were first assembled and operated with synthetic model leachates that represent the composition of acid- and methane-leachates that occur during MSW decomposition. The only PFAS detected in background for assembled bioreactors was PFOS at ≤ 11 ng/L. Initial data from the first month of operation indicated generation of PFAS in biologically-active reactors with concentrations increasing significantly above background concentrations in the first 10 days of operation. The most abundant PFAS in leachate was PFBS, the C4 carboxylate. PFBS concentrations of ~400 ng/L were a factor of three greater than those of PFOA (~150 ng/L). Perfluoroalkyl sulfonates remained at or near the background concentrations (~10 ng/L). Temporal trends in the concentration and composition of PFAS for biologically-active and control reactors and microcosms will be discussed.

239 Global environmental fate of C6-C10 perfluoroalkane sulfonic acid (PFSA) homologues from 1958 to 2030: sources and transport

Z. Wang, Swiss Federal Institute of Technology / Institut fur okologische Chemie, ETH Zürich / Institute for Chemical and Bioengineering; I.T. Cousins, Stockholm University / Applied Environmental Science (ITM); M. Scheringer, ETH Zürich / Institute for Chemical and Bioengineering; M. MacLeod, ITM – Stockhom University / Department of applied environmental science; K. Hungerbuehler, ETH Zürich / Institute for Chemical and Bioengineering

Long-chain perfluoroalkane sulfonic acids (PFSAs, $C_nF_{2n+1}SO_3H$, n = 6–10), especially perfluorooctane sulfonic acid (PFOS), are a group of global contaminants of high concern due to their persistence, bioaccumulative

potential and global presence in the environment, biota, humans and food items. However, the scientific understanding of their sources and cycling in the environment is still limited. Several recent studies reveal new emission pathways for PFOS and its precursors to the environment that are not included in previous emission inventory and environmental fate modeling studies. Additionally, previous environmental fate studies have been restricted to PFOS and its precursors although several shorter and longer homologues, either as impurities or active ingredients, have also been produced and released into the environment over many years. Hence, in this study, we aim to update and extend the current global emission inventory of PFOS to all C_6–C_{10} homologues and their potential precursors, based on an extensive analysis of new data sources. We estimate that 2700–7000 tonnes and 3600–6000 tonnes of PFOS precursors and PFOS are emitted between 1958 and 2015, respectively. Our new estimates are much higher than the previously estimated values (700–4000 tonnes and 350–3300 tonnes for PFOS precursors and PFOS, respectively). In order to fully evaluate the plausibility of our emission inventory, we have conducted a series of modeling exercises where we combine the new emission inventory and a global-scale multimedia mass-balance model, CliMoChem, and compare model results with field measurement, particularly in outdoor air, precipitation and ocean surface water. Furthermore, we employ the model (i) to understand the fate and transport of C_6–C_{10} PFSA homologues to remote polar regions and (ii) to evaluate whether and under what circumstances the degradation of PFOS-related side-chain fluorinated polymers can be an important source of PFOS in the foreseeable future, so as to provide information for future risk reduction measures.

240 Distribution of Perfluoroalkyl Compounds in the Atmosphere and the Transportation to Ground Environment at Dalian, China

W. Liu, School of Environmental Science and Technology, Dalian University of Technology

The occurrence of perfluoroalkyl compounds (PFAAs) in the atmosphere is critical in influencing their regional and global distribution and transportation. In the present study, particulate matters (PM) were collected from 2007-2012 at Dalian, to study the temporal trend of the PFAAs contamination in the atmosphere. Four perfluorosulphonates (C4, 6, 8, 10) and nine perfluorocarboxylates (C5-12, C14) were analyzed by HPLC-MS/MS, where internal standard calibration was employed for quantification. The sum of PFAAs concentrations (ΣPFAAs) were detected in the range of 59.7-861.5 pg/m3 (237-6978 ng/g PM). Relatively high ΣPFAAs were observed in 2007-2008, while atmospheric PFAAs levels decreased since 2009 and remained at comparable level in 2009-2012. In addition, the PFAAs levels in the ground environment nearby a preserved area, where precipitation might act as main pollution sources, were measured to evaluate the transportation of PFAAs to environmental water and soil via rain scavenging. Both the surface microlayer water (50 μm) and surface water (0-20 cm), surface soil (0-20 cm) and deep soil (50-60 cm) were collected before and after the rain event. The effect of precipitation on PFAAs levels in the ground environment seemed to be complicated, where influencing factors possibly include contamination background level and the precipitation amount. In summer, PFAAs concentrations in the environmental water decreased after the rainfall due to the dilution effect. In contrast, an increase of PFAAs concentration in the water was observed in autumn/winter. As for the soil analysis, a significant increase of PFAAs level in deep soil after rain was observed, with a decrease in surface soil, suggesting the transportation of PFAAs from surface to deep soil via the washout effect of rainwater. And the transportation of PFAAs in the soil via wet precipitation possibly acts as a contamination source for the underground water.

241 Isomer Specific Binding of Perfluorinated Alkyl Substances to Human Serum Albumin and Transplacental Transfer

S. Beesoon, University of Alberta / Div. of Analytical and Environmental Toxicology, Department of Laboratory Medicine and Pathology; D.G. Hemmings, University of Alberta / Obstetrics and Gynecology; J.W. Martin, University of Alberta / Laboratory Medicine and Pathology

PFOS and PFOA are among the most environmentally relevant members of the family of perfluorinated alkyl substances (PFAS). Prior to 2001, both these compounds were manufactured by an electrochemical fluorination method that yielded a mixture of linear and various branched isomers which still persist in the environment. Analysis of paired maternal and fetal blood have shown that the transplacental transfer efficiencies (TTE) of branched PFOS and PFOA isomers are higher than the corresponding linear isomer, but the reason(s) for this have not been studied. We hypothesized two mechanisms that would lead to enhanced TTE of branched PFOS and PFOA isomers: 1) that the binding affinity of branched PFOA and PFOS to human serum albumin would be less than the linear isomers and 2) that the branched isomers can cross the syncytiotrophoblast, the placental layer at the maternal-fetal interface, more efficiently than linear molecules. We first carried out a series of binding affinity tests using the Centrifree® Ultrafiltration device which separates albumin-bound PFOS and PFOA from the free molecules. Ultrafiltrates were analysed by an isomer specific liquid chromatography-MS/MS method. Dissociation constants of linear and three individual PFOS and PFOA isomers indicated that linear PFOS and PFOA were more strongly bound to human serum albumin compared to their corresponding branched isomers. Secondly, we designed an in-vitro model of the placental layer in direct contact with maternal blood by culturing a monolayer of human placental syncytiotrophoblasts on cell inserts. The maternal side of the model was challenged with a mixture of linear and branched isomers of PFOS and PFOA. Cell culture medium on both maternal (apical) and fetal (basal) sides of the insert cultures were collected and analysed as above. Results confirmed that the TTE of branched isomers was greater than for the corresponding linear isomer. Thus, two mechanisms were identified which, together or on their own, can explain consistent observations from human biomonitoring studies.

242 What can we learn about the fate of PFASs from retrospective temporal trend analysis in environmental and human samples? A Swedish case study

U. Berger, Stockholm University; A. Bignert, Swedish Museum of Natural History; A. Glynn, National Food Agency; K.E. Holmstrom, SWECO; J.H. Johansson, Stockholm University; E. Nyberg, A. Roos, Swedish Museum of Natural History; S. Ullah, Stockholm University

Per- and polyfluoroalkyl substances (PFASs) are a huge group of man-made chemicals with a variety of physical and chemical properties and partitioning behavior. Some PFASs have been produced and emitted to the environment for more than 60 years. The perfluoroalkyl acids (PFAAs) are a subgroup of PFASs with extraordinary persistence, while other PFASs (so called precursors) can be transformed in the environment and/or metabolically to form persistent PFAAs. Taken together, this results in a highly complex interplay of compounds in different environmental processes. Despite intense research during the last twelve years, our understanding of environmental chemistry and fate of PFASs, their bioaccumulation, and human exposure remains fragmented. To investigate these processes experimentally in the lab or in field studies is challenging due to analytical limitations such as the number of PFASs that can be included in a certain type of analysis and the very low levels of many PFASs in various environmental matrices. Limitations of theoretical studies are thus often the lack of accurate analytical input data as well as uncertain property estimates for PFASs. In this presentation we take an indirect approach to shed light on poorly understood processes. We investigate to which extent temporal trend monitoring studies of PFAAs (and to a limited extent also precursors) can be exploited to draw conclusions on environmental fate and bioaccumulation of as well as human exposure to PFASs. During the last years we have conducted a number of retrospective time trend studies on PFASs in different Swedish environments (terrestrial, limnic, marine) and matrices (fish, bird eggs, mammals, humans, food items). Each of the investigated matrices showed distinct changes over time in both PFAS concentrations and patterns, reflecting the unique "position" of the sample matrix relatively to the environmental processes that the PFASs undergo. Comparing these differences in temporal trends between the studies allows hypothesizing on emissions (e.g., to air or water), environmental transport processes (e.g., retention in soil), bioaccumulation (e.g., elimination of PFAAs in different species), and human exposure (e.g., exposure pathways and relative importance of precursors). These hypotheses will be put in the context of current paradigms of environmental chemistry and fate of PFASs.

243 Levels of unidentified organofluorine and commercial fluorosurfactants in human blood

L.W. Yeung, University of Toronto / Chemistry; S.A. Mabury, University of Toronto / Department of Chemistry

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) are anthropogenic chemicals and have been used in various industrial and daily applications for over 5 decades. Many of them are resistant to hydrolysis, photolysis, biodegradation, and metabolism. Their unique oil-repellency and high surface activity render them excellent surface protectors and surfactants. A previous study demonstrated that quantifiable PFASs accounted for approximately 48% on average (range: 30-90%) of the extractable organofluorine (EOF) in Chinese blood, indicating the presence of significant amount of unidentified organofluorines. Polyfluoroalkyl phosphate diesters (PAPs), perfluoroalkyl phosphonates (PFPAs), perfluorinated phosphinates (PFPiAs), and fluorotelomer sulfonates (FTSAs) are commercial fluorosurfactants that have been newly identified and reported in different environmental matrices. These chemicals might account for a portion of the unidentified organofluorine. In the present study, a single LC-MS/MS method using a water/methanol gradient containing 0.1% aqueous ammonium hydroxide was developed to analyze a suite of 48 known PFASs in Chinese (n=47) and German blood (n=23). PFPAs and monoPAPs that had been previously reported as suffering severe tailing on a C18 reverse phase column were separated using this method. An ion pair method was used to extract PFASs in blood samples; known PFASs and EOF were analyzed using a Waters Acquity UPLC coupled to a XeVo TQ-S (MS/MS) and Total organofluorine – combustion ion chromatography (TOF-CIC), respectively. Among the newly identified commercial fluorosurfactants, diPAPs: 6:2, 6:2/8:2, and 8:2; and FTSAs: 6:2 and 8:2 were detected in 40% of the German samples, and only 8:2 FTSA was detected in three of the Chinese samples. A mass balance analysis between known PFASs and EOF were conducted. Known PFASs accounted for 48-90% of the EOF, where PFOS was the major constituent (over 80%) of the known PFASs in Chinese blood. In German samples, in the periods of 1982-1999 and 2006-2009, known PFASs were 91% on average (range 63-100%) and 60% (26-100%) of the EOF, respectively. These results suggested that unidentified organofluorines at significant concentrations, similar to PFOS, were present in some cities in China and comparatively higher levels of unidentified organofluorines were observed after year 2000 in Germany.

Fate and Effects of Metals: Regulatory and Risk Assessment Perspective

244 Draft Reassessment of the 1988 Ambient Water Quality Criteria for Aluminum

D.M. Eignor, USEPA / Office of Water; C. Voros, Great Lakes Environmental Center; K.K. Taulbee, Great Lakes Environmental Center

The US Environmental Protection Agency (EPA) is in the process of updating its 1988 recommended aquatic life criteria for aluminum under Clean Water Act Section 304(a). The 1988 criteria account for the influence of pH on toxicity, similarity of acute toxicity of fish and invertebrates, and the greater sensitivity of invertebrates in chronic toxicity tests. The document recommended an acute criterion of 750 µg/L and a chronic criterion of 87 µg/L when the pH is between 6.5 and 9.0. Over the past 25 years, new acute and chronic toxicity data for both freshwater and saltwater organisms have been published. Sixteen freshwater species are represented in the current dataset of acceptable data for acute toxicity to aluminum. There is no freshwater taxonomic group that is uniformly more sensitive to the acute effects of aluminum. The acceptable chronic freshwater data indicate fish are slightly more sensitive than invertebrates. There are limited saltwater acute data and no acceptable saltwater chronic data. One of the technical issues being addressed in the reassessment is the correction factor for hardness and pH for acute aluminum toxicity. Previous research has demonstrated that aluminum toxicity is affected by both hardness and pH, and the independent and combined effects of hardness and pH on aluminum toxicity were evaluated using all acceptable studies in the acute dataset. There was a statistically significant relationship between acute toxicity and hardness that applied to all species in the dataset except for rainbow trout. For rainbow trout, the slope of the relationship between hardness and acute aluminum toxicity was significantly shallower than for all other species, and data from this species were not included in the pooled hardness correction slope. In contrast, the effects of pH on acute Al toxicity could not be included in a multiple regression model for these data, because a pooled pH correction slope could not be calculated for tested species. An analysis of covariance (ANCOVA) was performed to evaluate whether a pH correction slope would provide any statistically significant additional information to acute Al toxicity. The ANCOVA results suggested that after accounting for the effects of hardness, pH conveyed no statistically significant additional effect on acute Al toxicity. EPA is incorporating these new toxicity data and additional statistical analyses in the draft aluminum criteria recommendation under development.

245 Chronic Classification of Nickel Concentrates Based on Transformation/Dissolution Characteristics

J.M. Skeaff, CanmetMINING, Natural Resources Canada; R. Beaudoin, CanmetMINING, Natural Resources Canada

The EU (European Union) hazard classification scheme for metals and sparingly soluble metal compounds under the 2nd ATP (Advances to Technical Progress) to their CLP (Classification, Labelling, and Packaging) regulation calls for a derivation of chronic or long-term aquatic hazards by comparison of metal concentrations released at 0.01, 0.1 and 1 mg/L substance loadings under T/D (Transformation/Dissolution) conditions with a chronic ERV (Ecotoxicity Reference Value). Since, in an operational laboratory, it is generally not feasible to examine the T/D characteristics of metals and metal compounds at such low loadings, the only reasonable alternative appears to be a scaling approach in which seven-day T/D data from 100 and 10 mg/L loadings, and seven- and 28-day T/D data from 1 mg/L loadings would be extrapolated to determine the 28-day metal concentrations at the 0.01 and 0.1 mg/L loadings. In this presentation, we show how we validated the extrapolation approach for a nickel matte and provide worked examples as applied to nickel concentrates designated as A, B, C and D. We analysed T/D solutions taken at specified intervals for the total dissolved concentrations of Ni, Fe, Cu, Co and As. The Ni contents of the concentrates varied in the range 7%-29%. We found that, among all five analytes, Ni was released into the T/D solutions in the highest concentrations at pH 6. For instance, at pH 6 and the 1 mg/L loading of the one concentrate, the 168-hr values of Ni(aq), Cu(aq), Co(aq) and As(aq) were about 36, 0.7, 0.4 and 0.9 mg/L, respectively. These values of Ni(aq) and Cu(aq) represented about 27% and 22% of the metals in the Ni concentrate, respectively. Using a regression analysis-scaling approach, we derived 672-hr values of Ni(aq) for each of the four Ni concentrates at loadings of 0.1 and 0.01 mg/L for use in establishing chronic classification proposals. Comparing these derived Ni(aq) values against the ERV of 2.4 microg/L for dissolved Ni, we found that the A, C and D concentrates would all classify as Aquatic Chronic 2 under both the UN GHS and the EU CLP schemes, and the B concentrate would classify as Aquatic Chronic 3 under both schemes.

246 Attenuation of the Potential Impacts of Copper Roof Runoff by Stormwater Best Management Practices

B. Labarre, S.M. Lev, D.R. Ownby, Towson University / Urban Environmental Biogeochemistry Laboratory; K.J. Rader, Mutch Associates, LLC, Manhattan College / Civil and Environmental Engineering; R.E. Casey, Towson University / Urban Environmental Biogeochemistry Laboratory

Within the last 20 years, concerns have been raised by regulators over diffuse and non-point sources of metals including releases from copper roofs during storm events. A copper roof runoff tool is in development for use by regulators and other stakeholders to quantify the potential ecological impacts of copper roof runoff in receiving waters. It considers three key components of copper runoff fate and transport: 1) emission from roofs as runoff during rain events; 2) attenuation of runoff prior to release to receiving waters; and 3) bioavailability and fate in receiving waters. Emission rates and bioavailability are quantified using the copper roof emission algorithm developed at the Royal Institute of Technology in Sweden and the biotic ligand model (BLM), respectively, both of which have been validated. Understanding attenuation processes between the downspout and receiving water represents a data gap. The goal of this study is to collect data to fill this gap and provide information with which to test the copper roof runoff tool. Specifically, this study investigates the potential for two different BMPs to decrease both the copper quantity and the potential for biological stress in copper roof runoff. A 10 x 20 ft copper roof structure was built along with two biofiltration swales and two bioretention planters. The roof was sectioned into quarters; each drains into one of the BMPs. Flow and chemistry data were collected from seven storms. The BMPs consistently remove approximately 88-99% of

copper (total and dissolved). Total copper concentrations in BMP influent averaged 980 µg/L while BMP outflows averaged 50 µg/L for planters and 20 µg/L for swales. Compared to roof runoff, water exiting the BMPs had increased concentrations of copper-binding ligands, principally dissolved organic carbon. BLM-predicted *D. magna* LC50 values in effluent were increased 9 to 513 times the respective influent values (e.g., chronic predicted values were 0.12 µg/L for influent and 14.7 µg/L for effluent). Thus, toxicity potential was reduced both by the decrease in copper concentrations as well as the reduction in bioavailability. While BMP effluent values may still exceed some water quality criteria, it is clear the BMPs are improving water quality before it encounters biological receptors. This attenuation data, when integrated into the copper roof runoff tool, will allow more accurate assessment of the potential impacts in receiving waters.

247 Dynamic Speciation and Toxicity During Aging of Cu-spiked Sediments in a Flow-through Mesocosm

D. Costello, A. Harrison, University of Michigan / School of Natural Resources & Environment; D. Marsh, Wright State University; R. Mendonca, University of Michigan; G. Burton, University of Michigan / School of Natural Resources & Environment and Cooperative Institute for Limnology & Ecosystem Research; C. Hammerschmidt, Wright State University

In sediments, the partitioning of metals to solid-phases can reduce the bioaccessible (and potentially toxic) fraction of metal. Under anoxic conditions, reduced sulfur (e.g., AVS) and organic carbon are the primary binding fractions for metals; however, these fractions may not be as important under oxic conditions, like those common to surficial sediments in lotic ecosystems. Our research aims to improve metal bioavailability models for stream ecosystems by assessing the role of oxic sediments in sequestering metals. Two reference sediments, with differing binding capacities, were spiked and equilibrated under anoxic conditions with five concentrations each of Cu. These sediments were then aged in a flow-through mesocosm while concurrently exposing *Hyalella azteca* to those sediments to measure changes in toxicity as the sediment ages. Frequent temporal sampling produced a fine scale understanding of geochemical and toxicological dynamics in the sediment as surface sediments oxidized. Through time, oxygen penetration depth increased and oxidized surface sediment (2-5 mm) developed. In the surface sediment, total Cu remained stable, AVS declined, and simultaneously extracted Cu increased through time, which suggests oxidation of CuS but retention of remobilized Cu in the sediments. Although current equilibrium partitioning models (e.g., SEM-AVS) measured an increase in Cu bioavailability though time, our toxicity tests (7 day, H. azteca growth) measured a decline in toxicity as these sediments oxidized. Our data suggest that Fe and Mn oxide surfaces may sorb any Cu released from oxidized CuS, thus limiting the bioavailability of the metal. These data suggest that the current bioavailability models may be inadequate for sediments with oxidized surface sediments and future research should focus on developing bioavailability models that include Fe and Mn oxides.

248 Evaluation of Possible Freshwater Guidelines for Lead Using Multiple Linear Regression

D.K. DeForest, L. Tear, Windward Environmental LLC; B.G. Church, Windward Environmental LLC / Department of Environmental Sciences; J.R. Elphick, Nautilus Environmental; K.V. Brix, University of British Columbia / Biology, University of Miami, RSMAS

Biotic ligand models (BLMs) are now available for several metals. In Europe BLMs have been widely applied in ecological risk assessments and both regional and national water quality guidelines are under development that will incorporate BLMs, while in the United States the USEPA has recommended BLM-based water quality criteria for Cu in freshwater. However, despite the availability of BLMs, most US states and several other regulatory jurisdictions have been slow to adopt the BLM into the development of regulatory criteria or guidelines. Many regulatory jurisdictions have hardness-based criteria for metals in which the pooled slope of the relationship between toxicity and hardness is used to adjust metals criteria as a function of site-specific hardness. We evaluated the possibility of developing freshwater guidelines for Pb using a multiple linear regression (MLR) approach that considers the potential importance of additional water chemistry parameters on Pb bioavailability (e.g., DOC, pH). The resulting guideline equations are similar to the commonly used hardness-based equations. Because water chemistry influences the bioavailability of Pb to organisms differently (i.e., the important water chemistry parameters for one species may differ for another species), consideration must be given to the MLR model(s) that are most relevant to a guideline that is intended to be protective of a large variety of organisms over a wide variety of water chemistries. This evaluation demonstrated that current hardness-based criteria or guidelines may be significantly under-protective or over-protective depending on water chemistry and that the MLR-based approach may provide an alternative approach for developing water quality criteria or guidelines that more appropriately account for site-specific bioavailability.

249 Derivation of ecologically relevant effects threshold concentrations for lead in surface waters: a European experience

P. Van Sprang, ARCHE; C. Nys, University of Ghent; R. Blust, University of Antwerp; J. Chowdury, ILZRO; K. De Schampelaere, University of Ghent

In the REACH framework, lead (Pb) specific information on environmental toxicity and exposure/fate for key environmental compartments (water, sediment, soil) was compiled in order to assess the potential environmental risks related to the production and use of Pb in the European Union. The methodologies and concepts used in the present study represent the state-of-the-art about Pb specific aspects related to incorporation of bioavailability and large dataset handling. The proposed assessment encompasses the development of a quality-screened database containing 173 individual chronic toxicity data points for 25 different freshwater species and takes the effects of 1) Pb precipitation and 2) Pb speciation/competition on Pb toxicity fully into account by the use of newly developed Pb-total/dissolved translator and chronic Biotic Ligand Models (BLM) for 3 different trophic levels (algae, invertebrates and fish). As such, the approach allowed to normalize the freshwater Pb effects database towards the geochemical conditions prevailing in the EU surface water under scrutiny, and revealed that the safe toxic threshold for the freshwater environment (HC5-50) was expected to vary between 8.8 and 29.6 µg dissolved Pb/L. The statistical best fitting approaches that were used for the derivation of the freshwater HC5-50 from the species sensitivity distributions will be further discussed. Finally, the conservatism and uncertainties considered for the derivation of HC5-50 will be highlighted and a way forward in order to reduce the uncertainties associated with metal risk assessments in general will be proposed.

250 Total mercury concentrations in sharks of the Mexican Pacific Coast and its health risks

P. Ramirez Romero, R. Terrazas Lopez, U.A.M. Iztapalapa / Hidrobiologia; M. Ramirez Islas, INECC / CENICA; J. Padilla Torres, I. Arellano Lopez, U.A.M. Iztapalapa / Hidrobiologia

Mercury is a non-essential metal that is bioaccumulated and biomagnificated, this translates into a health risk for top predators, including human beings. The objectives of the present study was to quantify total mercury concentrations in sharks captured in different fishing localities of the Mexican Pacific Coast between 2009 and 2012 and to evaluate human health risk due to consumption of shark meat. A total of 98 samples of shark dorsal muscle were obtained in four localities, transported frozen to the laboratory in Mexico City and analyzed with AA spectrophotometry. Also, fish consumption surveys were applied to fishermen and their buyers to calculate shark consumption in men, women of reproductive age and children. In general, shark samples presented high total mercury concentrations (up to 3.95 ppm), the highest concentrations were found in the South Mexican Pacific samples and the lowest in those from the middle region. A good linear relation between mercury and shark size was found for female *Carcharinus falciformis*, which was attributed to their feeding habits since pregnant females feed closer to the coast and therefore closer to mercury sources. Human health risk analysis showed that in general people are not at risk since culturally people consume low quantities of marine products and even less shark; however shark meat was not considered a good option for females in reproductive age and children. It is necessary to continue monitoring mercury in sharks and other marine products to prevent toxic effects in the vulnerable population.

251 Critical Review of Mercury Sediment Quality Values for Protection of Benthic Invertebrates

J.M. Conder, M.M. Grover, ENVIRON International Corp; P.C. Fuchsman, ENVIRON International Corp; V.S. Magar, ENVIRON International Corp / Ecology & Sediment Management; M.H. Henning, ENVIRON International Corp

Sediment Quality Values (SQVs) are commonly applied to characterize the need for sediment investigation, understand causes of observed effects, and derive management strategies to protect benthic invertebrate communities. Among the 40-plus SQVs for mercury that we compiled for this study, nearly all are "co-occurrence" SQVs derived from databases of paired chemistry and benthic invertebrate effects data from field-collected sediment samples. Co-occurrence SQVs are not derived in a manner that reflects cause-effect, concentration-response relationships for single chemicals such as mercury, because multiple potentially toxic chemicals and other stressors often co-occur with mercury in the datasets used to derive SQVs. We assembled alternative lines of evidence, specifically, results of more than 20 laboratory studies with mercury-spiked sediments and toxicity and/or benthic community studies at mercury-contaminated sites (e.g., chloralkali facilities, mercury mines). Co-occurrence SQVs (0.05-2.7 mg/kg) were generally one to two orders of magnitude lower than LOECs, NOECs, and EC50s observed in mercury-spiked toxicity studies (0.08-10.5 mg/kg) and mercury site investigations (0.3-1,200 mg/kg). Additionally, there is a high degree of overlap between co-occurrence SQVs and naturally-occurring mercury levels in sediment to which benthic invertebrates have adapted over the millennia. Mercury co-occurrence SQVs do not reflect effect thresholds for benthic invertebrates and should not be used to characterize or manage risks at sediment sites; the spiked sediment and mercury site data compiled in our review provide a more useful alternative information source. Further research is needed to refine mercury effect thresholds, provide better tools for measuring mercury exposure and bioavailability to invertebrates, and advance theoretical understanding and modeling capability for predicting mercury bioavailability and toxicity in sediment.

Characterization and Processes of Atmospheric Pollutants

253 Atmospheric Polychlorinated Biphenyl Congeners and Synthetic Musk Fragrances in Chicago and Lake Michigan

Z.L. Rodenburg, University of Iowa / Dept. of Civil and Environmental Engineering; D. Hu, The University of Iowa / Department of Civil & Environmental Engineering, IIHR-Hydroscience and Engineering; D. Hu, University of Iowa / Chemical and Biochemical Engineering / Department of Civil & Environmental Engineering, IIHR-Hydroscience and Engineering, Public Policy Center and School of Urban and Regional Planning; Y. Ma, School of Public and Environmental Affairs; M. Venier, Indiana University / School of Public and Environmental Affairs; R.A. Hites, Indiana University / School of Public & Environmental Affairs; K.C. Hornbuckle, University of Iowa / Civil and Environmental Engineering, IIHR-Hydroscience and Engineering

Polychlorinated biphenyl (PCB) concentrations in Chicago air near Lake Michigan declined rapidly following the production ban of Aroclors over 30 years ago. However, since the early 1990s, concentrations of these chemicals have decreased only slightly. Synthetic musk fragrances (SMFs), alternatively, are still used in many consumer products, although their atmospheric concentrations in urban and suburban areas are not particularly well characterized, spatially or temporally. In order to better understand contemporary atmospheric concentration trends for these two classes of chemicals in a large metropolitan area, an extensive passive air sampling network has been established and operating throughout Chicago and along an urban-rural gradient extending beyond its suburbs since December, 2010. Furthermore, during September, 2010, an active field sampling expedition was conducted on Lake Michigan aboard the EPA R/V Lake Guardian approximately 5 km off the coast of Chicago. Passive samples were collected on polyurethane foam discs (PUF), and high-volume active air and water samples were collected using Amberlite XAD-2 resin as an adsorption media and quartz fiber filters (QFF) to collect particles in both phases. Air samples were extracted via Accelerated Solvent Extraction (ASE) and water samples via sohxlet prior to analyzing for 209 PCB congeners (sum of 158 peaks), six polycyclic musks (HHCB, AHTN, DPMI, ATII, ADBI and AHMI) and two nitro musks (musks ketone and xylene) using gas chromatography tandem mass spectrometry (GC/MS/MS). Sample concentration data will be used to determine spatial and temporal variability among sampling sites, identify potential emission sources, and calculate instantaneous PCB congener fluxes across the airwater interface at the Lake Michigan location. The analysis also includes a comparison of concentration data for PCBs and SMFs with those for polycyclic aromatic hydrocarbons (PAHs) and organohalogen and organophosphorus flame retardants collected with duplicate samplers and analyzed at Indiana University.

254 Differences in spatial and temporal variations of atmospheric PAH concentrations between North America and Europe

L. Liu; M. Venier, Indiana University / School of Public and Environmental Affairs; P. Kukucka, Masaryk University / RECETOX; R.A. Hites, Indiana University / School of Public & Environmental Affairs

Atmospheric concentrations of high molecular weight polycyclic aromatic hydrocarbons (PAHs) obtained at five sites (both urban and remote) near the North American Great Lakes, as part of the Integrated Atmospheric Deposition Network (IADN), and at three remote sites around Europe, as part of the European Monitoring and Evaluation Programme, were analyzed. The primary objectives were to reveal the spatial distributions, long-term temporal trends, and seasonal variations of atmospheric PAH concentrations; to investigate the differences between these two regions; and to give plausible reasons for the regional differences (if any). Atmospheric PAH concentrations at the urban sites in Chicago and Cleveland near Great Lakes were 8–100 times (depending on PAH compounds and sampling sites) greater than those at the remote sites except for Kosetice in the Czech Republic. Atmospheric PAHs concentrations at Kosetice, also a remote site, were slightly lower than those at Chicago, but 5–70 times higher than those at other remote sites (Sturgeon Point, Sleeping Bear Dunes, Eagle Harbor, Sweden, and Norway). Significant long-term decreasing trends of all these PAH were observed at the urban sites, with halving times of 5.4–9.3 and 6.0–13.4 years, respectively. For the other sites, either less significant or no long-term decreasing trends were observed. Clear seasonality was observed at the remote sites, with the highest PAH concentrations observed in the winter (January or February). Variations in regional anthropogenic activities are likely to be the plausible reasons for the spatial and temporal differences of atmospheric PAH concentrations, which we observe.

255 Using Polyethylene Passive Samplers to Analyze Spatial Gradients of Legacy and Emerging Contaminants in the Atmosphere of the Great Lakes Region

C. McDonough, URI Graduate School of Oceanography; M. Abd El-Hamid Khairy, University of Rhode Island / Graduate School of Oceanography; R. Lohmann, University of Rhode Island / Graduate School of Oceanography

Polyethylene passive samplers (PEs) are a cost effective and efficient means of measuring hydrophobic organic contaminants (HOCs) in air or water at many locations simultaneously, facilitating analysis of spatial distributions. The applicability of PEs in measuring ubiquitous contaminants such as polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) is well documented. Less is known about the applicability of PEs to studies of emerging contaminants. Here, extracts from atmospheric and aqueous PEs deployed in the Lake Erie and Lake Ontario region were analyzed using gas chromatrography/mass spectrometry (GC/MS) and tandem mass spectrometry (GC/MS/MS) to measure PAHs and polybrominated diphenyl ethers (PBDEs), respectively. Negative chemical ionization (NCI) mass spectrometry was also employed to attempt to identify halogenated organics. Spatial gradients in levels of PAHs, PBDEs and emerging contaminants are analyzed to identify useful proxies and possible sources. Initial results suggest that population is an effective proxy for PAH concentrations in the Great Lakes region. PAHs are well correlated with population within a 20 km radius surrounding each sampling site (r^2 = 0.71 for Σ16 PAHs),), while PBDEs are less so (r^2 = 0.29 for Σ12 BDEs).

256 Prediction, Identification, and Mutagenicity of Novel Nitro-PAHs Formed via Atmospheric Heterogeneous Reactions on Beijing Particulate Matter

S. Simonich, Oregon State University / Depts. of Chemistry and Environmental & Molecular Toxicology; N. Jariyasopit, Oregon State University / Department of Environmental & Molecular Toxicology; M. McIntosh, T. Yu, R. Dashwood, Oregon State University; K. Zimmerman, J. Arey, R. Atkinson, UC-Riverside

The heterogeneous reactions of five higher molecular weight polycyclic aromatic hydrocarbons (PAHs), benzo[a]pyrene-d_{12} (BaP-d_{12}), benzo(k) fluoranthene-d_{12} (BkF-d_{12}), benzo[g,h,i]perylene-d_{12} (BghiP-d_{12}),

dibenzo(a,i)pyrene-d_{14} (DBaiP-d_{14}), and dibenzo[a,l]pyrene (DalP), with NO_2, NO_3/N_2O_5, O_3, and OH radicals were investigated in a 7000 L indoor Teflon chamber. Quartz fiber filters (QFF) were used as the reaction surface and substrate and the analyses of parent PAHs and Nitro-PAH (NPAH) products was conducted using electron impact gas chromatographic mass spectrometry (GC/MS) and negative chemical ionization GC/MS. In addition, ambient PM samples collected from Beijing, China were exposed in the same indoor chamber under simulated trans-Pacific atmospheric transport conditions and the formation of NPAHs was studied. NPAHs were most effectively formed during the NO_3/N_2O_5 exposure and, for all exposures, there was no significant formation of 2-nitrofluoranthene or 2-nitropyrene, suggesting that heterogeneous reactions predominated. The reacted and unreacted filter extracts were tested in the Ames assay, using *Salmonella* strains TA98 (with and without metabolic activation), to determine changes in mutagenic activities upon exposures. In parallel to the laboratory experiments, a theoretical study was conducted to assist in determining the formation of NPAH isomers based on the OH-radical initiated reaction. The thermodynamic stability of OH-PAH intermediates was used to indicate the position of highest electron density and the most stable NPAH products were synthesized to confirm their identity. NO_2 and NO_3/N_2O_5 were the most effective oxidizing agents in transforming PAHs deposited on filters to NPAHs, under the experimental conditions. Reaction of BaP-d_{12}, BkF-d_{12} and BghiP-d_{12} resulted in the formation of several mono-nitro PAH isomer product, while the reaction of DalP and DaiP-d_{14} resulted in the formation of only one mono-nitro PAH isomer product. In addition, the degradation of particulate matter (PM)-bound PAHs by heterogeneous reaction with OH radicals, O_3, NO_3/N_2O_5 was also studied. The importance of this research with respect to atmospheric long-range transport of PM-bound PAHs will be discussed.

257 Utilization of a passive sampling device to assess air-water flux of PAHs and OPAHs before, during and after the Deepwater Horizon oil spill

L.G. Tidwell, Oregon State University / Environmental & Molecular Toxicology; S.G. O'Connell, Oregon State University / Environmental and Molecular Toxicology; S.E. Allan, Environmental and Molecular Toxicology, National Oceanic and Atmospheric Administration / Office of Response and Restoration; G. Wilson, Oregon State University; K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology

The Deepwater Horizon (DWH) oil spill introduced an estimated 4.1 million barrels of crude oil into the aqueous environmental compartment of the Gulf of Mexico. The oil released from the well head was composed of 3.9% polycyclic aromatic hydrocarbons (PAHs) by weight, an estimated 2.1 X 10^{10}g of PAHs were released into the water. Freely dissolved PAHs in the aqueous environment can volatilize to the atmospheric vapor phase. PAHs with ketone or quinone groups (OPAHs) are a largely unmonitored class of natural and anthropogenically occurring compounds that are emerging as contaminants of concern. OPAHs may be formed from PAHs in both aqueous and atmospheric environments through multiple mechanisms, including direct and indirect photolysis. Low density polyethylene (LDPE) based passive sampling devices were used to collect air and water samples of the vapor and dissolved phase respectively at four sites in four states along the coast of the Gulf of Mexico. LDPE passive sampling devices sequester both PAHs and OPAHs. Sampling was conducted from May 2010 to October 2011. Samples were analyzed by GC/MS for thirty three parent and alkylated PAHs as well as twenty one OPAHs. Σ_{33} PAH concentrations in air ranged from 1.02 to 24.1 ng/m^3. Σ_{33} PAH water concentrations ranged from 2.99 to 174.41ng/L. Σ_{21} OPAH concentrations in air were 0.193 to 26.6 ng/m^3. Σ_{21} OPAH concentrations in water were 0.349 to 635.37 ng/L. Qualitative assessment of OPAH flux was undertaken and flux direction was determined. Quantitative determination of net flux of bioavailable PAHs was undertaken. The magnitude of the flux of PAHs across the air water interface was greatly increased during the DWH incident.

258 Improvements on the Latin American Atmospheric Passive Sampling Network (LAPAN)

G. Fillmann, Universidade Federal do Rio Grande / Institute of Oceanography; K. Miglioranza, Universidad Nacional de Mar del Plata; R. Barra, EULA Chile Environmental Science Centre, University of Concepcion, Chile / Aquatic Systems Research Unit; P.M. Ondarza, Universidad Nacional de Mar del Plata / Ciencias Marina-Lab de Ecotoxiclogia; N. Gamboa, PUC-PE; B. Johnson-Restrepo, University of Cartagena; G. Eguren-Iriarte, Environmental Sciences and Ecology Institute. Science School. de la República University / Maestria en Ciencias Ambientales; R. Fernandez-Malve, Universidad Central de Venezuela; G. Anze, Simbiosis; C. Resabala-Zambrano, Escuela Superior Politecnica del Litoral; F. Wania, University of Toronto at Scarborough / Department of Physical & Environmental Sciences; G. de Avila Araujo, P.G. Costa, Universidade Federal do Rio Grande

A Latin American Atmospheric Passive Sampling Network (LAPAN) has been set up to create better conditions to study atmospheric contaminants within South American countries. It will also enable signatory countries to comply with Stockholm Convention demands on the identification of main sources and provide monitoring comparable data of POPs contamination. This will help to identify areas of concern and to evaluate the effectiveness of the control actions adopted by the member countries of the Stockholm Convention to eliminate the release of POPs to the environment. Thus, XAD2-based passive atmospheric samplers have been installed in South American countries since 2010 covering remote (background), urban, industrial and rural areas. Nowadays researchers from Argentina, Bolivia, Brazil, Colombia, Chile, Ecuador, Peru, Uruguay e Venezuela are collaborating with the LAPAN network, which aimed to assess local, regional and global sources using integrated data and to appraise the spatial and temporal distribution of POPs, among other contaminants. The XAD2-based passive atmospheric samplers has the advantage of being cheaper and easier to run, making it possible to increase the present spatial and temporal resolution run by GAPS (*Global Atmospheric Passive Sampling Network*) and requested by the Guidance on the Global Monitoring Plan (GMP) for POPs. The main LAPAN results will be available for the second Global Monitoring Report which will be produced for the COP7 – Conference of the Parties of the Stockholm Convention on Persistent Organic Pollutants that will take place in 2014.

259 Identification and Measurement on Organophosphorus Flame Retardants in Chicago's Atmosphere

M. Venier, Indiana University / School of Public and Environmental Affairs; Y. Ma, School of Public and Environmental Affairs; N.T. Petrich, The University of Iowa / Environmental Engineering – CGRER; A. Salamova, Indiana University / School of Public and Environmental Affairs; D. Hu, University of Iowa / Chemical and Biochemical Engineering / Department of Civil & Environmental Engineering, IIHR-Hydroscience and Engineering, Public Policy Center and School of Urban and Regional Planning; S.N. Spak, University of Iowa / Civil & Environmental Engineering; K.C. Hornbuckle, University of Iowa / Civil and Environmental Engineering, IIHR-Hydroscience and Engineering; R.A. Hites, Indiana University / School of Public & Environmental Affairs

Since polybrominated diphenyl ethers (PBDEs) and several other halogenated flame retardants have been either banned or retired from the market, the use of organophosphorus flame retardants has spiked in recent years. Here we report the levels of several commonly used organophosphorus flame retardants [tributyl phosphate, *tris*(2-chloroethyl)phosphate, triphenyl phosphate, *tris*(1-chloro-2-propyl)phosphate, tri-*o*-tolyl-phosphate, tri-*p*-tolyl-phosphate, tri(butoxyethyl)phosphate, *tris*(1,3-dichloro-2-propyl) phosphate, *tris*(2-ethylhexyl)phosphate, *tris*(4-butylphenyl)phosphate, *tris*(3,5-dimethylphenyl)phosphate, *tris*(2-isopropylphenyl)phosphate, and *tris*(2,3-dibromopropyl)phosphate] in the atmosphere of the greater Chicago, Illinois, area. Passive samplers equipped with polyurethane foam (PUF) absorbents were deployed for six weeks since the middle of 2012. Samples were collected from surrounding rural areas, from downtown Chicago, and from two water-intake cribs located about 5 km east of the Lake Michigan shoreline. Atmospheric concentrations of these compounds are 1-2 orders of magnitude higher than those of brominated flame retardants, which we also measured in this study. The levels of the organophosphorus flame retardants are expected to further increase in the future, given that the demand for these compounds will increase as the polybrominated aromatic flame retardants are phased out of production and sale.

Molecular, Genetic, Multi-Generational and Evolutionary Ecotoxicology: Part B

260 Genetic architecture of PCB tolerance in eight Atlantic killifish populations

E.R. Waits, USEPA / NERL; B. Rinner, USEPA; D.A. Proestou, USEPA / Atlantic Ecology Division; D.E. Nacci, USEPA / ORD, NHEERL, Atlantic Ecology Division

Atlantic killifish (*F. heteroclitus*) resident to estuaries contaminated by persistent organic pollutants exhibit heritable resistance to dioxin and like-compound (DLC) toxicity. Previously, we identified several quantitative trait loci (QTL) associated with susceptibility to a related prototypical compound, PCB-126. In this study, we evaluated the main-effect QTL for association with DLC susceptibility in eight wild Atlantic killifish populations known to be differentially sensitive. We found significant genetic structure attributable to population level differences in DLC tolerance and local contamination levels rather than geographic proximity or isolation by distance. A locus-by-locus analysis of molecular variance attributed up to 73% of the genetic variance at these loci to genotypic differences among tolerant and sensitive populations. The results of this study validate the QTL previously identified. Once the identity of each QTL is elucidated, these data will provide insight into the evolutionary basis of evolved tolerance to chemical contaminants in wild populations.

261 Environment induced genome variation influences population-level outcomes

J.R. Shaw, Indiana University / The School of Public and Environmental Affairs and The Center for Genomics and Bioinformatics; J.K. Colbourne, University of Birmingham / School of Biosciences

Understanding inter-individual variation in response to toxic exposures (i.e, the dose-response relationship) is central to the field of toxicology. These differences in response are described by variation in environments, which includes toxicant exposures, and genomes that combine to give rise to phenotypic variation within populations. In this talk we draw from recent population genomic studies to explore how toxicant exposure contributes to genome variability, influences the fate of genomic variation in populations, and over micro-evolutionary timescales drives population-level outcomes. These studies make use of the sentinel freshwater species, *Daphnia*, because their populations are defined by the boundaries of ponds and lakes, they are sensitive to modern toxicants in the environment, and are used to assess the ecological impact of environmental change. Their short generation time, large brood sizes, and ease of laboratory and field manipulation have assured *Daphnia's* importance for setting regulatory standards by environmental protection agencies, testing chemical safety, monitoring water quality, and as a model for environmental genomics research. We take advantage of maturing genomic tools that include array comparative genomic hybridization (aCGH), full-genome resequencing of over 50 individuals, and both neutral and selection driven mutation accumulation lines. We report exposure-induced alterations in the magnitude and distribution of single nucleotide polymorphisms and larger structural variants in genomes that include differences gene copy number (CNV). We reveal a method for measuring the contributions of this environment induced genome variation on phenotype, and through spatial and temporal studies determine that CNV play a major role in establishing the toxic-response of a population. Finally, we discuss the importance of understanding multi-generational outcomes and the evolutionary forces that drive them to the applied goal of environmental protection, detailing a path towards adverse outcomes forecasting. These investigations both benefit from and contribute to the *Daphnia* Genomics Consortium.

262 P-glycoprotein-mediated metal tolerance in the Cd-, Cu-, and Zn-exposed intertidal copepod, Tigriopus japonicus

J. Lee, Hanyang University / Dept of Chemistry, Hanyang University Graduate School

The intertidal copepod, *Tigriopus japonicus* shows a strong tolerance to metal exposure, compared to other invertebrates. To better understand its mechanism of metal resistance, we measured protein activity and the transcript expression profile of *T. japonicus* P-glycoprotein (*TJ-P-gp*). In this study, we firstly identified *T. japonicus P-gp* gene with the conserved motifs/domains, and characterized preliminary efflux activity and membrane topology of *T. japonicus* P-gp protein, supporting that it has a transport function for chemicals. To check whether the efflux activity of TJ-P-gp protein would be modulated by metal treatment, we exposed copepods to three metals (Cd, Cu, and Zn), and observed dose- and time-dependency on efflux activity of TJ-P-gp protein with or without 10 mM of verapamil and zosuquidar (LY335979), P-gp-specific inhibitors, for 24 h over a wide range of metal concentrations. As a result, three metals significantly induced the activity of TJ-P-gp in a concentration-dependent manner with transcriptional upregulation of *TJ-P-gp* gene within 24 h. In the present paper, we demonstrated that the copepod, *T. japonicus* has a strong resistance against metal exposure with induction of P-gp activity and transcriptional upregulation of the *TJ-P-gp* gene as a cellular metal defense system. We also suggest that *TJ-P-gp* gene provides an early signal as a potential biomarker for metal pollution. This finding gives a better understanding on molecular defense mechanisms involved in P-gp mediated metal detoxification in the copepod, *T. japonicus*.

263 Constitutive and benzo[a]pyrene-mediated changes in DNA methylation in zebrafish

K.L. Willett, University of Mississippi / Environmental Toxicology Research Program; X. Fang, Yale University; J. Corrales, University of Mississippi; C.E. Thornton, University of Mississippi / Department of Pharmacology and Environmental Toxicology Research Program; W. Wu, Department of Biology, / University of Florida,; L. Ballard, B. Scheffler, Genomics and Bioinformatics / USDA ARS

Epigenetic changes have emerged as a mechanism for multigenerational effects of environmental contaminants. Benzo[a]pyrene (BaP) is an environmentally relevant carcinogenic and endocrine disrupting compound that causes multigenerational effects in mammals. We hypothesized that BaP exposure would persistently alter zebrafish DNA methylation status during development. In one exposure, adult zebrafish (2 males x 4 females, *N*=3 replicate tanks per treatment) were exposed to control or 42.0 ± 1.9 µg/L BaP for 7 days. Eggs were collected and raised in normal conditions or continuously exposed to BaP until 3.3 and 96 hpf when DNA was isolated. CpG methylation status was investigated using sodium bisulfite-mediated PCR in either the promoter (12 genes), the first exon (5 genes), or across the transcription start site (4 genes). PCR products ranged between 375 – 488 nt long with 12 – 36 CpGs sites. PCR products were pooled by treatment and libraries prepared for Illumina 2000 deep sequencing followed by Bismark analysis of CpG, CHH and CHG methylation status. CpG islands tended to be either very highly methylated (>85%; 7 genes) or very hypomethylated (< 10%; 11 genes). Constitutively between 3.3 and 96 hpf the three genes with the highest change (increase) in methylation were *dazl*, *nqo1*, and *sox3*. At 3.3 hpf, BaP treatment decreased methylation (>20%) compared to controls in *bdnf*, *p21*, and *sox3* while increased methylation (>35%) in *dazl* and *nqo1*. At 96 hpf, BaP decreased methylation in *sox3*, *sox2*, *p53* and increased methylation in *cfos* and *mlh1*. In a second exposure, adult zebrafish (2 females x 2 males, N=10 replicate tanks per treatment) were fed 2% body weight/day flake food treated with 0, 11.6, 110, 1086 µg BaP/g flake (equivalent to 0, 0.23, 2.2, and 22 µg BaP/g fish/day) for 22 days. DNA was isolated from gonad, liver and hearts of parents and their adult F1 offspring for more global methylation analysis. In summary, BaP exposure affects DNA methylation patterns in zebrafish embryos and larvae, which could play a role in the observed developmental defects. Supported by NIEHS R21ES019940.

264 Effects of multigenerational cadmium exposure on life history, stressor tolerance traits, and population structure in Daphnia magna

D.A. Kimberly, C.J. Salice, Texas Tech University / Environmental Toxicology

Ecotoxicology attempts to relate the effects in individuals to changes in population size or structure. To date, the science relies on short term tests and implicitly assumes that populations are static. The goals of this study were aimed at understanding the effects of long term exposure to a chemical stressor and how key life history traits change through time in *Daphnia magna*. Daphnia were exposed to low and high concentrations of cadmium for 120 days in a longitudinal, quasi-natural selection design, where abundance of neonates, juveniles and adults was recorded every three days. Every three weeks, neonates were randomly selected to perform standardized, 21-day assays of reproductive endpoints such as time to first reproduction, clutch size, and number of clutches in the same exposure conditions as the longitudinal experiment. The purpose of these 21-day assays was to evaluate how specific life history traits may vary through time while also providing opportunity to compare assay results to population changes through

time in the longitudinal study. An aged-based matrix population model was used to place results from the 21-day assays into a population context. Lastly, we employed a cadmium and temperature challenge at the end of the longitudinal exposure on daphnia one generation removed from exposure conditions to determine whether cadmium tolerance and life history traits may have been altered by the long-term exposure. Overall, there was a strong exposure-response relationship on daphnid abundance and population structure during the longitudinal experiment and results indicated a decrease in carrying capacity with higher cadmium concentrations. In general, the 21-day assays tracked apparent patterns in the longitudinal experiment closely; for example, population growth rates followed an exposure response pattern similar to those of the longitudinal study. Daphnia exposed to high cadmium during the longitudinal experiment had lower mortality during the cadmium and temperature challenge than did those exposed to low cadmium but not as low as control treatments. These data indicate that long-term exposures to stressors can significantly alter life history and stressor tolerance traits, sometimes in non-linear and unpredictable ways. Thus, additional research on the manifestation of adverse effects from multi-generation exposures and their ecological implications is warranted.

265 Chained to the past: parental toxicant exposure alters offspring stressor tolerance and life history traits

S. Plautz, BASF / The Institute of Environmental and Human Health; C.J. Salice, Texas Tech University / Environmental Toxicology

Transgenerational effects of toxicant exposure can have significant effects on offspring fitness and stressor tolerance. Parental effects, a common type of transgenerational effect, are influences on offspring phenotype that occur without changes in the genetic sequence and that result from parental phenotypes or environments. Parental toxicant exposure can alter offspring tolerance not only to the toxicant to which parents were exposed, but also to other stressors. To further explore the effects of parental toxicant exposure on offspring stressor tolerances in both the short and long term, we exposed adult *Biomphalaria glabrata* snails to 0, 16, or 50 µg/L cadmium for seven days. We then transferred snails to clean water and collected egg masses. To test the short term stressor tolerance of offspring, when hatchlings were ≤48 h old, we exposed them to cadmium, copper, heat, cold, and salinity survival challenges and recorded mortality. Parental cadmium exposure did not affect offspring copper or salinity tolerance, but cadmium tolerance was greater in the offspring of snails exposed to 16 than 50 µg/L cadmium. Cold tolerance was greater in the offspring of controls than those exposed to 16 or 50 µg/L cadmium, while heat tolerance was greater in the offspring of snails exposed to 0 or 50 µg/L cadmium than those exposed to 16 µg/L cadmium. To test the long term stressor tolerance of offspring, 3-day-old hatchlings were raised to 8 weeks old in a parental cadmium (0, 16, or 50 µg/L) x offspring cadmium (0 or 2 µg/L) x temperature (25 or 30°C) factorial design. Offspring survival was unaffected by temperature or parental or offspring cadmium exposure, while the age at first reproduction was greater in snails directly exposed to cadmium (2 µg/L) than in controls. The number of eggs laid per snail per day was greater in controls than in snails exposed to cadmium, and greater in snails exposed to 25°C than 30°C. The interaction of temperature and parental cadmium exposure also had a marginal effect on egg mass production. Overall, offspring stressor tolerance in both the short and long term can be altered by parental toxicant exposure and this may have important implications for offspring fitness if offspring are exposed to stressful conditions themselves and thus for population persistence.

266 Advancing the Systems Biology Approach in Ecotoxicology Studies with Non-Gel Based Phosphoproteomics

L.C. Smith, University of Florida / Department of Physiological Sciences; C.M. Lavelle, Center for Environmental Health and Toxicology; C. Silva-Sanchez, University of Florida / Interdisciplinary Center for Biotechnology Research; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology; T. Sabo-Attwood, University of Florida / Department of Environmental & Global Health, Center for Environmental and Human Toxicology

The field of ecotoxicology has benefited greatly by advancements in the "omics" disciplines, especially in regards to determining the molecular mode of action of environmental contaminants. The study of protein phosphorylation in ecotoxicology has traditionally been limited to 2-Dimensional Gel Electrophoresis (2-DE) methods which are useful for identifying multiple differentially phosphorylated protein isoforms; however 2-DE methods do not offer site specific information of phosphorylation events. This is a limitation of 2-DE as the function of a phosphoprotein is largely determined by the site at which it is phosphorylated. To date, non-gel based methods such as mass spectrometry (MS) have been primarily employed in the field of ecotoxicology to evaluate contaminant-mediated changes in the levels of specific protein biomarkers or quantification of global proteome changes; however, MS-based methods have recently emerged that allow for identification of site-specific changes in the phosphoproteome. This information is useful for mapping intracellular signaling pathways mediated by the activity of protein kinases and phosphatases that are modulated by contaminants. To demonstrate the utility of an MS-based approach in comparison to traditional 2-DE methods in analyzing changes in the phosphoproteome, we aqueously exposed male fathead minnows to two constituents of the birth control pill, either 5 ng/L ethynyl estradiol or 100 ng/L levonogestrel. Liver, brain and gonad tissues were harvested after 30 minutes, soluble protein was isolated and divided for analysis by LC-MS/MS and 2-DE using the Isobaric Tagging for Relative and Absolute Quantification (iTRAQ) and ProQ Diamond detection, respectively. For LC-MS/MS studies, proteins were fractionated, enriched with ZrO_2 spin columns and quantified by iTRAQ. Preliminary results have identified the gonad as the tissue with the greatest change in phosphorylation status to these potent estrogenic contaminants. These phosphoproteomic methods support investigations of the rapid signaling events and cascades mediated by protein phosphorylation/dephosphorylation events in response to environmental contaminants. Overall, analysis of the phosphoproteome is a powerful tool in elucidating complex mechanisms of action of toxicants.

267 Perspectives on Biological "Read across:" Challenges and Opportunities for Ecological Risk Assessments of Pharmaceuticals

B.W. Brooks, Baylor University / Department of Environmental Science; K.A. Connors, Baylor University / Institute of Biomedical Studies,

Assessing ecological risks of contaminants of emerging concern is inherently challenged by toxicokinetic and toxicodynamic data scarcity. Pharmaceuticals, which are designed to be biologically active, present additional complications as biologically relevant effects may occur through endpoints not typically evaluated in standardized toxicity assays or whole effluent toxicity testing. Unlike the vast majority of industrial chemicals, a wealth of information fortunately exists about chemical properties, pharmacology and toxicology of pharmaceuticals in mammalian systems. Thus, a unique opportunity exists to incorporate omparative pharmacology and toxicology approaches in prospective and retrospective ecological risk assessments. Comparative pharmacodynamic studies, especially those that leverage an Adverse Outcome Pathway (AOP) framework, have been increasingly reported by our group and others to conceptual link mammalian pharmacological information to predict effects in ecologically-relevant species. However, the challenges associated with using similar applications of comparative pharmacokinetics in fish are starting to be explored. Here we present several case studies from our recent work to critically examine the utility of leveraging data sets among vertebrates to develop an advanced understanding of comparative environmental hazards posed by therapeutics. Further, examining biological "read-across" with pharmaceuticals and AOPs promise to improve ecological risk and hazard assessments of other industrial contaminants.

Occurrence, Fate, Transport, and Risks of Veterinary Pharmaceuticals in the Terrestrial Environment

268 Influence of trenbolone acetate dose on serum 17β-trenbolone concentrations

T.R. Brown, Texas Tech University / Department of Animal and Food Sciences; B.R. Blackwell, Texas Tech University / Environmental Toxicology; P.R. Broadway, Texas Tech University / Department of Animal and Food Sciences; S.L. Parr, Feedlot Health Management Services; P.N. Smith, Texas Tech University / Environmental Toxicology; B.J. Johnson, Texas Tech University / Department of Animal and Food Sciences

British × Continental steers (*n* = 168; 7 pens/treatment; initial BW = 362 kg) were utilized to evaluate the effect of dose of trenbolone acetate (TBA) and estradiol-17β (E_2) and on serum 17β-trenbolone (17β-TBOH) concentrations. Steers received one of three treatments: (1) no implant (NI); (2) Revalor-S (REV-S; 120 mg TBA + 24 mg E_2); (3) Revalor-XS (REV-XS; 200 mg TBA + 40 mg E_2). Blood was collected from 2 steers per pen (n = 84/d) on d 0, 3, 7, 14, 28, 56, 84, 112, 132, 147, 153, and 164, relative to

implanting. Serum concentrations were analyzed by LC-MS/MS. Serum 17β-TBOH concentrations for REV-S and REV-XS followed a typical biphasic release rate, in which an initial peak at d 3 ($P < 0.05$; 674.55 pg/mL, 681.25 pg/mL) was observed, followed by a depletion curve out to d 164, respectively. However, a secondary peak at d 112 was observed for REV-XS ($P < 0.05$; 718.99 pg/mL) when compared to REV-S and NI (172.03 pg/mL, 11.1 pg/mL), respectively.

269 Characterization of steroidal metabolite excretion from implanted cattle

T.R. Brown, Texas Tech University / Department of Animal and Food Sciences; B.R. Blackwell, Texas Tech University / Environmental Toxicology; P.R. Broadway, J.C. Brooks, B.J. Johnson, Texas Tech University / Department of Animal and Food Sciences; G.P. Cobb, Baylor University / Department of Environmental Science; P.N. Smith, Texas Tech University / Environmental Toxicology

The use of anabolic implants is common today throughout all stages of beef production: from the use of low-dose combination implants in suckling calves to the use of various implant strategies in commercial feedlots. Despite the vast utilization of anabolic implants in beef production, the time course and excretion profile of endocrine active steroidal metabolites has not been well characterized. Thus, British x Continental steers (n = 16; 8 head/treatment; initial BW = 310 kg) were utilized to determine the effect of implant status on the changes in steroidal metabolite circulation and excretion over the entire finishing period. Steers received one of two treatments: (1) no implant (NI); (2) Revalor-XS (REV-XS; 200 mg TBA + 40 mg E_2). Blood, urine, and feces were collected from individual animals on d 0, 1, 3, 7, 14, 28, 44, 56, 70, 84, 112, 122, 140, 161, 165, and 182-d post-implantation. Urine and fecal samples were subjected to enzymatic hydrolysis with β-glucuronidase to determine free and conjugated metabolites. Trendione, 17α-trenbolone, 17β-trenbolone, estrone, 17α-estradiol, and 17β-estradiol concentrations were determined by LC-MS/MS analysis. The primary metabolites found in urine and fecal samples were 17α-trenbolone and 17α-estradiol, with the majority of the urinary fraction being conjugates. Preliminary analysis indicates a greater propensity for biliary excretion, resulting in increased concentrations in fecal samples when compared to urine samples.

270 Degradation of steroid metabolites in urine and feces from implanted cattle

B.R. Blackwell, Texas Tech University / Environmental Toxicology; T.R. Brown, B.J. Johnson, Texas Tech University / Department of Animal and Food Sciences; G.P. Cobb, Baylor University / Department of Environmental Science; P.N. Smith, Texas Tech University / Environmental Toxicology

The majority of beef cattle finished on feedyards are administered steroid implants to improve growth rate and feed efficiency. These steroidal compounds are excreted in urine and feces, thus land application of cattle manure to agricultural operations could introduce these endocrine active compounds to the environment. To assess the degradation of steroids excreted from the bovine system, feces and urine was collected from steers recently implanted with a Revalor-XS implant. Manure and urine were homogenized and divided into 50 mL polypropylene tubes alone (urine or feces) or in combination (slurry). Samples were aged in an incubator without light at 21°C for up to 112 days. Samples were sacrificed at given intervals with half subjected to enzymatic hydrolysis with beta-glucuronidase to determine free and conjugated metabolites. LC-MS/MS analysis was performed to determine concentrations of 17α-trenbolone, 17β-trenbolone, trendione, 17α-estradiol, 17β-estradiol, and estrone. Deconjugation of steroid metabolites occurred within 1-3 days for all matrices. Trenbolone degradation occurred at a much slower rate as compared to previous studies in spiked soils, with half-lives on the order of days as opposed to hours. Estrogen degradation varied by sample matrix, but metabolism to estrone occurred in all matrices. These data will be summarized and discussed as they pertain to the environmental degradation of steroids in cattle wastes.

271 Occurrence of steroid growth promoters associated with particulate matter originating from beef cattle feedyards

B.R. Blackwell, K.J. Wooten, Texas Tech University / Environmental Toxicology; M. Buser, Oklahoma State University; B.J. Johnson, Texas Tech University / Department of Animal and Food Sciences; G.P. Cobb, Baylor University / Department of Environmental Science; P.N. Smith, Texas Tech University / Environmental Toxicology

Veterinary growth promoters are frequently utilized in the United States for livestock production. In beef production, anabolic steroid growth promoters are administered to increase growth rate and feed efficiency in an estimated 90% of cattle. Transport of these endocrine active compounds has been demonstrated in airborne particulate matter (PM) emanating from beef cattle feedyards. To better assess occurrence and transport of these steroid growth promoters from beef cattle feedyards, PM samples were collected from five feedyards across the Southern High Plains, USA, over the course of two years. Each feedyard was sampled four times per year for a total of eight sampling events per feedyard. Sixteen low volume air samplers equipped with total suspended particulate (TSP), PM_{10}, or $PM_{2.5}$ sampler heads were deployed with each feedyard for a period of 6-10 days. PM was collected on 47 mm Zefluor filters. Filters were collected after a set time period and transported to the laboratory under dry ice. PM mass was determined gravimetrically and filters were subjected to steroid analysis. LC-MS/MS analysis was performed for target analytes including metabolites of the synthetic androgen trenbolone acetate, estradiol metabolites, and the synthetic progestin melengestrol acetate. Preliminary analyses indicate estrogen detections at a greater frequency compared to androgens and progestins. Several steps within the sampling and sample handling process have been identified to have the potential to increase steroid degradation prior to analysis. These data, along with potential sampling biases, will be presented and detailed as they pertain to environmental occurrence and transport of steroid growth promoters in PM.

272 In vitro endocrine activity of particulate matter collected near beef cattle feedlots

K.J. Wooten, B.R. Blackwell, Texas Tech University / Environmental Toxicology; A.D. McEachran, Texas Tech University / The Institute of Environmental and Human Health, Environmental Toxicology; P.N. Smith, Texas Tech University / Environmental Toxicology

Beef cattle feedlots contain large numbers of cattle in relatively small spaces, and thus generate significant amounts of organic waste. Feedlot waste has potential to enter the environment as airborne particulate matter (PM) due, in part, to the arid conditions that exist where many beef cattle feedlots are located. Included in the waste are both natural hormones and synthetic hormones which are administered to cattle to increase feed efficiency. Limited research has been published on the potential effects of these hormones after they become airborne in association with PM. In this study, airborne PM was collected downwind and upwind of five feedlots between April and June 2012. Particulate matter samples were screened for potential androgenic and estrogenic activity using *in vitro* transcriptional activation assays, and selected hormones were quantified using LC-MS/MS. As expected, the mass of PM collected downwind was significantly greater than that collected upwind ($p < 0.0001$). Androgenic activity *in vitro* was greater ($p < 0.0001$) in downwind samples than upwind, and this activity was supported by more frequent detections of trendione, 17α-, and 17β-trenbolone in downwind samples. Unexpectedly, *in vitro* estrogenic activity was similar ($p=0.33$) for upwind and downwind samples. These results were supported by numerous detections of estrone, 17α-, and 17β estradiol in both upwind and downwind samples. Further sampling is currently underway to investigate additional potential sources of estrogenic activity in upwind samples, as well as to determine the impacts of distance from the feedlot on observed *in vitro* endocrine activity.

273 Potential Ecological Risks of Trenbolone Acetate, a Model Endocrine-Active Veterinary Drug

G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory

Trenbolone (TRB) acetate is registered in the US as a veterinary drug for use as a growth enhancer in livestock. Usually administered as a slow-release implant, the parent acetate is converted to two metabolites, 17α- and 17β-TRB, which bind to and activate the mammalian androgen receptor (AR) thereby producing the desired anabolic response. Both steroid isomers are excreted by livestock, and can enter aquatic systems by direct and indirect routes, including via transport associated with dust particles. Studies with a variety of species have shown that the two isomers can impact reproduction in fish, with the more potent (and better studied) 17β-TRB causing effects on some endpoints at water concentrations approaching 1

ng/L. *In vitro* studies have shown that 17β-TRB binds to the fish AR; the types of responses observed *in vivo* are consistent with activation of the AR, including weight gains and phenotypic masculinization of females. Both 17α- and 17β-TRB cause adverse effects on reproduction through inhibition of vitellogenesis in female fish, by depressing sex steroid synthesis, likely through feedback inhibition. Different field studies have reported water concentrations of 17α- and 17β-TRB in the vicinity of some animal feeding operations to be comparable to those that can cause reproductive effects in the lab which, based on modeling, would be sufficient to cause population-level impacts. This presentation will provide an overview/synthesis of the environmental occurrence and effects of TRB acetate and its metabolites in the context of assessing potential risks to animals associated with aquatic environments. The contents of this abstract neither constitute nor necessarily reflect USEPA Policy.

274 Effects of 17α-trenbolone and Melengestrol Acetate Alone and in Combination on Xenopus laevis Larvae Growth and Development

B.E. Finch, Oregon State University / Environmental and Molecular Toxicology; B.R. Blackwell, Texas Tech University / Environmental Toxicology; D.R. Faust, Mississippi State University / Wildlife, Fisheries, and Aquaculture; K.J. Wooten, Texas Tech University / Environmental Toxicology; J.D. Maul, Texas Tech University / The Institute of Environmental and Human Health / Department of Environmental Toxicology; S.B. Cox, Texas Tech University / Dept. of Environmental Toxicolgy; P.N. Smith, Texas Tech University / Environmental Toxicology

Beef cattle feedlots administer synthetic hormones to promote rapid muscle growth via ear implants or as a feed additive. The Food and Drug Administration has approved the use of the synthetic hormones trenbolone acetate (TBA), melengestrol acetate (MGA), and zeranol in beef production. Trenbolone acetate is metabolized in cattle into 17β-trenbolone, trendione, and 17α-trenbolone (17α-TB), whereas MGA is reported to be excreted in an unmodified form. Of trenbolone metabolites, 17α-TB has been detected most frequency and in the highest concentration. Synthetic hormones, including TBA metabolites and MGA, have been detected in beef cattle feedlot waste, effluent, particulate matter, and in neighboring streams. Therefore there is a potential for aquatic organisms to be exposed to synthetic hormones emanating from beef cattle feedlots. Amphibians may especially be at risk as a result of their life history and sensitive stages during metamorphosis. This study examines the effects of environmentally relevant concentrations of 17α-TB and MGA alone and in combination on *X. laevis* larvae growth and development. Beginning on post-hatch day 2, *X. laevis* larvae were exposed to 17α-TB (10, 100, and 500 ng/L), MGA (1, 10, and 100 ng/L), or a combination of both (1+10, 10+100, 100+500 ng/L MGA/17α-TB, respectively). Exposure to 1 ng/L MGA + 10 ng/L 17α-TB and 10 ng/L MGA + 100 ng/L 17α-TB combination treatments resulted in significant increases in all growth metrics compared to controls. Contrarily, *X. laevis* larvae total body mass and snout-vent length were significantly reduced in the 100 ng/L MGA and 100 ng/L MGA + 500 ng/L 17α-TB treatments. Significant reductions were also observed in total body mass, snout-vent length, and total length following exposure to 500 ng/L 17α-TB. Stage of development was significantly increased in 1 ng/L MGA + 10 ng/L 17α-TB treatment, while 500 ng/L 17α-TB resulted in a significant reduction in development. Trends indicate reductions in growth metrics with increasing concentrations of MGA, 17α-TB, or a combination of both. Results suggest exposures to environmentally relevant concentrations of growth promoters from beef cattle feedlots may have implications on amphibian fitness.

275 Aerial dispersal of antibiotics and antibiotic resistant bacteria from industrial beef cattle production facilities

A.D. McEachran, Texas Tech University / The Institute of Environmental and Human Health, Environmental Toxicology; B.R. Blackwell, Texas Tech University / Environmental Toxicology; J. Hanson, S. Cox, Research and Testing Laboratory; K.J. Wooten, G. Mayer, P.N. Smith, Texas Tech University / Environmental Toxicology

Large scale animal feeding operations rely on extensive use of veterinary pharmaceuticals, including growth hormones and antibiotics, for the production of meat-producing animals. Antibiotics are used to treat and prevent disease as well as to promote growth in beef cattle. Upon excretion, antibiotics enter the environment via runoff, leaching, land application of manure, and potentially via airborne particulate dispersal. Selection for antibiotic resistant bacteria occurs in the presence of antibiotics, and has been documented in proximity to feeding operations and agricultural facilities. While environmental entry and co-occurrence of antibiotics and antibiotic resistance are documented in water and soil matrices, the potential for broad dispersal of antibiotics and antibiotic resistant bacteria via airborne particulate matter (PM) derived from beef cattle feedyards has not been evaluated. Here we report that PM emanating from large scale beef cattle feeding operations contains veterinary antibiotics, potential human pathogens, and genes encoding bacterial resistance to antibiotics at levels significantly different from those occurring immediately upwind of feedyards. Concentrations of antibiotics in airborne PM immediately downwind of feedyards ranged from 0.5-4.6 μg/g of PM, similar to concentrations reported within indoor, contained large scale swine production facilities. Additionally, levels of confirmed nucleotide sequences of eight genes encoding for resistance to tetracycline and sulfonamide antibiotics were significantly greater in PM downwind of feedyards as compared to upwind. Furthermore, overall microbial community structure of PM was significantly different downwind of feedyards as compared to PM collected upwind. These results demonstrate that antibiotics used in beef cattle production enter the environment via a previously uncharacterized pathway, airborne dispersal of PM. These results also confirm the occurrence of pathogenic microbial species and presence of antibiotic resistant bacteria in airborne PM collected immediately outside feedyard boundaries. Such broad dispersal of antibiotics, microbes, and antibiotic resistance via airborne PM has serious implications for the spread of antibiotic resistance and consequent impacts on environmental, human and livestock health.

Multi-sector Applications and Harmonization of Life Cycle Assessment and Environmental Product Declarations for Sustainability

276 Why Globally Harmonized Life Cycle Impact Indicators and Methods are Needed

B.W. Vigon, SETAC; O. Jolliet, University of Michigan / School of Public Health; R. Frischknecht, TreezeLtd.; S. Valdivia, UNEP / Secretariat ILCI

Until now the development of life cycle impact indicators and methods has been a research effort carried out to explore the possibilities of impact characterization within the bounds of the ISO 14040 and 14044 standards. However, such an approach, while encouraging creativity and expansion of the procedural basis for impact assessment, has severe limitations in a globally practiced methodology, whether for product improvement, information dissemination or policy implementation. The challenge now is to create a consensual list of environmental impact category indicators embedded in a consistent methodological framework. Such a set (or subset) of consensual environmental impact category indicators can be used in environmental product information schemes, corporate reporting of multinational companies, international and/or national environmental policy and common LCA work commissioned by governments and companies. This presentation will discuss the advantages and disadvantages of a harmonized system and provide a vision and plan for how such a system can be realized. First steps to organize working groups and to prioritize the activities will be described. The work is being supported as a multi-year flagship project within the UNEP/SETAC Life Cycle Initiative.

277 GHG Protocol: A common framework for multi-impact corporate inventories

L. Draucker, World Resources Institute / GHG Protocol

We rely on our environment for the resources we need to live, work, and prosper. Because our environment is so interconnected, stress on one aspect of the environment can have negative impacts on others. For example, many of the adverse impacts of climate change, such as drought and wildfires, are putting resources like water and viable crop land at risk. Quantifying and ultimately reducing the impacts that a company's activities have on all aspects of the environment makes good business sense. By assessing multiple impacts, companies have more angles from which to assess their operations and performance, which may offer more innovative and actionable reduction solutions. For example, accounting for risks to local air pollution can show the co-benefits of reducing GHG emissions, which in turn could improve financing prospects for reduction projects. Additionally, accounting for multiple environmental impacts allows companies to avoid burden shifting if, for example, investing in a GHG reduction project increases water risk. Several initiatives exist or are being developed to facilitate corporate

reporting on multiple environmental impacts; however, these initiatives lack a common accounting framework and consistent reporting elements. While product-level GHG standards, such as GHG Protocol's Product Life Cycle Standard, build upon a general, multi-impact standard [1], no such general multi-impact standard or framework exists for corporate-level accounting. Furthermore, many standard-setting initiatives are only single-impact and being developed independently of each other. This effectively limits a company's ability to comprehensively evaluate how its activities or decisions affect multiple natural systems, including consideration of co-benefits and burden shifting. All of this creates additional confusion for companies seeking to systematically account for, report, and reduce their environmental footprint. This presentation will highlight the conclusions and next steps of research conducted by the GHG Protocol on how the scope 1, 2, and 3 framework can be utilized as a common basis for multi-impact corporate inventories. It will also identify differences between the GHG Protocol corporate approach and the EU Commission's OEF, noting how we can reduce confusion in the market place through clarity and harmonization. [1] ISO 14040:2006 and 14044:2006

278 The Life Cycle Assessment Harmonization Tool and the Improvement of OpenLCA

T.R. Hawkins, USEPA

As ORD life cycle assessment researchers, we[a] receive regular requests for technical support for sustainability analyses being conducted throughout the Agency. There is an incredible need for data and consensus-based modeling approaches to support systems-level analyses. The tool development activities described here address this need by adding key functionality to openLCA, a free, open source LCA modeling software package, and by providing a tool for harmonizing disparate data for incorporation into LCA models. These tools address two key bottlenecks for the broader application of LCA, the lack of low-cost, user-friendly LCA software and the challenge of reconciling data sources for use in a common model. The objective of this project is to set in place the data architecture and modeling infrastructure required to support a wide array of quantitative assessments from a systems-perspective. Here we present two ongoing efforts. The first focuses on improving the user experience and providing enhanced modeling capabilities for openLCA. The second is the development of the LCA Harmonization Tool, which facilitates the incorporation of multiple datasets within a common LCA model. GreenDelta initiated development of openLCA in 2006. It is a freely available, fully open source software developed in Java on the Eclipse platform with data managed in a MySQL server and made publicly available under the Mozilla Public License 1.1. The LCA Harmonization Tool assists users to define clear relationships between life cycle inventory, life cycle impact assessment, and other data sources to allow for their incorporation in LCA models. While the demand for this tool grew out of efforts to reconcile a broad array of data sources into a common LCA database, the tool will be made publicly available as an open source plug-in for openLCA. The tool builds upon an LCA ontology developed in connection with the Earthster Project and is being developed in collaboration with USDA partners involved in the development of the LCA Digital Commons. The effort has been structured to address both near- and mid-term objectives. In the near-term, this tool will facilitate the development of more robust LCA models and comparison between LCA models constructed with different data sources through provision of a free tool for public use. In the mid-term, this tool provides a pipeline for the incorporation of a diverse array of datasets into an EPA LCA Database.

279 Why it is important for Industry to participate in Standard Setting processes

S. Giese-Bogdan, 3M Company

In recent years, many organizations around the world have started creating standards for Life Cycle Assessment (LCA) inventory generation. In this presentation we will show why it is important for Industry to have a voice in this standard setting process. Development of standards in this space is not easy with many stakeholders and viewpoints involved. Standard setting organizations strive to obtain a high level of scientific validity. The challenge is to balance this with impact of implementation of standards. The goal should be to have the highest level of scientific validity while enabling broad implementation. With case studies of standards from Europe and the US we will show how a scientifically sound approach proposed in a standard might result in a standard that may be difficult or impossible for Industry to implement. We will further show examples on how constructive feedback and incorporation of voices from all sectors (Academia, Government and Industry) can result in a standard that is still scientifically sound, leads to useful information and can be broadly implemented.

280 Incorporating Life Cycle Assessment in the Alternatives Assessment Process

C.D. Robertson, Hewlett Packard

Industry faces ever increasing regulatory and consumer pressure to remove substances of concern from products. Many of these concerns stem from end-of-life processes that release substances that are toxic to human health or the environment. Alternatives assessment, green chemistry and pollution prevention are all terms to describe the process of selecting safer chemicals based on their hazard profile. Alternatives assessment has emerged as an important tool to identify sustainable materials. Life cycle assessment can inform this process and this presentation will discuss the integration of LCA into the alternatives assessment process. The work performed by the Business-NGO Working Group (BizNGO) evaluating alternatives to the flame retardant DecaBDE in electronics will be presented. BizNGO provides a forum for business, regulatory and NGO stakeholders to develop frameworks that bring sustainable materials to the marketplace. The DecaBDE Alternatives project was established to create a pilot report to meet the requirements of the State of California's Safer Consumer Products alternatives assessment regulation. Released by the Department of Toxic Substances Control (DTSC) this legislation is a key component of California's Green Chemistry Initiative. Adverse multimedia life cycle impacts are a key consideration of the regulation meant to prevent regrettable substitution and burden shifting. The wide range of endpoints accounted for by LCA addresses most of the adverse impact areas identified by the Safer Consumer Products regulation. LCA is well suited to evaluate the environmental impact of complex systems from extraction, manufacturing, use and end-of-life. LCA can also identify hot-spots or areas where environmental impact is greatest. Evaluation of LCA results may lead to deselection of alternatives or areas where mitigation is needed. A key discussion point is the need to balance the benefits in energy or emissions identified by LCA with the use of substances of concern. Through the combination of hazard based alternatives assessment and life cycle assessment, the most sustainable materials can be identified, ultimately leading to safer products in the marketplace.

281 Life Cycle Impact Studies of Home and Personal Care Products for Sustainability Measurement and Reporting

C. Helt, S. Herron, C. Mars, K. Dooley, The Sustainability Consortium

Life Cycle Assessments (LCAs) are used to evaluate the environmental impacts of specific consumer products from cradle to grave. The Sustainability Consortium (TSC) utilizes LCA results to support the identification of sources of greatest impact along a product supply chain to help identify improvement opportunities for consumer goods categories. In order to quickly determine the largest sources of environmental impacts across a representative range of products within a given category, TSC utilizes a modified approach to traditional LCA that we term Life Cycle Impact Screening (LCIS). LCIS enables assessment of impacts for a 'generic' product bill of materials plus manufacturing and transport, where variations across a product category are then assessed with a sensitivity analysis. Two recent applications of LCIS analyses were performed on skin-care products and non-aerosol air fresheners. Generic formulations were determined for these categories using product surveys, literature reviews, and expert input from TSC member companies. The results for skin-care products identified palm oil production, plastic packaging, and transportation to be the most impactful activities across the product category. Evaluation of non-aerosol air fresheners revealed the largest impacts to be from electricity used for plug-in devices, as well as from plastic packaging and transportation. The results of these analyses are presented within the context of TSC's Knowledge Product development for Home and Personal Care products. Future application of market share data will provide further insight into the relative contribution that each specific product type has on the product category's overall environmental impact.

282 A Government and Industry Application of LCA and Novel Sustainability Metrics: Part I

A. Weisbrod, Procter & Gamble (P&G) / Global Product Stewardship; J. Bare, USEPA; M. Ceja, Procter & Gamble; W. Ingwersen, D. Young, USEPA; M. Gausman, Procter & Gamble

The USEPA's Office of Research and Development is partnering with Procter & Gamble (P&G) to advance the state of the sustainability science, by developing innovative methods that can be applied to future sustainable supply chain design of consumer products. The problem being addressed is how to help sustainability practitioners to be more productive, accurate, and improve analyses of environmental, economic, and social factors. The first milestone in this collaborative research is to develop a baseline assessment of the current environmental, social, and economic impacts for Bounty® paper towel manufacturing and supply webs. The extensive data feeding the four methods presented in this talk come from two P&G facilities and represent two generations of papermaking and converting technologies. Part I of this talk will cover development of an Industrial Production Systems Assessment (IPSA), which is a standardized allocation method, and Fisher information, which measures production and community dynamics. Part II of this talk will cover the Life Cycle Assessment (LCA) and Green Net Value Added (GNVA), a full cost accounting method that accounts for the value of environmental damages along a supply chain. Insight for engaging different functional experts on interdisciplinary teams will also be presented. Results are used to identify potential product and/or production changes that could improve environmental profiles, costs, resource use and efficiency. Results are also used to determine what to communicate about the product and/or processes' sustainability.

283 A Government and Industry Application of LCA and Novel Sustainability Metrics: Part II

J.C. Bare, USEPA / MS-466; A. Weisbrod, Procter & Gamble (P&G) / Global Product Stewardship; W. Ingwersen, USEPA; M. Ceja, Procter & Gamble; D. Young, USEPA; M. Gausman, Procter & Gamble

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Wildlife Exposure and Effects Relative to Emerging Anthropogenic Substances

284 In ovo exposure to organophosphorous flame retardants: Survival, development, neurochemical and behavioral changes in white leghorn chicken hatchlings

M.A. Bradley, Department of Environmental Health Sciences; J. Rutkiewicz, K. Mittal, University of Michigan; K.J. Fernie, Environment Canada / Ecotoxicology and Wildlife Health; D.E. Crump, Environment Canada; N. Basu, McGill University / Department of Environmental Health Sciences

Organophosphorous flame retardants (OPFRs) are contaminants of emerging concern owing to the phase-out of some polybrominated diphenyl ethers (PBDEs, such as PentaBDE) in Europe and some US states. Despite the potential for these chemicals to be neurotoxic, they have received little research attention until recently, particularly in the realm of avian toxicology. Here, the purpose was to characterize the neurodevelopmental toxicity of two relevant OPFRs, TCrP (tricresyl phosphate; a non-halogen-containing OPFR) and TDCPP (tris (1,3-dichloro-2-propyl) phosphate; a halogen-containing OPFR) in an avian embryo/hatchling model. We injected white leghorn chicken eggs with a range of TCrP (0, 10, 100, and 1000 ng/g) or TDCPP (0, 10, 100, 1000, 50000 ng/g) concentrations at incubation day 0. Hatching success was unaffected by TCrP, but TDCPP showed a dose-dependent reduction in hatching success (with the exception of 100% hatching success in the 1000 ng/g exposed eggs). On 7-9 day old hatchlings, we assessed behaviors in an open field (using Noldus EthoVision ® XT 9 software), righting reflex, angled balance beams, gait patterns, and wing flap reflex; these data will be presented in full. Cerebrum from 10 day old hatchlings was sent for histopathological analyses. Brain tissues from 10 day old hatchlings were also analyzed for acetylcholinesterase (AChE) activity and acetylcholine receptor (both nicotinic [nAChR] and muscarinic [mAChR]) levels. Preliminary neurochemical analysis of TCrP-exposed birds shows apparent reductions in AChE activity and lower mAChR levels than control birds. TDCPP-exposed birds showed no differences from control birds in AChE activity (nAChR and mAChR activity remains to be tested in TDCPP-exposed birds). Preliminary behavioral analysis indicates that, among TCrP-exposed birds, those exposed to 1000 ng/g performed more poorly than non-injected control birds in righting reflex; however, vehicle-injected control birds also performed more poorly than non-injected control birds, and no differently than the 1000 ng/g-exposed birds. In the angled beam test, birds exposed to 1000 ng/g TCrP performed similarly to control birds. We will present data concerning a range of neurodevelopmental measures in birds exposed to selected OPFRs, and these findings should enable us to better predict ecological risks.

285 Prediction of exposure and food web transfer of pharmaceuticals in ospreys (Pandion haliaetus) nesting in Chesapeake Bay

R.S. Lazarus, USGS Patuxent Wildlife Research Center / Beltsville Laboratory; B.A. Rattner, USGS-Patuxent Wildlife Research Ctr; B.W. Brooks, Baylor University / Department of Environmental Science, Center for Reservoir and Aquatic Systems Research; B. Du, Baylor University / The Institute of Ecological, Earth, Environmental Sciences; P.C. McGowan, U.S Fish and Wildlife Service Chesapeake Bay Field Office; V.S. Blazer, USGS-Leetown Science Center / USGS; M. Ottinger, University of Maryland / Department of Animal and Avian Sciences

Ospreys are a well-known sentinel of environmental pollution, yet no studies have examined pharmaceuticals in the fish-osprey food web. An integrative modeling approach is being undertaken to evaluate bioaccumulation potential of a suite of pharmaceuticals and an artificial sweetener using hypothetical screening-level exposure scenarios. Theoretical concentrations in water reflecting low-flow "effluent dominated" or high-flow "dilution dominated" scenarios are being used in combination with pH specific bioconcentration factors to predict uptake in fish. A first-order kinetic exposure model is being applied to estimate the average daily dose and cumulative 45-day dose of pharmaceuticals received by a nestling osprey. To complement this assessment, predicted doses are being compared to actual plasma concentrations detected in 40-45 day old nestlings sampled from locations near wastewater treatment plants, combined sewer overflows, and urban non-point sources in Chesapeake Bay. Water, and blood plasma from fish and nestling osprey samples were evaluated for 24 analytes (antibiotics, antihypertensives, antidepressants, anti-inflammatories, antihistamines, analgesics, stimulants, antidepressants, antilipemics, antiseizures, anticoagulants, and sucralose). Of the 18 analytes detected in water, 8 were also found in fish plasma, but only 1 in osprey plasma. Gizzard shad (*Dorosoma cepedianum*) contained 6 of 8 analytes compared to 4 of 8 in catfish (*Ictaluridae* sp.), and 3 of 8 in both striped bass (*Morone saxatilis*) and carp (*Cyprinus carpio*). The antihypertensive diltiazem was the only analyte that could be traced from water to fish plasma to osprey plasma. Concentrations in water (range: ND-0.0079 μg/L) and fish (ND-1.79 μg/L) were low. Diltiazem was detected in 6 of 149 fish plasma samples collected from dominant prey species of osprey. However, this antihypertensive was detected in all 47 osprey plasma samples (0.62-8.48 μg/L), albeit at levels below human therapeutic concentrations (40-200 μg/L). There were no differences in plasma diltiazem concentrations in osprey samples among sites, but values were greater in 2011 versus 2012 ($p < 0.0001$). We hypothesize that patterns of accumulation in fish and osprey plasma may relate to individual dietary preferences, and differences in diltiazem clearance rate and metabolism.

286 You are what you eat: Bioavailability and consequences of antimicrobials in two species of wild birds exposed to municipal biosolid

J. Sherburne, Boise State University; E.T. Furlong, USGS; C.A. Kinney, Colorado State University – Pueblo; J. Forbey, Boise State University; D.W. Kolpin, USGS; A. Dufty Jr, Boise State University

A major goal in the field of toxicology is to determine the effects of potentially harmful and persistent environmental contaminants on biota and the environment. Despite the implications of elevated levels of antimicrobials in urban ecosystems, few studies address the bioavailability and consequences of these contaminants on higher trophic levels. Triclocarban (TCC) and triclosan (TCS) are two of the most utilized antimicrobial compounds in the world and they can be introduced into ecosystems through a variety of sources including the application of biosolids to agricultural fields. We hypothesized that antimicrobials found in biosolids would be bioavailable and would negatively impact bird populations. To test these hypotheses, we compared the transfer of antimicrobials from land-applied biosolid to the eggs of two bird species between an Experimental (biosolid applied over a seven year period) and Control (no application of biosolid) agricultural site. We also tested the impact of antimicrobials on egg morphometrics (egg length, egg mass, and egg shell thickness) and nesting success of birds. We used Liquid Chromatography – Mass Spectrometry to quantify the concentration of antimicrobials in biosolid, soil, earthworms, and eggs of the American kestrel (*Falco sparverius*) and the European starling (*Sturnus vulgaris*). Antimicrobials were significantly higher in soil, earthworms and eggs of starlings on the Experimental site compared to the Control site. Concentrations of antimicrobials of kestrel eggs did not differ between the Experimental and Control site. There was no correlation between antimicrobial concentrations and egg morphometrics for either bird species. Nesting success for kestrels was significantly lower on the Experimental site than the Control site, whereas nesting success of starlings did not differ between sites. This study demonstrated that antimicrobials from biosolids can be transferred to eggs of secondary and tertiary consumers and contaminants from biosolids should be further investigated as a potential factor influencing the nesting success of birds.

287 River birds exposed to persistent organic pollutants on urban streams experience developmental impairment

C.A. Morrissey, University of Saskatchewan / Biology; D. Stanton, Cardiff University / School of Biosciences; M.G. Pereira, Centre for Ecology & Hydrology; J. Newton, Scottish Universities Environmental Research Centre (SUERC) / NERC Life Science Mass Spectrometry Facility; C.R. Tyler, The University of Exeter; I. Durance, Cardiff University; S. Ormerod, Cardiff University / School of Biosciences,

Many urban European streams are recovering from gross pollution from industrial, mining and sewage during the 20th century. However, associated recolonisation by clean water organisms can potentially result in exposure to persistent legacy or novel toxic pollutants whose endocrine disrupting effects are still poorly understood in riparian birds. We conducted a study to investigate pollutant loads in eggs, adults and nestlings of a river passerine, the Eurasian dipper (*Cinclus cinclus*), from 33 rivers in South Wales and the English borders (UK) which varied in catchment land use from rural to highly urbanized (range 0-77% urban land cover, 36-3100 people/km^2). Concentrations of total PCBs and PBDEs in eggs were positively related to urban land cover and human population density while legacy organochlorine pesticides such as DDE were found in higher concentrations at rural sites. Levels of PBDEs in urban dipper eggs (range 136-9299 ng/g lw) were among the highest reported in passerines, and increasing egg PCB and PBDE contaminant levels reflected depressed thyroid hormone levels in nestlings at levels sufficient for adverse effects on growth and development. With the exception of dieldrin, persistent pollutants such as PCBs and other organochlorine pesticides have declined negligibly in the past 20 years in dipper eggs despite discontinued use. In additional work, we are developing an ELISA method to assess vitellogenin induction in free living dippers and other birds to assess potential for exposure and effects of effluents containing chemicals with estrogenic (or anti-androgenic) properties. This work is the first to evaluate the exposure and potential for endocrine disrupting effects of complex wastewater effluents on birds living in urban environments.

288 Dirty Birds: Utilizing a Novel Analytical Approach to Investigate Persistent Contaminant Exposure to Black Skimmers (Rynchops niger) in San Diego Bay

C. Millow, San Diego State University / Department of Biology; E. Hoh, San Diego State University / Graduate School of Public Health; R. Lewison, San Diego State University / Department of Biology

Southern California's fragmented and urbanized coastal wetlands provide refuge for over 300 bird species, including the Black Skimmer (*Rynchops niger*). Critical habitats like San Diego Bay and Tijuana River Estuary are known to harbor high levels of contaminants from urban runoff. Persistent organic pollutants (POPs), including anthropogenic organohalogen compounds like DDT, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), occur throughout southern California's waterways and are known causes of numerous impairments in wildlife. Exposure to POPs has been the traditional focus of targeted contaminant analysis and is well documented in a wide range of fauna. An emerging area of research focuses on the bioaccumulative implications of non-targeted (naturally-occurring, unregulated, or emerging) compounds, yet little is known about their impacts to wildlife. By analyzing non-targeted compounds alongside targeted POPs, we may comprehensively assess true toxin burdens. Black Skimmers exhibit poor reproductive output throughout the United States, especially in southern California. Previous studies show skimmers accumulating contaminants in higher amounts than similar seabirds, possibly because they forage exclusively for top-schooling fish along coastal wetland margins susceptible to concentrated urban runoff. Also, as long-lived upper trophic level consumers, skimmers serve as valuable ecological sentinels. Using a novel GC´GC/TOF-MS non-targeted analytical approach, we identified and quantified both targeted POPs and non-targeted compounds of interest in presumed-viable and non-viable Black Skimmer eggs. Targeted compounds discovered in all egg samples included PCBs, PBDEs, DDT and its metabolites, and polycyclic aromatic hydrocarbons (PAHs). Mass spectrometry combined with a "halogenomic" analysis of non-targeted compounds revealed 29 novel contaminants of interest structurally similar to anthropogenic POPs. This novel ecotoxicological methodology, as well as qualitative and quantitative results, will be presented.

289 In-situ and sex-dependent accumulation of PBDEs, HBCDs and several alternative flame-retardants in smallmouth bass (Micropterus dolomieu)

M.J. La Guardia, R.C. Hale, Virginia Institute of Marine Science / Environmental & Aquatic Animal Health

In-situ mollusk bioaccumulation factors for BFRs: polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs) and the alternative-PBDEs (alt-PBDEs) 2-ethylhexyl 2,3,4,5-tetrabromobenzoate (TBB), 2-ethylhexyl 2,3,4,5-tetrabromophthalate (TBPH) and 1,2-bis (2,4,6-tribromophenoxy) ethane (BTBPE) have been previously identified downstream from a textile processing facility. That effort indicated that HBCD and PBDEs with 4 -6 bromines had similar tendencies to accumulate; but that accumulation propensities for the alt-PBDEs were lower. To evaluate in-situ BFR accumulation in a teleost consumed by humans, the carnivorous smallmouth bass (*Micropterus dolomieu*) was collected (n=22) at this BFR contaminated site, 47 km downstream and 18 km upstream for whole body and muscle tissue analysis. Whole body ΣBFR concentrations ranged from 389 – 25,550 ng g^{-1} lipid weight normalized (lw). BDE-47 and -99 were the major PBDE congeners detected, constituting >73%, by weight. TBPH was the only alt-PBDE observed, detected in one fish at 62 ng g^{-1} lw and two others slightly above their detection limit (1.0 ng g^{-1} lw). HBCD was detected in 16 of 22 fish; major isomer observed was α-HBCD. Whole body concentrations ranged from 5 – 423 ng g^{-1} lw. The range of ΣBFR muscle tissue concentrations was 70 – 15,100 ng g^{-1} lw. BDE-47 and -99 contributed >66% of the total by weight. The mean ΣPBDE male tissue levels exceeded females by 5:1. A positive trend was observed between male ΣPBDE concentration and length, but an inverse relationship was observed for females. Most notably, females that exceeded 254mm in length had the lowest PBDE body burdens. This size is considered a minimum for spawning in this species. Hence, the break in the ΣPBDE distribution may result from maternal transfer of lipophilic contaminants (e.g., PBDEs) during egg production and their depuration during spawning. These results demonstrate that: 1) male smallmouth bass had higher PBDEs and HBCDs burdens than mature females; 2) biomagnification of TBB, TBPH and BTBPE are considerably lower than PBDEs and HBCDs in these

piscivorous fish; 3) PBDEs and HBCD are transferred across the food-web and thus should be considered in pollutant consumption advisories.

290 Temporal Trends of Persistent Organic Pollutants and Vitamins in Northern Fur Seals from St. Paul Island, Alaska

J.R. Kucklick, National Institute of Standards & Technology / Chemical Sciences Division/ Hollings Marine Laboratory; J.L. Reiner, Environmental Health Sciences, NIST / Chemical Sciences Division; P.R. Becker, National Institute of Standards and Technology / Chemical Sciences Division; J. Hoguet, National Institute of Standards and Technology / Analytical Chemistry Division; J.M. Keller, National Institute of Standards and Technology (NIST) / Analytical Chemistry Division; A. Moors, NIST / Hollings Marine Laboratory; J. Ness, D. Peterson, R. Pugh, T. Ragland, C. Rimmer, J. Rhoderick, M. Schantz, NIST

The goal of this work was primarily to define temporal trends of legacy persistent organic pollutants (POPs) and current-use halogenated contaminants in a high trophic level marine predator from the North Pacific Ocean—a region with limited information on POP temporal trends. In addition, vitamin concentrations were assessed as a possible response variable that may be related to contaminants. To accomplish this goal, liver and blubber samples from 50 juvenile male northern fur seals (*Callorhinus ursinus*) were collected on St. Paul Island, Alaska from four different seal rookeries between 1987 and 2007 as a part of the Alaska Marine Mammal Tissue Archival Project (AMMTAP). Samples were analyzed for vitamins, polychlorinated biphenyl (PCB) congeners, organochlorine pesticides, brominated flame retardants, and perfluorinated alkyl acids (PFAAs). With the exception of mirex, all legacy compounds showed statistically significant declines with sampling year consistent with trends observed in marine mammals from other northern areas such as the Canadian Arctic. Overall, concentrations of legacy POPs are similar to levels seen in seal samples from other areas of the North Pacific Ocean and the Bering Sea and are considerably lower (factor of 10 or greater) than observed in marine mammals from temperate regions of the United States. The current-use brominated flame retardants, polybrominated diphenyl ether (PBDE) and hexabromocyclododecane (HBCD), showed exponential increases with sampling year although PBDE concentrations may be reaching a plateau. Likewise, PFAAs also show exponential increases with sampling year indicating continued release and incorporation into the North Pacific food web. Concentrations of retinol (vitamin A) and a- and g-tocopherol (vitamin E) in liver were not related to POP concentrations, year of sampling, or the rookery where the seal sample originated. POP concentrations do not appear to influence vitamin concentrations as observed in other wildlife studies. Further monitoring of northern fur seals is warranted because of increasing concentrations of current-use POPs and declining northern fur seal populations.

291 Clarifying the Relationship Between Persistent Contaminant Concentrations and Age in Biomonitoring of Long-Lived Organisms

M.J. Binnington, University of Toronto Scarborough / Dept. Physical & Environmental Sciences; F. Wania, University of Toronto at Scarborough / Department of Physical & Environmental Sciences

Biomonitoring of persistent organic pollutants (POPs) in long-lived organisms frequently reveals significant relationships between POP levels and age. While it is tempting to interpret these as changes in contaminant burden in an organism as it grows older, cross-sectional body burden-age trends (CBATs) obtained during the biomonitoring of a population should not be confused with data gleaned from sampling the same individuals repeatedly over time to produce longitudinal body burden-age trends (LBATs). In order to clarify the extent to which CBATs and LBATs for wildlife species may deviate from each other, and what role lifespan and gender play in this regard, we used mechanistic bioaccumulation models to estimate longitudinal exposures of polar cod (*Boreogadus saida)*, ringed seal *(Pusa hispida)*, bowhead whale (*Balaena mysticetus)*, and aboriginal Arctic humans to PCB congener 153 under a realistic time-variant emissions scenario. Resultant LBATs for successive male and female age cohorts were then "sampled" at specific timepoints to produce CBATs analogous to those from biomonitoring investigations. As found previously for human biomonitoring, the year of sampling relative to the year of peak environmental exposure was the most critical parameter in determining CBAT shape. Further, differences in reproductive behaviors contributed significantly to variation in species CBATs by sex, as well as variation between species. The impact of gender in mammals increased with increasing frequency of pregnancies, length of the lactation period, and lipid content of milk. CBATs were similar for organisms with widely different lifespans, ranging from 6 years for cod to 160 years for bowhead whales, as long as equivalent age scales were used. The extent to which CBATs resembled LBATs was dependent on the relative length of a species' life span and the time scale of notable changes in environmental exposure. When exposure changed only marginally during a species' lifespan, CBATs start to resemble LBATs. Whereas cross-sectional age trends in relatively short-lived species can thus often be interpreted as revealing information on contamination of individuals as they age, this is not possible for species that live long enough to experience changing environmental exposure conditions during their life time. Accounting for both age cohort and sex effects is essential when interpreting age trends in biomonitoring studies of long-lived pollutants and long-lived species.

Tennessee Valley Authority Kingston Fly Ash Recovery Project: Part B

292 Evaluation of Effects of the Kingston Ash Release on Benthic Invertebrate Communities

T.F. Baker; J.G. Smith, Environmental Sciences Division

A dike failure at the Tennessee Valley Authority's (TVA) Kingston Fossil Plant in 2008 released an estimated 5.4 million cubic yards of coal ash into the adjacent Emory River, with some ash deposits extending downstream into the Clinch and Tennessee Rivers. Assessments of the impact of the ash release on river ecology have included intensive post-spill surveys of benthic invertebrate communities, analysis of sediments for concentrations of target analytes, and characterization of the physical substrate to evaluate whether there were any correlations between ash-related sediment characteristics and benthic community structure. Some ash-related contaminants such as arsenic and selenium were highly correlated with the composition of ash in the sediments and were frequently elevated. Although benthic community differences among sites clearly exist, these differences appear to be primarily related to differences in substrate type rather than ash or ash-related contaminants. Benthic invertebrates in the Emory River in the immediate area of the spill were undoubtedly impacted by the initial ash deposits and later dredging operations, but the invertebrate community results suggest there will be no persistent adverse impacts from the ash spill.

293 Benthic Invertebrate Community Risk Characterization for Residual Coal Ash-Related Metals and Metalloids in Watts Bar Reservoir, Tennessee

N.L. Bonnevie, A.R. Stojak, M.A. Beauchemin, ARCADIS / Environmental Sciences Assessment and Planning; D. Buys, ARCADIS; R.M. Sherrard, Tennessee Valley Authority / Kingston Ash Recovery Project; T.F. Baker,

Benthic invertebrates are frequently used as indicators of sediment/habitat quality. Therefore, the evaluation of potential risks to benthic invertebrates was a critical element of the Baseline Ecological Risk Assessment (BERA) conducted to evaluate the impacts of residual fly ash in Watts Bar Reservoir, Tennessee. Potential risks to the benthic community were evaluated using data collected from approximately 20 miles of the river system where residual ash was present or potentially present. Multiple lines of evidence were independently evaluated. Data on water, sediments, and porewater chemistry and other (physical) characteristics were collected to assess chemical composition and to document other factors that could influence the distribution and abundance of benthic species (e.g., TOC, grain size). The results of toxicity tests and evaluation of tissue data provide information to evaluate the potential bioavailability and toxicity of the COPECs present. Measures of *in situ* biological effects, such as benthic community structure, provide information on actual alterations of resident communities that may be related to sediment chemistry and/or general habitat conditions. The information obtained from each line of evidence was qualitatively combined in an overall weight of evidence approach evaluating potential risks to the benthic community. Based on the overall weight of evidence, the ash release has resulted in potential impacts to the benthic invertebrate community, particularly in lower reaches of the Emory River. These risks appear to be primarily associated with ash and ash-related COPECs, especially arsenic. This conclusion is based on the finding of concentrations of ash-related COPECs in sediments and benthic tissues at concentrations potentially associated with adverse effects; combined with the observation at many locations of statistically significant reductions in biomass of test organisms in laboratory toxicity tests. The risk was determined to be moderate, based on the fact that the benthic community surveys conducted did not indicate definite impacts attributable

to the ash release, with observed differences between sampling locations appearing more likely related to variation in available substrate and habitat rather than the presence or absence of ash.

294 Deterministic and Probabilistic Wildlife Risk Assessment Using Dietary Exposure Modeling

C.B. Meyer, T.H. Schlekat, D.S. Jones, ARCADIS

We used dietary exposure models as one line of evidence to assess risk to wildlife from exposure to constituents of potential ecological concern (COEPC) associated with residual coal fly ash in river sediment at the Tennessee Valley Authority (TVA) Ash Recovery Site. Receptors of interest used were: wood duck, mallard, killdeer, great blue heron, muskrat, raccoon, mink, tree swallow, and gray bat. We used concentrations of representative food items, sediment, and water measured at the site in the dietary exposure models. Receptor intake assumptions were identified from the literature and ingestion rates were calculated using allometric equations. We divided modeled exposure doses by diet-based screening toxicity reference values (TRVs) for each COPEC to obtain screening hazard quotients (HQs). Seven COPECs (Se, As, Al, Pb, Mn, Cu, and Fe) had screening HQ > 1 and were greater than screening HQs in reference reaches; they were carried forward into a refined analysis. We refined HQs for the metals by refining the bioavailability factors using speciation, depuration, and sequential extraction of metals data. We also refined the TRVs with a thorough review of laboratory and field studies, selecting conservative TRVs that reflected the receptors and COPEC form. Further, risk was evaluated stochastically to determine the probability of exceeding a target HQ. Parameters that were varied stochastically included: concentration in biotic and abiotic media, body weight of the wildlife receptor (and consequently the allometrically-calculated ingestion rates), bioavailable fraction, and TRVs. Body weights were set at a normal distribution, while bioavailable fractions and concentrations in the diet and surface water were set at lognormal distributions. The distribution used for the refined TRVs varied, set to a triangular distribution if only a best estimate and range of TRVs was available, or set to the distribution that best fit the TRVs (normal, lognormal) if many TRVs were available. The deterministic and probabilistic models indicated that risk is negligible for herbivorous birds and all mammals other than bats. Model results show low risk from Se for bats and from As and/or Se for insectivorous and omnivorous birds. The most sensitive factor in the probability model was the uncertainty of the TRVs, followed by the variability in prey tissue concentration, which was about one-third as important at influencing the probabilities as the TRV.

295 Ecological Risk Analysis of Residual Coal Ash and Associated Metals and Metalloids in a River Reservoir System

D.S. Jones, ARCADIS; S.J. Young, ARCADIS / Environmental; A.R. Stojak, N.L. Bonnevie, ARCADIS / Environmental Sciences, Assessment, and Planning; L.W. Fontenot, ARCADIS / Risk Assessment and Ecological Sciences; B. Fulton, C.B. Meyer, , ARCADIS; J.S. Meyer, Arcadis US, Inc., ARCADIS; D. Buys, D.K. Rigg, T.H. Schlekat, ARCADIS; N. Carriker, TVA

The December 2008 Kingston ash release discharged approximately 5.4 million cubic yards of coal ash slurry into the adjoining river system. While the released ash itself is primarily composed of fine silica particles similar to sand, it also contains trace amounts of arsenic, chromium, copper, lead, mercury, nickel, selenium, thallium, vanadium, zinc, and other elements that occur naturally in the coal. The initial ash release at Emory River mile (ERM) 2.5 traveled upriver as far as ERM 5.75, with some ash also being transported downstream into the Clinch River and Tennessee River. In response to this event, the Tennessee Valley Authority (TVA) Kingston Ash Recovery Project was executed in three phases. Phases 1 and 2 focused on mass removal of ash from the Emory River and impacted embayments, as well as closure of the failed dredge cell. Phase 3 included comprehensive human health and ecological risk assessments of the estimated 500,000 CYs of residual ash that were not removed during Phase 1 or were transported downstream during storm events. The Baseline Ecological Risk Assessment (BERA) evaluated a wide-ranging list of potentially exposed and sensitive aquatic and terrestrial receptors. TVA collected an extensive suite of biological and environmental data, yielding multiple lines of evidence (LOEs) for most receptors. A weight of evidence (WOE) paradigm was used to evaluate multiple LOEs in the risk characterization process. Potential risk was categorized as Negligible, Low, Moderate, or High. Confidence in the risk determination was categorized as being Low, Moderate, or High. The final characterization of risk also included a determination for each assessment endpoint as to whether risk management actions were recommended, which provided a means for further characterizing the likelihood and magnitude of impairment of the assessment endpoint. Overall, the estimated risks related to residual ash and ash-related constituents were estimated to be: 1) primarily low and, at most, moderate; 2) associated with direct exposures of benthic invertebrates to ash, arsenic, and selenium in surface sediment, and dietary exposures of birds to arsenic and selenium via consumption of benthic invertebrates; and 3) localized and diminished with increasing distance from the release area. These risk drivers were considered along with other factors in the EE/CA in order to support the risk management decision-making process for the river system.

296 Development of Remedial Goals for Coal-Ash Associated Arsenic and Selenium in Support of Long-Term Site Monitoring

A.R. Stojak, ARCADIS / Environmental Sciences, Assessment and Planning; D.S. Jones, ARCADIS; S.J. Young, ARCADIS / Environmental; C.B. Meyer, D.F. Ludwig, T.H. Schlekat, ARCADIS; R.M. Sherrard, Tennessee Valley Authority / Kingston Ash Recovery Project; N. Carriker, TVA

The 2008 Kingston Fossil Plant ash release discharged 5.4 million cubic yards (cy) of fly ash into the Emory River, with subsequent migration of part of the ash into the Clinch and Tennessee Rivers. Approximately 3.5 million cy of sediment and ash were removed from the Emory River during a Time-critical Removal Action. This phase of clean-up was completed in December 2010 and as a result, approximately 500,000 cy of residual ash remain in the Emory, Clinch, and Tennessee Rivers. The residual ash and the ash-related metals were studied as part of an investigation of all three rivers to evaluate the need for additional remedial actions in Watts Bar Reservoir. Human Health and Ecological Risk Assessments used an extensive suite of biotic and abiotic data to identify receptors at risk. Estimated risks were mostly low, at most moderate, and limited to reaches near the release. The baseline ecological risk assessment recommended risk management actions for benthic invertebrates exposed to ash, arsenic, and selenium in surface sediment, and birds exposed to arsenic and selenium via consumption of benthic invertebrates and emergent insects. In August 2012, an Engineering Evaluation/Cost Analysis (EE/CA) was completed which evaluated alternative actions for residual river-system ash. As a result from the EE/CA, Monitored Natural Recovery was selected as the best case action. The EE/CA required long-term monitoring remedial goals for biota and sediments. These goals were based on site-specific sediment toxicity tests and dietary exposure models for tree swallow and killdeer. Results from these studies are considered conservative in nature, and appropriate for providing a range of monitoring goals. Toxicity test endpoints causing 25% inhibition (IC_{25}) on endpoints (survival, emergence, and biomass) for *Hyalella azteca* and *Chironomus dilutus* were calculated for arsenic, selenium and ash content. To generate robust RGOs, the outcome for both test organisms at IC_{25} for all three endpoints is averaged. Tissue monitoring endpoints (TMES) are risk-based concentrations in tissue of prey items that result in hazard quotients (HQs) equal to one. TMEs were developed for larval mayflies as representative of prey items for killdeer and adult mayflies as representative of prey items for tree swallows. TMEs were selected based on consideration of reference concentrations and HQs, with the goal of monitoring reduction in risks over time.

297 Kingston Ash Recovery Environmental Monitoring and Assessment—A Case Study in Adaptive Management

N. Carriker, M. Cagley, R. Crawford, TVA; R.M. Sherrard, Tennessee Valley Authority / Kingston Ash Recovery Project; D.H. Yankee, TVA; M.E. Stack, Jacobs Engineering / Federal Operations; M. Houck, Jacobs Engineering; P. Clay, TVA Ash Recovery, Restoration Services, Inc; D.S. Jones, ARCADIS; S.J. Young, ARCADIS / Environmental

Over the nearly five years of the Kingston Ash Recovery Project, environmental monitoring and assessment activities have changed as project needs have changed. From initial emergency response monitoring to evaluate potential immediate public health threats to the recently-approved Long-Term Monitoring plan, to assess effectiveness of the Monitored Natural Recovery remedy for the approximately 500,000 cubic yards of ash remaining in the river system, monitoring and assessment of air quality, water quality, ash movement, and ecological impacts have been periodically re-assessed and modified to provide timely information for decision-making. This presentation reviews the evolution of these monitoring and assessment programs and

the adaptive management process used to ensure optimal use of available resources.

298 Risk Management related to Restoration Decisions and NRDA for the TVA Kingston Fly Ash Recovery Project; Roane County, TN

M. Cagley, TVA / Environmental Permitting and Compliance

As sampling plans were developed over the life of the project, each plan addressed the goal of gathering information regarding potential damages to the flora and fauna in areas of and adjacent to the site of the release. This was in addition to each plans primary purpose which was to evaluate the current state of the river and the organisms which the river supported. Data gathered was used to guide the design for restoration of the impacted areas in order to restore those areas and associated wildlife to their pre-spill or better conditions in a timely manner. This included riparian and open water areas, forested peninsulas as well as areas which were previously lawns. Data was also used by the trustees to calculate ecological and human use losses as part of the Natural Resource Damage Assessment. This data informs the baseline condition of the areas, the potential future damages and the value given to enhancements above baseline conditions.

Transitioning Nanosafety Science to Solutions

299 Comparing the fate and ionic silver and nanometallic silver in soils – implications for ecotoxicity tests and risk assessment

M. McLaughlin, CSIRO/University of Adelaide / School of Agriculture, food and wine, CSIRO / Land & Water; D.G. Navarro, CSIRO / Advanced Materials Transformational Capability Platform, Land and Water; K. Langdon, CSIRO Land and Water; J. Kirby, CSIRO / Advanced Materials Transformational Capability Platform, Land & Water; R.S. Kookana, CSIRO / Land and Water; C. Doolette; L. Settimio, University of Adelaide; A. Kumar, CSIRO Land and Water

Increasing use of both ionic and nanosilver compounds as biocidal agents in consumer products has rekindled interest in the fate and toxicity fo this metal in soil. Ionic silver is potentially toxic to a range of soil organisms but undergoes rapid transformation in soils to forms that are less soluble and less toxic, largely related to the properties of silver which mean that it binds strongly to sulfur-containing groups on soil organic matter and may undergo redox reactions which change speciation markedly. Nanometallic silver on the other hand may undergo dissolution in soils to ionic silver, but also undergoes aggregation (both homo- and hetero-) in soils to be retained strongly on the solid phase. Furthermore, the main expsoure pathway for nanometallic silver into the terrestrial environment is via wastewater treatment systems and through this process most of the material is converted to silver sulfides, either as nanosized or larger particles or larger aggregates. Hence the fate of nanometallic silver in the terrrestrial envireonment is closely linked to the fate of silver sulfide compounds in soils. These transformations need to be considered in the design of ecotoxicity testing of silver compounds for risk assessment, so that exposure pathways and expsoure forms of silver to soil organisms are environmentally relevant. We will present examples of these transformations in soils and rates of reaction, as well as information on the fate of nanosilver sulfide compounds in soils, and relate this to information required for the risk assessment of nanosilver in the terrestrial environment.

300 Bringing it all together: Comparing a standard ERA with a more informed, nano-specific ERA for ZnO and Ag nanoparticles

C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology, Centre for Ecology & Hydrology / Microbial Ecology and Ecotoxicology, NERC Centre for Ecology & Hydrology / Ecotoxicology; A. Crossley, K. Jurkschat, Oxford University Begbroke Science Park / Department of Materials; M. Hassellov, Goteborg University / Department of Chemistry and Molecular Biology; G. Cornelis, University of Gothenburg / Department of Chemistry and Molecular Biology; J. Hammes, Goteborg University; J.A. Gallego-Urrea, University of Gothenburg / Chemistry; S. Loureiro, Universidade de Aveiro / Departamento De Biologia; P.V. Silva, Universidade De Aveiro / department of Biology & CESAM; E. Lahive, W. Tyne, M. Diez-Ortiz, NERC Centre for Ecology and Hydrology; M. Matzke, Centre for Ecology & Hydrology (NERC) / Hails Section; D. Spurgeon, Centre for Ecology & Hydrology; A. Johnson, CEH Wallingford; R. Williams, Centre for Ecology and Hydrology; V. Keller, Centre for Ecology & Hydrology; E. Dumont, NERC Centre for Ecology and Hydrology; S. Lofts, Centre for Ecology & Hydrology / Shore Section; C. van Gestel, Vrije Universiteit Amsterdam / Institute of Ecological Science; P. Waalewijn-Kool, VU University; F. Dondero, University of Piemonte Orientale / DiSIT; T. Backhaus, University of Gothenburg / Dep. of Biological and Environmental Sciences; M. Faust, Faust & Backhaus Environmental Consulting; L. Walker, Centre for Ecology & Hydrology

The basic foundation of nanotechnology is that engineering the size and shape of materials at the nanometer scale produces distinct, novel properties with potential functional and commercial value. The specific properties of nanomaterials (NM) and their resulting unique environmental behaviour and effects have led to the concern that current environmental risk assessment (ERA) methods, endpoints and approaches may not be adequate. This presentation will compare and contrast the outcome of an ERA based on standard PEC modelling and PNEC estimates with the best possible ERA that can be derived using new information and data generated in the EU FP7 project NanoFATE. The project is a multi-disciplinary effort involving analytical chemists, ecotoxicologists, material scientists and fate and exposure modellers aiming to assess the environmental risks posed by engineered nanoparticles (ENPs). NanoFATE challenges the applicability of current fate and risk methodologies in order to identify possible improvements required for ENP ERA. The work is undertaken using both commercial ENPs from high-volume products for which recycling is not an option, e.g., fuel additive, personal care and antibacterial products (CeO_2, ZnO, Ag of varying size, surface and core chemistries) and purpose-made model ENPs for mechanistic work. These are being followed through their post-production life cycles i.e. from environmental entry as "spent product", through waste treatment to their final environmental fates and potential toxic effects. The analysis of different ERA options will start from simple, worst-case assumptions and then progressively include more data to increase realism. On the hazard side internal kinetics, long-term exposure effects and more sensitive and relevant endpoints (multiple species, functions and communities) will be included. Exposure assessment will successively include greater spatial and temporal resolution of PEC modelling, NM aging and bioavailability. The presentation will focus on key issues in both terrestrial and aquatic settings using Ag and ZnO NMs as examples. The relative impact and importance of the additional data generally improving the ERA realism vs. the nano-specific additional data and information will be discussed and related to the resources required.

301 The importance of considering transformations when evaluating risks of engineered nanomaterials in agroecosystems

J.M. Unrine, University of Kentucky; O.V. Tsyusko, University of Kentucky / Department of Plant & Soil Sciences; S. Rathnayake, D. Starnes, B. Collin, P. Bertsch, A. Whiltey, University of Kentucky

Recent life cycle and environmental material flow analyses suggest that agricultural soils are a primary sink for a large proportion of engineered nanomaterials that are used in consumer products and industrial processes. Until recently, the vast majority of ecotoxicological studies of nanomaterials have been conducted on aquatic organisms. Currently, there is an increasing body of research aimed at uptake and toxicity of nanomaterials in terrestrial ecological receptors; however, many studies fail to take into account biogeochemical transformations of nanomaterials that occur in wastewater streams, during wastewater treatment, after subsequent introduction into natural soils and within organisms after their uptake or due to interactions with organisms. The majority of these studies also use unrealistic exposure scenarios and concentrations. This presentation will summarize major research efforts underway which have begun to unravel the nature of these transformations and their implications for toxicity, environmental fate and ecological risks. There are profound chemical and physical transformations of nanomaterials in wastewater and during wastewater treatment, including sulfidation of Ag and sulfidation and phosphatation of ZnO. Interactions between these nanomaterials and organic matter also profoundly affects their behavior and toxicity. The transformations also tend to negate the properties that affect fate of nanomaterials in soils conferred by coatings applied to nanomaterials during manufacturing, potentially simplifying the risk assessment process. Transformations of materials may occur during uptake by detritivores and plants that alter the bioavailability of nanomaterials during subsequent trophic transfers. Overall, most of the biogeochemical transformations that would occur during wastewater treatment tend to reduce toxicity to a variety of species. However, a recent study conducted using a realistic exposure scenario in outdoor terrestrial mesocosms demonstrated effects on plants and microbial community function that were greater for Ag nanomaterials, added using biosolids as a vehicle, than for Ag salts. This

highlights the importance of addressing potential risks posed by nanomaterials to agroecosystems and suggests that we need to redouble our efforts to address this important environmental exposure pathway.

302 Fate and transformations of silver nanoparticles in lake mesocosms

L. Furtado, Trent University / Environmental and Life Sciences / Environmental & Resource Studies; E. Hoque, Environmental & Resource Studies; B. Cheever, Trent University / Department of Biology; D.M. Mitrano, EMPA Technology & Society Lab / Chemistry and Geochemistry; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; H. Hintelmann, Trent University / Chemistry; C.D. Metcalfe, Trent University / Environmental & Resource Studies

Silver nanoparticles (AgNPs) are an emerging contaminant that could put aquatic ecosystems at risk. Once released into natural waters, AgNPs can remain in nanoform, heter- or hom-agglomerate, or dissociate into dissolved silver (dAg). These transformations will dictate the fate and ecological impacts associated with the release of AgNPs. Thus, it is imperative to understand AgNP transformations in natural water matrices at environmentally relevant concentrations using suitable analytical methods. In this study, sequential filtration, including ultra-filtration, Cloud Point Extraction (CPE), Single-Particle-Inductively-Coupled Plasma Mass Spectroscopy (SP-ICP-MS), and Asymmetric Flow Field Flow Fractionation with on-line ICP-MS (AF4-ICP-MS) techniques were used to characterize transformations of AgNPs added to mesocosms in the Experimental Lakes Area (ELA) of Ontario, Canada. Mesocosms (2 m diameter x 2 m depth) were deployed in Lake 239 at ELA to evaluate the fate of 50 nm capped AgNPs. In two mesocosms spiked with a single dose of polyvinylpyrrolidone (PVP) capped AgNPs at a nominal concentration of 80 ug/L, total Ag levels declined relatively slowly, with a $t_{1/2}$ of ~20 days. SP-ICP-MS indicated that the mean size of AgNPs declined over time from 50 to 30 nm over 21 days, indicating either particle dissolution or precipitation of larger particles. AF4-ICP-MS results are consistent with particle dissolution as there was an increase over time of particles in the 1-10 nm size range. However, very little dAg was detected using ultrafiltration, likely due to binding of Ag^+ to inorganic ligands or accumulation by planktonic biota. CPE results were difficult to interpret, but AgNP "weathering" may have contributed to an observed decrease in extractable AgNP. These results indicate that the AgNPs were relatively stable in the mesocosms. This stability may have been due to the low ionic strength, moderately high NOM and circum-neutral pH of the lake water. The various analytical techniques will be discussed with respect to their utility as robust and sensitive methods for studying AgNP fate at environmentally relevant concentrations.

303 The influence of soil properties on the bioavailability and toxicity of ZnO nanoparticles to the earthworm, Eisenia fetida

E. Lahive, Centre for Ecology & Hydrology (NERC); D.J. Spurgeon, C. Svendsen, M. Matzke, M. Diez Ortiz, L. Heggelund, S. Lofts, A. Lawlor, Centre for Ecology and Hydrology (NERC)

Metal and metal oxide nanoparticles (NPs) present a series of challenges for terrestrial ecotoxicology. Both chemical transformations of the NPs (e.g.dissolution) and the chemistry of the soil may modify the organism exposure and response in NP–dosed soils. In this study we investigated how soil properties influence the bioavailability and toxicity of zinc oxide (ZnO) NPs and ionic zinc to the earthworm *Eisenia fetida*. Worms were exposed to both a 30nm diameter uncoated ZnO NP and to ionic zinc references, zinc nitrate ($Zn(NO_3)_2$) or zinc chloride ($ZnCl_2$). Five soils were used for this study, one of which was pH–adjusted to three different pH levels using calcium carbonate, giving seven exposure media in total. Soil organic matter (SOM) content ranged from 1.8–16.7% and soil pore water pH from 4.5–8.3. Earthworms were exposed to the nominal concentration range 100–4400 mg Zn/kg, with survival and reproduction assessed after 28 and 56 days, respectively. Soil pore waters were extracted from all soils at the beginning (t0) and end (t56) of the experiment, ultrafiltered (10kDa) and analysed for zinc, pH and dissolved organic carbon. Tissue zinc concentrations were measured in depurated worms after 28 days of exposure. Based on nominal soil concentrations of zinc, the reproduction EC50s in the various soils ranged from 277-1002 mg Zn/kg for ionic Zn and from 705–>2200 mg Zn/kg for ZnO NPs. When EC50s were based on pore water zinc concentrations, they were generally higher in the ionic Zn-spiked soils than in the ZnO NP-spiked soils, suggesting that the effects in the ZnO NP exposures were due to both Zn NPs and ionic Zn released by dissolution. However, direct comparison of toxic effects was confounded by the contrasting pH effects of dosing the soils with NPs (pH increases) and ionic Zn (pH decreases). Worms exposed to ZnO NPs accumulated significantly more zinc in their tissues than the ionic Zn exposed worms at comparable effect levels. This study has shown evidence for NP-specific effects in earthworms exposed in a range of soils. Furthermore, we have seen that soil properties influence the bioavailability and toxicity of zinc, whether dosed as a NP or in an ionic form. The results suggest that modelling toxicity in ZnO NP-dosed soils needs to consider the dissolution of the NPs to form ionic Zn and the influence of the soil chemistry on the bioavailability and toxicity of both the ionic Zn and the ZnO NPs.

304 Plant uptake of manufactured nanomaterials under environmentally realistic conditions and the importance of nanomaterial properties to bioaccumulation

J.D. Judy, J.M. Unrine, University of Kentucky; P. Bertsch, University of Kentucky / Plant and Soil Sciences

Manufactured nanomaterials (MNMs) are likely being discharged in rapidly increasing quantities into terrestrial ecosystems, primarily via land application of biosolids. Plant uptake of MNMs is a pathway by which humans and other higher trophic level organisms could potentially be exposed to MNMs. Despite this, few studies have examined how bioavailable and toxic MNMs will be to plants under environmentally realistic conditions or how the great diversity of MNM properties (e.g., size, composition, surface chemistry, etc.) might affect bioavailability and toxicity. We used the test organisms *Nicotiana tabacum* L. cv *Xanthi* (tobacco) and *Triticum aestivum* (wheat) to investigate plant uptake of 10, 30 and 50 nm diameter Au MNMs coated with either tannate (T-MNMs) or citrate (C-MNMs). Plants were exposed to MNMs hydroponically for 3 or 7 days for wheat and tobacco, respectively. Volume averaged Au concentrations were determined using inductively coupled plasma mass spectrometry (ICP-MS). Spatial distribution of Au in tissue samples was determined using laser ablation ICP-MS (LA-ICP-MS) and scanning X-ray fluorescence microscopy (μXRF). Both C-MNMs and T-MNMs of each size treatment bioaccumulated in tobacco, but no bioaccumulation of any treatment was observed in wheat. Characterization of post-exposure treatment suspensions revealed that the wheat plants had induced substantially more MNM aggregation during the exposure period. These results indicate that MNMs of a wide range of sizes and with different surface chemistries are bioavailable to plants and raise questions about the importance of plant species and root exudation to MNM bioaccumulation. To gain a greater understanding of the bioavailability and phytotoxicity of MNMs to plants under environmentally realistic conditions, we exposed *Medicago truncatula* (barrel clover), a model legume species, inoculated with *Sinorhizobium meliloti* to soils amended with biosolids. The biosolids were from pilot wastewater treatment plants where waste streams had been enriched with ZnO, TiO_2, and Ag MNMs, equivalent bulk (TiO_2) or dissolved metal ($ZnSO_4$ and $AgNO_3$) controls, or untreated biosolids. The results of this study including biomass production, nodulation rate, bioaccumulation (ICP-MS), and other key parameters will also be presented.

305 Tracking Nanoparticle Release from Polymer Nanocomposites Using spICPMS

J. Wang, Colorado School of Mines; R. Lankone, Johns Hopkins University; H. Fairbrother, Johns Hopkins University / Department of Chemistry; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry

Development of reliable nanometrology is critical to the life cycle assessment of nano-enabled products. One of the most important types of nano-enabled products are polymer nanocomposites. These incorporate nanoparticles(NPs) such as carbon nanotubes (CNTs) or nano-scale metal oxides, enhance polymer properties such as conductivity and load bearing capabilities. With increasing applications of polymer nanocomposites in automotive, aeronautical and packaging industries, there is concern that NPs will be released to the environment and raise the risk to environmental systems. In order to more fully understand how NPs are released from polymer nanocomposites, TiO_2–PS (polystyrene) and CNT (carbon nanotubes)–PCL (polycaprolactone) materials were prepared and subjected to degradation experiments over a period of 30 days. Chemical, thermal, and UV light treatments were used to degrade the nanocomposites. For polymers containing 0.5% NPs, the weight loss varies from 12% to 29%, depending on experimental conditions. Release of nanoparticulate TiO_2 was monitored by single particle ICPMS (spICPMS) measuring ^{47}Ti. Because of difficulties in detecting CNTs in complex environmental system by carbon analysis,

yttrium (^{89}Y), intercalated in CNT structure, was used. Released NPs were easily observed as pulses in the spICPMS data. The observed number of TiO2 and CNT particles increased with degradation time. The effect on quantitative determination of released NPs of data processing procedures for discriminating NPs from the background was examined.

306 Attempts to quantify TiO2 particle release from sunscreen in a natural swimming area: a feasibility study using spICPMS

R. Reed, Colorado School of Mines / Department of Chemistry and Geochemistry; F. Von der Kammer, University of Vienna / Department for Environmental Geosciences; A. Gondikas, University of Vienna / Environmental Geosciences; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; T. Hofmann, University of Vienna / Environmental Geosciences

Incorporation of nano-TiO_2 into commercially available sunscreens creates the potential for release to the environment during typical use and for subsequent exposure to organisms. Due to the low (ng/L) surface water predicted environmental concentrations of TiO_2 and the likely background of natural particles, detection and quantification of these materials is not feasible by widely used methods such as electron microscopy or light scattering. The feasibility of single particle inductively coupled plasma mass spectrometry (spICPMS) was investigated for analysis of titanium-containing particles in samples from a natural water body. Previous research has shown spICPMS to be a highly sensitive element-specific method capable of detecting ng/L concentrations for many metal nanoparticles, including TiO_2 in simple systems. Analysis of a reference nano-TiO_2 material (NM-104, OECD) by spICPMS was performed to optimize a method for the size-dependent characterization of TiO_2 nanoparticles. This method was then applied to analysis of titanium-containing particles in natural surface waters. To investigate the possible occurrence of nano-TiO_2 release from sunscreens in real-world systems, water samples were taken from bathing areas along the Old Danube in Vienna, Austria and analyzed by spICPMS for particle number concentrations and size distributions. This sampling site is a still-water lake which is used by thousands of bathers per day during the summer months, making it ideal for determining real-world exposure data for nano-TiO_2 released from sunscreens. Water, suspended particulate matter, and sediment samples were collected and analyzed throughout the year to determine background levels of Ti outside of the summer bathing season as well. Titanium-containing particle number concentrations measured by spICPMS over the course of the year showed a large increase during the bathing season of July and August (15,000 to 40,000 particles/mL) relative to the rest of the year (~5,000 particles/mL). Bulk analysis of suspended particulate matter element concentration ratios such as Ti/Al, used to control for natural variability, show an increase of ~25% during the summer bathing season.

PAH in the Environment: Advances in Assessment of Occurrence, Sources, and Human and Ecological Risks: Part B

307 Temporal and Spatial Patterns in Tumor Prevalence in Brown Bullhead (Ameiurus nebulosus) in the Tidal Potomac River Watershed (USA)

F.E. Pinkney, US Fish & Wildlife Service / Chesapeake Bay Field Office; J.C. Harshbarger, George Washington University Medical Center / Department of Pathology; M.A. Rutter, Penn State Erie – The Behrend College / Department of Mathematics

Liver tumors in bottom-dwelling fish are caused by exposure to polynuclear aromatic hydrocarbons (PAHs) in sediment. Tumor surveys are used to monitor freshwater, estuarine, and marine habitats, often tracking improvement after cleanups. We describe brown bullhead (*Ameiurus nebulosus*) tumor surveys conducted in 2009–2011 in the tidal Potomac River watershed, focusing on the Anacostia River (Washington, DC), a Chesapeake Bay Region of Concern. We report statistically significant decreases in liver tumor probabilities for a 280 mm Anacostia bullhead between the 1996 and 2001 samplings (female: 78.2%, male: 42.5%) and those in 2009–2011 (female: 42.6%, male: 13.3%). Liver and skin tumor prevalence were similar in Anacostia River (2009–2011), Potomac River in Washington, DC (2009) and Piscataway Creek (2011) collections, suggesting a regional rather than Anacostia problem. Despite the improvement, liver tumor prevalence in bullheads from the Anacostia and several Potomac watershed locations were significantly higher than estimated Bay-wide background (280 mm bullheads: liver—9.7% female, 2.3% male; skin—3.7% female, 2.4% male). Logistic regression, used to analyze the Chesapeake Bay bullhead tumor database from 1992–2011 (n=1404), showed that both liver and skin tumor prevalence were higher in females and increased with total length. Updated sediment data are needed to determine if there is a cause-effect relationship between reduced liver tumor prevalence in Anacostia River bullheads and actions that may have decreased PAH exposure. We recommend conducting tumor surveys on a 5-year cycle with sediment chemistry analyses from collection locations to monitor changes in habitat quality in this watershed.

308 Distribution and bioconcentration of polycyclic aromatic hydrocarbons among water, suspended particulate matter, and fish

H. Li, M. Yang, Guangzhou Institute of Geochemistry / State Key Laboratory of Organic Geochemistry; Y. Ran, Guangzhou Institute of Geochemistry / Chinese Academy of Sciences

The spatial distribution, partitioning, and bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) in water, suspended particulate matter (SPM), and fish samples from the Pearl River Delta (PRD) and the estuary (PRE) were examined. 15 USEPA priority PAHs in the water range from 10.95 ng/L to 48.08 ng/L. PAHs in SPM varied from 8.74 ng/L to 98.13 ng/L. PAHs in the fresh water fish range from 10.52 ng/g to 244.87 ng/g in carp, tilapia, and red grass carp, and in sea fish in the PRE range from 19.52 ng/g (*yellow drum*) to 142.81 ng/g (*blue pomfret*) in muscle, from 24.20 ng/g (*halfbeak*) to 856.38 ng/g (*hairtail*) in gill and from 32.43 ng/g (*halfbeak*) to 1094.40 ng/g (*tongue fish*) in viscera. Although the concentrations of aqueous and particulate PAHs are much lower in PRE than in DR or PR, PAHs in the tissues of different fish species are significantly higher in PRE than in DR or PR, possibly relating to the dilution effect of fish growth. Aqueous or particulate PAHs respectively show significant correlations with DOC, POC, and Chl a, suggesting that biological pumping effect and eutrophication regulate their distribution and bioaccumulation. The in-situ partitioning coefficients (log K_{oc}) estimated for PAHs are one order magnitude higher than the empirical log K_{oc}-log K_{ow} correlation, implying that the hydrophobicity of PAHs and the heterogeneity of organic matter are critical factors in their distribution. It is also observed that the bioconcentration factors (log BCF) in the different fish tissues firstly increase with the increasing logK_{ow}, and then reach a maximum value, and then decrease. The log BCF value is a little lower for the sea fish than for the freshwater fish. This trend indicates that the values of BCF may vary due to the diversity of fish species, feeding habits, and metabolism of PAHs in fish. In addition, PAHs are dominated by 3 and 4-ring compounds in the water and the SPM samples. The combustion of fossil fuels and coal is the major source of PAHs in the PRD, and the petroleum contamination could be a minor source in this region.

309 Absence of Predicted Toxicity from Creosote-Derived PAH in Sediments

N.L. Bonnevie, ARCADIS / Environmental Sciences, Assessment, and Planning; P.D. Anderson, Arcadis US, Inc.; M.A. Beauchemin, ARCADIS / Environmental Sciences Assessment and Planning; T.J. Iannuzzi, Arcadis US, Inc.

Accepted regulatory practice for sediment investigation at environmental sites is to analyze sediments for site-related constituents of potential concern and compare reported concentrations to sediment screening benchmarks. If these point-concentration benchmarks are exceeded, additional investigations and/or assessments are typically undertaken. This paper examines the toxicity of polycyclic aromatic hydrocarbons (PAHs) in sediments at several sites where creosote-derived PAHs were expected to be the primary source of potential toxicity. It also includes data from a large riverine system with multiple sources to demonstrate how the results may be impacted by other inputs. The data reveal a strong relationship between PAHs and observed toxicity in which toxicity increases with increasing PAH concentration. However, the relationship also reveals that all standard screening benchmarks used to evaluate the potential toxicity of PAHs in sediments greatly overestimate the toxicity of creosote-derived PAH to benthic invertebrates. Based on these data, actual allowable concentrations of PAHs in sediments could be much higher than currently available benchmarks would suggest.

310 Evaluating toxic potential of sediments in Taean near Hebei Spirit Oil spill site and contributions of PAHs and alkylated PAHs

S. Lee, Seoul National University / School of Public Health; S. Hong, Seoul National University / Division of Environmental Science and Ecological Engineering / School of Earth and Environmental Sciences; K. Noh, Seoul

National University / Graduate School of Public Health; X. Liu, Seoul National University / School of Public Health; J. Khim, Korea University / Division of Environmental Science and Ecological Engineering, Seoul National University / School of Earth and Environmental Sciences; U. Yim, Korea Institute of Ocean Science & Technology / Oil & POPs Research Group, Korea Ocean Research & Development Institute; W. Shim, Korea Institute of Ocean Science and Technology / Oil and POP Research Group; J.P. Giesy, University of Saskatchewan / Toxicology Centre and Department of Veterinary Biomedical Sciences; K. Choi, Seoul National University / School of Public Health

The *Hebei Sprit* oil spill occurred approximately 10 km off the coast of Taean in December 2007. Long-term consequences of the oil spill on the coastal area still remain largely uncertain. The sediments near the oil spill are contaminated with complex mixtures of pertroleum derived compounds. Therefore, total extracts as well as fractionized extracts were employed to identify compounds that are responsible for the observed *in vitro* toxicity. Polycyclic aromatic hydrocarbons (PAHs) and alkylated PAHs, common products of weathering, were also tested for *in vitro* toxicity to understand the contribution of these compounds to the observed sediment toxicity. Three cell lines of H4IIE-luc, MVLN bioassay, and H295R cell were employed to measure dioxin-like toxicity, estrogen receptor binding affinity, and steroidogenic pathway alteration, respectively. A total of 14 sediment samples were collected from 3 locations during 2007 to 2012, and extracted and analyzed for 19 PAHs and 25 alkylated PAHs. Five major PAHs, e.g., naphthalene, fluorine, phenanthrene, dibenzothiophene, and chrysene, and their alkylated forms were also tested. TEQs measured from the sediment extracts were over 0.9 ng/g dw. Ten out of 14 extracts showed increased E2/T ratio. However, there are neither temporal nor spatial pattern of toxicity change which may reflect heterogeneous nature of sediment. Aromatic fraction (F2) and polar fraction (F3) generally showed greater toxic potency in all three cells compared to aliphatic fraction (F1). The same observation was made from *in vitro* toxicity assays on crude and weathered oil. Among the study PAHs and alkylated PAHs, naphthalene and dibenzothiophene groups generally induced E2 production. Phenanthrenes showed greater ER binding potency but alkylated forms showed lesser binding affinity. Chrysene and alkylated chrysene showed relatively great AhR binding potency. TEQs estimated for PAHs in the sediment samples ranged between 0.01 and 0.33 ng TEQ/g and accounted for up to 7.4% of the total AhR binding potency in the sediment sample. Compounds that are responsible for the rest of AhR binding potency warrant further investigation.

311 Method for Estimating Dermal Skin Cancer Risk from Benzo[a]pyrene Exposure in Terrestrial Mammals via Soil Contact at Contaminated Sites

A. Knafla, Equilibrium Environmental Inc.

The carcinogenic potential of polycyclic aromatic hydrocarbons (PAHs), and in particular Benzo[a]pyrene (B[a]P) has been known for decades and these hydrocarbons are regulated at contaminated sites in order to protect the health of humans and ecological receptors. Most published guidance provides algorithms to estimate cancer risk from PAH exposure via oral or inhalation uptake, with consideration for the amount of PAH absorbed through the skin, which may contribute towards a systemic dose leading to systemic tumour development. An algorithm was developed that allows for skin cancer risk to be estimated following dermal contact with soil containing B[a]P. Using a reasonable and not-overly conservative approach, it was determined that skin cancer risk exceeded the risk of systemic tumours following oral and dermal absorption exposure. This is supported by published research with small mammals in a laboratory setting where skin painting of B[a]P in various delivery vehicles promoted increased DNA adduct levels in skin and an increased incidence of carcinomas and papillomas in the epidermis. The relative incidence of systemic tumours (e.g., of the lung, liver, etc.) following dermal exposure was more than an order of magnitude lower. This is due in part to the relatively fast metabolic capacity of the skin that may rapidly transform the dose of B[a]P that has penetrated through the stratum corneum and into the epidermis, into its reactive metabolic forms, which subsequently can produce DNA adducts and ultimately carcinoma and papilloma development. This work suggests that terrestrial mammals in addition to humans, exposed chronically to B[a]P in soil, may be at increased risk of developing skin cancer. The importance of considering simultaneous exposure to ultraviolet radiation is discussed, since publisehd work suggests this may promote B[a]P related tumour development.

312 Quantitative Chemical Source Attribution of Sediments at Former MGP Sites

D. Mauro,

The ability to identify the sources of contamination in environmental samples has improved greatly over the past decade or so. Chemical fingerprinting, as it is sometimes called, has benefited from advancements in analytical instrumentation, development of new techniques, and the accumulated information from the analysis of tens of thousands of samples. However, urban environments are complex and quantitative source apportionment can be difficult to achieve with confidence. Regardless, for sites with mixed impacts, such as urban sediments near former MGP sites, the ultimate goal is always to determine the amount of each source in each sample with some known level of confidence. This presentation will discuss the meaning of terms such as similarity, dissimilarity, and match. It will explore the sources of variability that affect the confidence associated with stating that the chemicals in a particular sample came from a particular source. It also will examine several methods for estimating the amount of each contaminant source at the site and putting a level of confidence on that estimate. The appropriate application of methods such as simple statistics and similarity metrics, multivariate similarity metrics, and so-called rules of thumb will be explored and illustrated using data from MGP sites.

313 Receptor Models for PAH Source Characterization: Opportunities and Limitations

K. O'Reilly, Exponent, Inc; S.M. Mudge, Exponent International Limited; P.D. Boehm, Exponent, Inc. / Environmental & EcoScience Group

Multivariate receptor models are seeing increased use in the characterization of potential sources of polycyclic aromatic hydrocarbons in sediments. Focusing initially on single watersheds, they have been used recently to evaluate multiple sediment systems across the country. While these tools are powerful, the application of receptor models is constrained by the models' underlying assumptions. A particular challenge is distinguishing among sources of pyrogenic PAH that have similar chemical profiles. While alignment with all model assumptions may not be possible in many situations, understanding how violations influence the results is critical. Because results are being presented as evidence in cost allocation cases and to support PAH source control policies, it is important that practitioners present modeled results and associated uncertainties in a way that is understandable to a lay audience. Focusing on published case studies, this presentation will describe the appropriate use of receptor models to characterize PAH sources and will demonstrate how slight changes in input parameters can result in important differences in model outputs. Comparative results from various models based on the same inputs will be presented.

314 Research-Based Input Parameters for Risk Assessment of Coal-Tar-Based Pavement Sealants

B.H. Magee, ARCADIS; J. Keating-Connolly, Arcadis US, Inc.

Recent publications have presented human health risk estimates for children and adults with presumed exposure to potentially carcinogenic PAHs in residences adjacent to where coal-tar-based pavement sealants have been used. These published reports assert that use of coal-tar-based sealants is associated with human health risks in excess of widely accepted regulatory levels of concern. A range of risk estimates will be presented based on alternate estimates for these key input parameter values in the risk calculations: PAH concentrations in house dust, PAH bioavailability, and benzo(a) pyrene and coal tar toxicity. Data supporting the use of alternate estimates for these input parameters will be summarized. Published data on concentrations of PAHs in house dust from studies at residences adjacent to where coal-tar-based pavement sealants have been used will be compared to reported PAH concentrations in house dust and soil from sources other than pavement sealants for locations throughout the United States. The relative source contribution of PAHs to house dust attributable to coal-tar-based pavement sealants will be factored into alternate risk calculations. Reported risk estimates rarely account for relative bioavailability of PAHs in an exposure medium that includes a coal tar matrix and supporting soil/dust structure that would reduce the absorbed dose of PAHs. The scientific literature on absorption of PAHs from coal tar and coal tar in soil or dust will be reviewed to assess a range of potential relative bioavailability factors and their subsequent effects on risk calculations. Risk estimates for the exposure scenarios evaluated are also influenced by assumptions regarding the potential carcinogenicity of benzo(a)pyrene and coal tar mixtures. A range of

risk estimates will be presented based on toxicological data for benzo(a)pyrene [B(a)P] as B(a)P toxic equivalents and coal tar mixtures from published guideline-compliant toxicological studies.

Emerging Approaches for Rational Design of Chemicals with Minimal Biological Activity

315 Second Generation Design Guidelines for Reduced Aquatic Toxicity Using Mechanism-based Electronic Parameters

A. Voutchkova-Kostal, George Washington University; J. Kostal, Sustainability A to Z; P. Anastas, Yale University / Center for Green Chemistry and Green Engineering; J. Zimmerman, Yale University

The ability to minimize aquatic toxicity of commercial chemicals at the design stage has substantial economic and animal welfare implications. Until recently this has been considered unrealistic, but research published in recent years shows that Lipinski-like empirical rules for reduced acute and chronic aquatic toxicity are indeed possible. Here we report second generation design guidelines, which include ionization at pH 7.4 to improve the representation of bioavailability. In addition, we show that by improving the accuracy of the computational method for calculation of HOMO-LUMO energy gaps we can improve the sensitivity of this model. Our revised approach uses AM1/Monte Carlo simulations to identify the lowest-lying conformers in water, followed by geometry optimizations using the mPW1PW91 density functional and the MIDIX+ basis set. The empirical guidelines capture >85% of the compounds designated as "safe" by (EPA category 3 and higher) and only 5% in category 2. Additional electronic parameters that model nucleophilic, electrophilic and radical reactivity were calculated. When segragated by mode of action, the eletcronic parameters show excellent correlations (R>0.8) with mol-based measures of toxicity.

316 Designing Acid Corrosion Inhibitors with Reduced Hazards for Oil and Gas Applications

D. Woloch, T. McLean, M. Muellner, M. Malwitz, W. Blake, Nalco Champion, An Ecolab Company

There has been an unprecedented growth in oil and gas production especially in the US. In parallel to this growth there is increasing public concern over the safety of chemicals used for oil and gas applications. In response to public concern, stringent regulations for industrial chemicals have been introduced in certain US states and globally. The heightened demand for using chemicals with reduced hazards has led chemical suppliers to reevaluate their product portfolios and develop less hazardous innovative technologies. To this end, Nalco Champion is using EPA predictive modeling programs and an internal prescreening scorecard system called eVerified to aid in the design of novel chemistries. The EPA predictive models were used in early product development to determine ecological and toxicity properties of new compounds. The eVerified scorecard identifies individual components in a final formulation that may contribute to the overall hazard of a product. These prescreening tools were used to design and develop an acid corrosion inhibitor. ACIs play an important role in protecting well integrity during a well stimulation process called acidizing. The results of the predictive modeling were compared with experimental results and eVerified scorecards were used to compare existing technologies with new ones within a product line. The development of an effective and cost-competitive, yet less hazardous, ACI validates the use of these prescreening tools during early product development.

317 Towards Prediction of LogP and Bioaccumulation Based on Experimental Spectroscopic Characterization

N. An, George Washington University / Department of Chemistry; F. Van Der Mei, The George Washington University; A. Voutchkova-Kostal, George Washington University

There is an evident need for tools that allow chemists to consider toxicity minimization during the design and synthesis of new commercial chemicals. To be adopted, such tools must be easily incorporated into the routine chemical synthesis and characterization workflow. Nuclear Magnetic Resonance (NMR) is the most widely used spectroscopic technique for structure characterization. Here we report the development of a Quantitative Spectra Property Relationship (QSpPR) model that predicts octanol-water coefficient (logP) and bioaccumulation from proton NMR spectra. The training set consisted of ~500 compounds with predicted proton NMR spectra and experimental logP and bioaccumulation values. The NMR spectra were used to derive descriptors that reflect chemical shift, integration, and peak broadness. QSpPRs for logP and bioaccumulation and were built with linear and non-linear regressions. Internal and external validations were performed to test the model robustness.

318 Reducing aquatic toxicity: Probabilistic Hazard assessment of sustainable molecular design guidelines

K.A. Connors, Baylor University / Institute of Biomedical Studies; A. Voutchkova, Yale University / Center for Green Chemistry and Green Engineering, George Washington University / Chemistry; P. Anastas, Yale University / Center for Green Chemistry and Green Engineering; J. Zimmerman, Yale University; B.W. Brooks, Baylor University / Department of Environmental Science

Basic toxicological information is lacking for the majority of industrial chemicals. In addition to increasing empirical toxicity data through additional testing, prospective approaches that aim to specifically design chemicals with reduced unintended toxicological activity are recognized as a critical component of green chemistry. Recent work by our research team has resulted in the derivation of a "rule of two," wherein chemicals with logP and ΔE values within a defined range are predicted to elicit low acute and chronic aquatic toxicity. The present study examined the potential benefit of reducing hazards presented by industrial chemicals if these two sustainable molecular design guidelines were employed. Probabilistic hazard assessment modeling indicated that utilizing these sustainable molecular design guidelines for logP and ΔE would appreciably decrease the number of chemicals that are of high and very high concern for acute and chronic toxicity to standard model organisms and endpoints. Additional property guidelines provide further opportunity to reduce concerns by accounting for the toxicological activities of specific chemical classes (e.g., aldehydes), chemical attributes (e.g., ionization) and adverse outcome pathways in representative species (e.g., receptor mediated responses).

319 Avoiding endocrine disruption in the design of new chemicals

T. Schug, NIEHS; R. Abagyan, UC San Diego; B. Blumberg, UC Irvine; T. Collins, Carnegie Mellon University; D. Crews, University of Texas, Austin; P. DeFur, Virginia Commonwealth University; S. Dickerson, University of Texas, Austin; T. Edwards, Louisiana Technical University; A. Gore, University of Texas, Austin; L. Guillette, Medical; T. Hayes, University of California, Berkeley; J. Heindel, NIEHS; A. Moores, McGill University; H. Patisaul, North Carolina State University; T. Tal, USEPA; K. Thayer, NIEHS; L. Vandenberg, Tufts University; J. Warner, Warner-Babcock Institute; C. Watson, University of Texas, Galveston; F. vom Saal, University of Missouri; T. Zoeller, University of Massachusetts, Amherst; K. O'Brien, Advancing Green Chemistry; J. Myers, Environmental Health Sciences

We propose here an endocrine disruption testing protocol for use by chemists in the design of new chemicals, the Tiered Protocol for Endocrine Disruption (TiPED). It starts with a chemist theoretically at "the drawing board" and consists of five testing tiers ranging from broad *in silico* evaluation through specific cell- and whole organism-based assays. To be effective at detecting endocrine disruption, a testing protocol must be able to measure potential hormone-like or hormone-inhibiting effects of chemicals, as well as the many possible interactions such chemicals may pose on signaling pathways. Accordingly, we have designed this protocol to broadly interrogate the endocrine system. Here we present the principles that should guide the science of testing new chemicals for endocrine disruption, as well as principles by which to evaluate individual assays for applicability, and laboratories for reliability. In a 'proof-of-principle' test, we ran 6 endocrine disrupting chemicals (EDCs) that act via different endocrinological mechanisms through the protocol using published literature. Each was identified as endocrine active by one or more tiers. We believe that this voluntary testing protocol will be a dynamic tool to facilitate efficient and early identification of potentially problematic chemicals, while ultimately reducing the risks to public health.

320 Rational selection of environmentally compatible detergents for production of biopharmaceuticals

J. Straub, F.Hoffmann-La Roche Ltd / Group Safety & Environment Protection; R. Shearer, Genentech Inc., a member of the Roche Group; M. Studer, F. Hoffmann-La Roche Ltd

Sustainable production is not only concerned with a 'green' final product but incorporates the whole process. In order to replace a detergent used in biotechnological production, environmental properties of potential

alternatives were searched in the public literature and, where insufficient information was retrieved, tested to assess biodegradability and model removal in the wastewater treatment plants serving three biotechnological production sites of the Roche Group. Predicted environmental concentrations (PECs) were calculated using realistic amounts of both detergents used, total wastewaters discharged, modelled removals for the wastewater treatment plants (WWTPs) and site-specific dilution factors by the receiving waters. Predicted no-effect concentrations (PNECs) were derived both for fresh and marine receiving waters. This resulted in a spreadsheet showing PECs, PNECs and PEC÷PNEC risk characterisation ratios for the WWTPs and receiving waters for the three production plants, in a comparative site-specific risk assessment. The spreadsheet also serves as a decision support for the biotechnological production developers, allowing a quick evaluation of the environmental compatibility of alternative detergents for all production sites in the Roche Group.

321 USEPA Alternatives assessments for flame retardants: Providing information in a multi-stakeholder forum to consider complex chemical management issues

E.T. Lavoie, USEPA / Office of Pollution Prevention and Toxics; M. Kawa, SRC Inc.; J.L. Tunkel, SRC, Inc. / Environmental Health Analysis; G. Howard, Dickinson College; J. Rhoades, SRC, Inc.

Flame retardants have become important additives in plastic, foam, and textile products in the last fifty years due to growth in plastics applications and flammability requirements. Flame retardants function through a range of modes of action and include a variety of chemistries: metal oxides; phosphates; bromine or chlorine substituted phosphates, diphenyl oxides and polymers; and large inorganic and organic polymers. The exposures and hazards associated with polybrominated diphenyl ethers (PBDEs) in particular have driven a focus on flame retardants that will require choices in policy to balance the risk-risk trade-off between fire safety and environmental safety that is debated by interest groups. While the challenge to find the right approach may continue for years, green chemistry principles argue the need to identify the least hazardous chemicals for uses in the mean time. Because exposure to flame retardant chemicals is inevitable at one or more parts of a product life cycle, selecting the least hazardous chemicals that perform the needed functional use is an important approach to reducing chemical risks. The USEPA Design for the Environment Program has provided information in several projects over the last ten years by assessing more than 50 flame retardants in four projects covering alternatives for pentaBDE, decaBDE, TBBPA, and HBCD used in furniture foam, textiles, electronics and polystyrene building insulation. These projects are voluntary and stakeholder informed. The alternatives assessments have not only identified relevant chemicals and provided environmental hazard profiles but also presented a forum for all stakeholder voices, assessed many chemicals at one time and importantly have yielded data not previously available to EPA or the public. The evidence shows that 1) flame retardants have inherent persistence 2) there is a spectrum of inherent hazards across the broad class of chemicals but 3) some flame retardants do have lower hazards (e.g., some metal oxides and some very large polymers). Providing data about flame retardant availability, uses, inherent hazards, and associated tradeoffs gives stakeholders the information needed to make informed decisions about chemicals they use, research and development priorities, policy approaches, and life-cycle impacts while flammability standards shift and scientific evidence is gathered.

Organic Flame Retardants Beyond PBDEs: What Have We Learned?

322 Identification and Occurrence of Analogues of Dechlorane 604 in Sediment and Fish from the Great Lakes

L. Shen, Brock University / Department of Chemistry; K. Jobst, E. Reiner, Ontario Ministry of the Environment; P.A. Helm, Ontario Ministry of the Environment / Environmental Monitoring and Reporting Branch; V. Taguchi, Ontario Ministry of the Environment; R. McCrindle, Wellington Laboratories; C. Marvin, S. Backus, Environment Canada; I. Brindle, Brock University / Department of Chemistry

The Dechloranes represent a group of halogenated norbornene flame retardants based on Diels-Alder chemistry. They include the high production volume compound Dechlorane Plus (DP), which replaced Mirex (also named Dechlorane) as a flame retardant, and Dechloranes (Dec) 602, 603, and 604. The latter compounds have been found in fish and sediment in the Great Lakes region and Dec 602 has recently been found in arctic biota. Dec 602 was found to be more bioaccumulative than Dec 603, Dec 604, and DP based on calculated biota-sediment accumulation factors. Dec 604 was found to have low concentrations in fish in our previous studies. This presentation highlights new findings for compounds that are related to Dechlorane 604, which consists of a tetrabromostyrene molecule bonded to hexachlorocyclopentadiene. Targeted analyses by GC-HRMS for Dec 602, 603, 604 and DP revealed several unknown peaks for mass fragments typical of retro Diels-Alder chemistry. Non-targeted investigative analysis by ultra-high resolution Fourier Transform Ion Cyclotron Resonance mass spectrometry with data analysis using Kendrick mass defect plots revealed debrominated analogues of Dechlorane 604 present in Lake Ontario lake trout. After confirmation with synthesized standards, semi-quantitative analyses indicated that the tribromophenyl hexachloronorbornene (triBr-Dec604) concentrations in Lake Ontario lake trout were approximately 50-200 times greater than the concentrations of 5-50 pg/g wet weight for Dec 604. The monoBr-Dec604 and diBr-Dec604 analogues were present at lower amounts. In contrast, triBr-Dec604 concentrations were generally lower than Dec 604 in sediment. Trends of triBr-Dec604 in sediment cores from Lake Ontario were the same as Dec 604, indicating that the compound was either present as an impurity or any production followed that of Dec 604. The results suggest that triBr-Dec604 is either considerably more bioaccumulative than Dec604, that the triBr-Dec604 is more stable and more likely to be accumulated than Dec604, or that debromination of Dec604 may be occurring in the food web. Further, the findings highlight the importance of considering degradation products / impurities for assessment of persistent and bioaccumulative compounds.

323 Novel Brominated and Organophosphorus Flame Retardants in the Arctic Atmosphere

A. Salamova, Indiana University / School of Public and Environmental Affairs; M.H. Hermanson, University Center in Svalbard / Arctic Technology / Environmental Chemistry; R.A. Hites, Indiana University / School of Public & Environmental Affairs

The use of most polybrominated diphenyl ethers (PBDEs) was banned by the European Union and phased out in the United States. PBDEs are now listed by the Stockholm Convention on Persistent Organic Pollutants in Annex A, which calls for elimination of their production and use. However, there is a continuing need for flame retardants due to stringent flammability standards both in the USA and Europe, and as a result, there seems to be a shift to using non-regulated flame retardants. Several replacement flame retardants have been recently detected in the environment, including brominated benzoate and phthalate esters and organophosphorus esters. Here we present the levels of these replacement flame retardants in atmospheric samples (particle phase) collected in Svalbard, Norway. The samples were collected using Hi-volume atmospheric sampler during 2012-2013. The analytes of interest include 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB), *bis*(2-ethylhexyl)-tetrabromophthalate (TBPH), *tris*(2-chloroethyl) phosphate (TCEP), *tris*(1-chloro-2-propyl)-phosphate (TCPP), *tris*(1,3-dichloro-2-propyl)phosphate (TDCPP), *tris*(2,3-dibromo-propyl)-phosphate (TDBPP), triphenyl phosphate (TPP), tri-*o*-tolyl-phosphate (TOTP), tri-*p*-tolylphosphate (TPTP), *tris*(3,5-dimethylphenyl)phosphate (TDMPP), *tris*(2-isopropylphenyl)phosphate (TIPPP), tri-*n*-butyl phosphate (TnBP), *tri*(butoxyethyl)phosphate (TBEP), *tris*(2-ethylhexyl)phosphate (TEHP), and *tris*(4-butylphenyl) phosphate (TBPP). The levels of the replacement flame retardants will be compared to the levels of PBDEs in the same samples to investigate the occurrence of these two groups of flame retardants in the Arctic atmosphere.

324 Dietary accumulation of two phosphate ester flame retardants TBEP and TCPP in juvenile rainbow trout (Oncorhynchus mykiss)

L. Bestvater, Freshwater Institute / Department of Chemistry; G. Tomy, University of Manitoba / Department of Chemistry; K. Pleskach, Freshwater Institute; K. Wautier, Freshwater Institute

Organophosphate flame retardants (OPFRs) have been used for many decades; however, due to the recent ban on PBDEs, their use has increased greatly in the past couple of years. Increasing use has led to their detection in many environmental compartments including ground water, surface water, wastewater, air, sediment, dust, and human biological samples, both in Europe and North America. Considering these FRs are now ubiquitous, there is very little information on their fate in the environment and their

effects on wildlife. We exposed juvenile rainbow trout to two OPFRs, i.e., tris (chloropropyl) phosphate (TCPP) and tris (2-butoxyethyl) phosphate (TBEP); that have been found in significant amounts in the environment. The aim of our study is to determine biological half-lives (persistence), bioaccumulation factors, and potential effects on the endocrine system (toxicity).

325 Tissue Distribution and In Ovo Transfer of Bioaccumulative Organophosphate Flame Retardants in Great Lakes Herring Gulls (Larus argentatus)

A.K. Greaves, Carleton University / Department of Chemistry; R.J. Letcher, Environment Canada / Science and Technology Branch, Ecotoxicology and Wildlife Health Division

As a result of the recent phase out of polybrominated diphenyl ethers (PBDEs), the usage of organophosphate flame retardants (OPFRs) in commercial products has increased. Recent investigations into the Great Lakes ecosystem of North America have shown repeated detection of tris(2-butoxyethyl) phosphate (TBEP) and tris(2-chloroisopropyl) phosphate (TCPP) in multiple fish species. Furthermore, studies have shown the detection of TBEP, TCPP, tris(2-chloroethyl) phosphate (TCEP), and triphenyl phosphate (TPhP) in the eggs of herring gulls (*Larus argentatus*) from across the Great Lakes. Herring gulls are a top avian predator species in the Great Lakes. With the bulk of biotic studies examining either hepatic or blood concentrations, extremely little is known regarding the pharmacokinetics of OPFRs in the body. In this study, eight female herring gulls and their eggs were collected from Chantry Island, Lake Huron. In total, eight tissue types were analyzed for OPFRs: liver, adipose, muscle, brain, blood plasma, red blood cells, and both the yolk and albumen of their eggs. In the liver, TCEP (1.18 ± 0.27 (standard error) ng/g wet weight; ww) and TBEP (0.77 ± 0.26 ng/g ww) were consistently detected. In their eggs, TBEP, TPhP, and tributyl phosphate (TBP) were all quantifiable in the yolk, at or near the quantification limit. TPhP was the only quantifiable OPFR in the albumen. The results of this study indicate that OPFRs are maternally transferred *in ovo* from mother to offspring. Furthermore, this is the first study to perform a comprehensive tissue/body compartment distribution study of OPFRs in any biotic species.

326 You are what you eat: Using biotelemetry to investigate the links between habitat use and contaminant profiles in avian species

M.L. Gentes, Université du Québec à Montréal / Département des sciences biologique; M. Patenaude-Monette, J.F. Giroux, University of Quebec in Montreal (UQAM) / Behavior and Animal Ecology Research Group (GRECA), Department of Biological Sciences; R.J. Letcher, Environment Canada / Wildlife and Landscape Science Directorate, Science and Technology Branch, Carleton University; J. Verreault, University of Quebec in Montreal (UQAM) / Environmental Toxicology Research Centre (TOXEN), Department of Biological Sciences

Flame Retardants (FRs) are chemicals added to furniture and electronics. While they increase fire safety, they have become ubiquitous contaminants worldwide. Increasing concern regarding endocrine disruption and neurotoxicity of Polybrominated Diphenyl Ethers (PBDEs) contributed to their international phase out, and recent research suggests that some of the replacement products (e.g, Bis-(2-ethylhexyl) Tetrabromophthalate (TBPH)) may also disturb hormone balance, notably the thyroid axis. Birds can become highly contaminated with FRs; however, the contaminant burden of individuals within a single colony can vary widely due to several biological and ecological factors. For instance, inter-individual variations can arise from differences in diet and habitat use – which could only be fully characterized if birds were to be followed in their foraging trips. These knowledge gaps may lead to critical misinterpretation of spatial and temporal trends of contaminant data. Biotelemetry is currently undergoing a spectacular evolution as a result of increasing miniaturization of GPS dataloggers, which makes it a promising new tool in ecotoxicology. In the present study, biotelemetry was used to investigate the links between foraging strategies and FR concentrations/proportions (liver and plasma) in an omnivorous bird (ring-billed gull (*Larus delawarensis*)) breeding downstream from Montreal (QC) – an area recently identified as a FR "hotspot" in Canada. Emerging FRs with the highest detection frequency (>80%) and highest concentrations were Bis(2-ethylhexyl)-2,3,4,5-tetrabromophthalate (TBPH) and Dechlorane Plus (DP). Other detected compounds included 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB) and hexachlorocyclopentenyl-dibromocyclooctane (HCDBCO). The proportion of BDE-209 (25% of sum PBDE) and its concentrations (57.2 ± 12.2 ng/g ww) were unexpectedly high for this mid-trophic gull species, exceeding levels previously reported for raptors. Analysis of gulls' foraging movements pinpointed local anthropogenic environments such as wastewater ponds and landfills as a major source of BDE-209. These results highlight the importance of documenting not only diet composition but also habitat use strategies to understand contaminant profiles in avian species, and stress the need for further restricting the use of BDE-209.To our knowledge, this work is among the first to ally biotelemetry and wildlife toxicology.

327 Variations in behaviour and energy expenditure in urban-breeding birds- associations with halogenated flame retardant exposure

S.C. Marteinson, University of Quebec in Montreal / Département des sciences biologique; J.F. Giroux, Université du Québec à Montréal / Département des sciences biologique; J.F. Helie, Université du Québec à Montréal / Geotop; M.L. Gentes, Université du Québec à Montréal / Département des sciences biologique; J. Verreault, Université du Québec à Montréal / Department of Biological Sciences

Exposure to environmental contaminants may affect the endocrine status, energy expenditure and related endpoints in vertebrates. Moreover, for wild individuals, the metabolism of toxicants may incur an additional energetic cost that could impact the behaviours important for their survival and reproduction. Because birds living in urban areas are exposed to high levels of contaminants, examining their exposure-related impact on the hormonal status and bioenergetic functions is highly relevant. We examined the plasma concentrations of established and emerging halogenated flame retardants (HFRs) in relation to field metabolic rate (FMR), time-activity budget behaviour and associated endocrinology (circulating thyroid hormones and corticosterone levels) in ring-billed gulls (*Larus delawarensis*) breeding in the greater Montreal area (QC). The doubly labeled water technique was used to determine FMR. Bird-borne GPS data-loggers were used for detailed behavioural assessment over a 2-5 d period from which the activity budget was divided into time spent in nest attendance, flying, foraging and resting on the colony. The plasma HFR profiles were largely dominated by PBDEs. Those congeners that were detected in all individuals, in descending order of mean concentration (ng/g ww), were BDE-209, -99, -47, -153, -100, -207, -201, -208, -154/BB-153. Several emerging or less studied HFRs were also detected at lower concentrations in some individuals including *syn-* and *anti*-dechlorane plus (DP), pentabromoethylbenzene (PBEB), hexabromobenzene (HBB), and bis(2-ethylhexyl)tetrabromophthalate (BEHTBP). The plasma sumPBDE concentrations were positively associated with the time spent in nest attendance and negatively associated with the time spent flying, but are not directly correlated with FMR or hormone concentrations. The field metabolic rate FMR was partially dependant on the proportion of time spent in these the activity budget behaviours. Further analyses on associations with emerging HFRs are currently underway, however, these preliminary results suggest that present suite of flame retardants may be associated with an impact on the behaviour of these urban-breeding birds.

328 Can we get beyond PBDEs: what have we learned??

M.L. Diamond, University of Toronto / Department of Earth Sciences; E. Goosey, University of Toronto / Earth Sciences; A. Golnoush, University of Toronto / Geography; A. Saini, University of Toronto / Department of Physical and Environmental Sciences; F. de Leon, University of Toronto / Dalla Lana School of Public Health; D. Serodio, University of Toronto / Department of Earth Sciences

Recall that production bans/voluntary cessation of penta and octa-BDEs were passed in 2003 and 2004 in the European Union and US, respectively. Deca-BDE will hopefully be out of production in North America by 2013. The masses of penta, octa and decaBDE in-use in foam furniture, personal vehicles, electronics and textiles from 1970-2020 was estimated at 45 000, 20 000 and 250 000 tonnes, respectively, with the largest mass of deca in-use in 2012. The legacy of decaBDE in products will remain in-use for decades past 2020. At the same time, numerous halogenated and phosphorus-containing flame retardants are replacing PBDEs in new products. The proliferation of many FRs will make tracking their inventories yet more difficult than that of PBDEs. The physical-chemical properties of many of these replacements suggest that they too may be problematic. What have we learned? Apparently not much has been learned from the PCB story in terms of the management of in-use inventories of toxic chemicals. As many wealthy countries are still working towards compliance with the Stockholm

Convention for PCBs, it is difficult to see how the in-use stock of PBDE-containing products can be managed in an environmentally sound way. So, what have we learned? That depends on who the "we" is. The chemical industry has learned to step up production of flame retardant chemicals "grandparented" into chemical management schemes such as TCSA and CEPA, environmental scientists have learned to be CSI detectives by identifying and evaluating "new" flame retardants, and government policy-makers have learned to be patient as political wrangling interests often supersedes policy built on "sound science". What we need to learn is to strengthen and to understand the science upon which flammability standard setting rests since these standards are a key source of the problem.

Fate and Effects of Metals: Marine Concerns

329 Adaptation of the freshwater transformation/dissolution protocol to marine systems

P. Huntsman-Mapila; J. Skeaff, M. Pawlak, R. Beaudoin, CANMET-Mining, Natural Resources Canada

Until now, the freshwater medium OECD 203 for ecotoxicity testing of fish and daphnia has been used in all Transformation/Dissolution (T/D) research on metals, metal compounds and alloys, domestically and internationally. Nonetheless, the composition of a marine medium is given in the T/DP section of the UN Globally Harmonized System for the Classification and Labelling of Chemicals (GHS), and by implication, a method for marine T/D testing is open for development and validation. Because many chemicals in commerce are transported through marine shipping, the hazard identification, classification and labelling (HCL) of metal-bearing substances in commerce for marine systems is the subject of increasing interest and may well be required in the near future. The analytical component of this project has been complex due to the necessity of determining ppb levels for trace metals in a high salt matrix. This complexity has required the use of a metal chelation step using an ion exchange resin prior to analysis of the marine medium on an ICP-MS. To date, we have examined the marine T/D characteristics of four substances: Ni metal powder, Cu oxide powder, Co oxide powder and a Ni-Co-Fe alloy, which were also examined under the freshwater T/DP validation study. The Cu oxide powder appears to be considerably more reactive in the marine media than in pH 8 freshwater with 100 mg/L loadings resulting in a $[Cu]_{aq}$ of 881 µg/L at 168 hr compared to 103 µg/L in freshwater. The nickel metal powder is slightly less reactive in the marine medium with 100 mg/L loadings of the Ni metal powder resulting in a $[Ni]_{aq}$ of 176 µg/L at 168 hr compared to 314 µg/L in freshwater. Cobalt oxide is similarly unreactive in both the marine and freshwater pH 8 medium: < 5 µg/L for the 100 mg/L loading at 168 hr. The Ni-Co-Fe alloy is considerably more reactive in the marine medium with $[Ni]_{aq}$ and $[Co]_{aq}$ of 365 and 192 µg/L for Ni and Co respectively compared to 7.3 and 2.2 µg/L for Ni and Co in freshwater, for 100 mg/L loadings at 168 hr. Comparisons of the T/D marine data with ecotoxicity reference values in marine water will be required for hazard classification purposes.

330 Effects of waterborne copper and its possible mode of toxic action in marine invertebrates

A. Bianchini, Fundação Universidade / Instituto de Ciências Biológicas, Universidade Federal do Rio Grande

It is well known that copper (Cu) is an iono/osmoregulatory toxicant in freshwater invertebrates and fish. The mechanism underlying its toxicity is based on a Cu-induced inhibition of the Na^+,K^+-ATPase activity, which is a key-enzyme involved in Na^+ uptake at the gills. This effect leads to reduction of gill Na^+ uptake with consequent impairment in the ion regulatory processes that ultimately leads to death. In contrast, the mechanism of acute Cu toxicity in osmoconforming (marine) invertebrates is still unclear. As no significant osmotic gradient must be maintained between the surrounding medium and body fluids in osmoconforming animals, it is unlike that osmotic disturbances would be related to the acute Cu toxicity in these animals. Although most of marine invertebrates are osmoconformers, they are still ionoregulators, especially when considering divalent cations (Ca^{2+} and Mg^{2+}). Therefore, Cu effects on the regulation of these ions cannot be disregarded. In fact, ionoregulatory (K^+, Ca^{2+} and Mg^{2+}) disturbances have been reported in marine invertebrates acutely exposed to waterborne Cu under isosmotic condition. However, it is shown that Cu exposure also induces disturbances in parameters related to respiration, oxidative status, energy metabolism, acid-base regulation and feeding in marine invertebrates. These effects have been reported in studies performed with both gill-breathing and non gill-breathing animals. Most studies were performed with crustaceans and mollusks. However, there are also reports in echinoderms, cnidarians, holothurians and foraminifers. In this presentation, a review of the waterborne Cu effects on biochemical and physiological parameters in marine invertebrates (crab, shrimp, isopod, copepod, mussel, clam, sea cucumber, sea anemone, and foraminifer) is provided. Also, the possible mechanism of acute Cu toxicity in these animals is discussed. Financial support: IDRC, ICA, CNPq, and CAPES.

331 Life-cycle Toxicity of Lead to Americamysis bahia in a Flow-through Exposure Including Detailed Investigation of Lead Analysis in Salt Water

S.D. Smith, Wilfrid Laurier University / Department of Chemistry; O. Vukov, Department of Biology, Wilfrid Laurier University / Biology; R. Nasir, Wilfrid Laurier University / Department of Biology; J. Cunningham, Wilfrid Laurier University / Biology; J. Chowdury, ILZRO; J. McGeer, Wilfrid Laurier University / Department of Biology

There is a paucity of data on effects of chronic exposure to lead (Pb) on sensitive marine invertebrates. Additionally, the measurement of Pb concentrations in marine environments potentially varies depending on the methodology applied which further complicates the assessment of potential risks of environmental Pb. The objective of this project was to study the effect of waterborne Pb on life-cycle toxicity to the marine mysid *Americamysis bahia* using a flow-through exposure system and to compare and validate analytical methods for measuring Pb in salt water at low concentrations. Spike recoveries and testing on parallel samples were utilized to compare Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Anodic Stripping Voltammetry (ASV) and graphite furnace atomic adsorption spectroscopy (GFAAS). ASV could be run on unmodified samples, for ICP it was necessary to run diluted samples to avoid salt effects. GFAAS required a pre-separation of the lead from the saltwater matrix using a lanthanum hydroxide precipitation technique. For 100 ppb spike recoveries GFAAS underestimated the most (recovery < 70%). ICP and ASV exhibit recovery at 90% with ICP having a tighter reproducibility than ASV (standard deviation of ±6.6 ppb for ICP versus ±16.6 ppb for ASV). ASV was subsequently utilized for all Pb determinations (total and dissolved) in the chronic 30-d mysid test (ASTM E1191- 03a). Survival, total offspring, offspring/female and day to first brood were assessed using a logistic model and when appropriate a model that included hormesis was included for comparison. The endpoints based on offspring production were more sensitive with total offspring EC values being somewhat more robust than offspring per surviving female. The latter endpoint tended to have higher variability due to the low numbers of surviving females. A logistic model estimation of EC20 and EC10 for the total number of offspring was 10.6 and 7.5 µg/L dissolved Pb respectively.(95% CI of 3.6- 32.0). A model that included hormesis effects at low concentrations was also used and under this approach the EC20 and EC10 for offspring were 11.8 and 9.9 µg/L (95% CI 6.2-22.5). The results suggest that *Americamysis bahia* is one of the most sensitive species for Pb in marine waters. This research is funded by NSERC through a partnership with ICA, CDA, ILZRO, NiPERA, IZA, Teck, Vale and Xstrata Zinc.

332 Influence of salinity and DOC on chronic Ni toxicity to the mysid Americamysis bahia

C.A. Cooper, R. Nasir, Wilfrid Laurier University; J. McGeer, Wilfrid Laurier University / Department of Biology; S.D. Smith, Wilfrid Laurier University / Department of Chemistry

The biotic ligand model (BLM) has been successfully used to predict metal toxicity in freshwater environments. However, this model has been primarily based on copper (Cu) toxicity data; and metal toxicity in marine and estuarine environments can differ considerably when compared to freshwater environments, due the different water chemistries. In addition, there is also a growing body of evidence that shows that dissolved organic carbon (DOC) concentration can have a protective effect against metal toxicity and that this effect can be DOC source dependent. Therefore, static chronic nickel (Ni) toxicity tests using mysids, *Americamysis bahia*, were performed to assess the effects of DOC concentration and DOC source in full strength seawater (30 ppt). The chronic test, based on an EPA test method, ran for a duration of 7 days and used mysids that were 7 days old at the start of the test. During these tests, mysids were exposed to a range of Ni concentrations, 3 different DOC concentrations (zero added up to 10 mg C L^{-1}) and to DOC from different sources. The DOC sources were chosen based on relative

concentrations of humic, fulvic and tryptophan, which were determined using fluorescence excitation emission matrix (EEM) spectra analysis. Total Ni and dissolved Ni concentrations were measured using anodic stripping voltammetry (ASV). The toxicity test end points were mysid mortality, length, dry weight and female brood pouch development. Under control conditions, zero added DOC, the Ni 'half maximal effective concentration' (EC50) on *A. bahia* female brood pouch development and length was 100 and 75 mg Ni L^{-1}, respectively. The 'median lethal concentration' (LC50) of Ni was 234 mg Ni L^{-1}. The potential for addition of DOC to demonstrate protective effects is evaluated. A comparison to a similar mysid study showed that Ni was less toxic than Cu. Data from this research will be used in the development of a marine and estuarine BLM, and ultimately criteria for the protection of organisms in these environments. This research is funded by NSERC through a CRD grant in partnership with ICA, CDA, ILZRO, NiPERA, IZA, Teck, Vale and Xstrata Zinc.

333 Modeling Metal Bioaccumulation in Killifish (Fundulus heteroclitus) in Three Contaminated Estuaries

J. Dutton, Adelphi University / Environmental Studies Program; N.S. Fisher, Stony Brook University / School of Marine and Atmospheric Sciences

Fish are exposed to metals through dietary and aqueous exposure pathways. Although studies have shown that the diet is the dominant exposure pathway for most metals, the importance of aqueous exposure should not be overlooked. The bioavailability of aqueous metals to fish is predominantly influenced by the salinity and dissolved organic matter concentration in the water. To further understand the influence of water chemistry on aqueous metal uptake, loss, and tissue distribution, we exposed killifish (*Fundulus heteroclitus*) to three metals (Cd, Cr(III), and Hg (as Hg(II) and methlymercury (MeHg))) and one metalloid (As(V)) in water collected from three contaminated estuaries with varying salinity and dissolved organic carbon (DOC) concentration using a radiotracer technique; two field sites were in the Chesapeake Bay (Baltimore Harbor (7.6 ppt) and Elizabeth River (19.5 ppt)) and one in San Francisco Bay (Mare Island (22 ppt)). Uptake rates from the dissolved phase were highest for MeHg (0.370-0.781 L g^{-1} d^{-1}) and lowest for As (0.0003-0.0006 L g^{-1} d^{-1}). Cd and MeHg uptake showed an inverse relationship with salinity, whereas As uptake increased as salinity increased. No clear relationship was observed between metal uptake and DOC concentration for any metal due to the narrow DOC concentration range between the three field sites (2.0-4.6 mg L). Loss rate constants were highest for As (0.046-0.096 d^{-1}) and lowest for MeHg (0.006-0.009 d^{-1}). Tissue distribution showed that Cd was predominantly associated with the gills, As, Hg(II), and MeHg with the body, and Cr was split between the head and body. As salinity increased the percentage of Cd associated with the gills decreased and increased in the viscera; marine fish drink to osmosregulate and drinking could be an uptake mechanism for Cd. The kinetic parameters calculated in the present study were entered into a biokinetic model to calculate the predicted body burden of each metal at steady-state and the percentage body burden attributed to dietary exposure on a site-specific basis. Calculated body burdens varied between field sites for all metals except Cr and were influenced by the role of salinity on metal uptake from the aqueous phase. The diet accounted for >97% of the body burden of Cd and MeHg, whereas aqueous exposure accounted for a large portion of the Hg(II) body burden (53-97%). Our data stresses the importance of not ignoring the aqueous phase as a source of metals to fish.

334 Dietary Metal Toxicity to the Marine Sea Hare, Aplysia californica

T.A. Jarvis, Valdosta State University / Department of Biology, ; T. Capo, Division of Marine Biology and Fisheries, National Resource for Aplysia, University of Miami Rosenstiel School of Marine and Atmospheric Science; G.K. Bielmyer, Valdosta State University / Department of Biology

Marine ecosystems are heavily influenced by metals, primarily due to inputs from various anthropogenic sources. In excess, metals may accumulate and potentially cause toxic effects in marine organisms. This study investigated the accumulation and effects of dietary metals in a macro invertebrate. The green seaweed, *Ulva lactuca* was exposed to two concentrations (10 and 100 mg/L) of five metals (Cu, Ni, Pb, Cd, and Zn) individually and concurrently in a mixture for 48 h. *U. lactuca* was then fed to the sea hare, *Aplysia californica* for two to three weeks depending on the exposure concentration. Body mass of *A. californica* was measured weekly, and at the end of the exposure duration organs were dissected and measured for metals. Metal distribution in *A. californica* organs varied with different metal species. *A. californica* fed the metal-exposed diets had significantly reduced body weight by the end of the exposure periods, as compared to controls; however, differences were observed, dependent on exposure concentration, duration, and exposure regime (metal mixture versus individual metal). These results highlight the significance of dietary metal exposure in lower trophic levels and support the use of *A. californica* as a sensitive bioindicator of metal pollution in marine systems.

335 The Abiotic Photo-degradation of Methylmercury in seawater

M. Kim, Seoul National University / Dept. of Health and Environment; A. Won, Seoul National University; K. Zoh, Seoul National University / Department of Environmental Health, School of Public Health

Photo-degradation is among the most important process in mercury cycling that maintains methylmercury (MeHg) at low concentration in water systems. Photochemical degradation is a known sink of MeHg in freshwater lakes, but less is known about its importance in marine waters. Relatively little research has been conducted on the fate and stability of MeHg in oceanic waters. In this study, we investigated the effects of the wavelength and light intensity on the photochemical degradation of MeHg in seawater. In addition, we assessed the production of dissolved gaseous mercury (DGM, Hg^0) in order that the fate of inorganic mercury species generated from MeHg photo-degradation is of great important of understand the exactly mechanism of MeHg photo-degradation in seawater. The results showed that UVB induced the higher MeHg photo-degradation than UVA. For light intensity, the first order reaction coefficient increased from 0.21 to 0.94 h^{-1} with UVB and from 0.16 to 0.52 h^{-1} with UVA, respectively. Rates of MeHg photo-decomposition decreased with increasing salinity while the emission of Hg^0 was increased with decreasing salinity. This result may be due to changes in MeHg complexation, reflecting a shift from thiol based dissolved organic matters complexes that facilitate MeHg photo-degradation at low salinity to chloride complexes that are less susceptible to photolytic decomposition at higher salinity. Therefore, the wavelength and light intensity have a significant effect on MeHg photo-degradation and Hg^0 emission. The chloride complexes result in different photo-degradation mechanisms and pathways.

336 Determination of chemical contaminants from military bombing ranges near the ocean in Hawaii

D. Klein, Texas Tech

The US Military has had practice bombing ranges in Hawaii for decades. Some like Makua, Oahu, Hawaii and Pohakuloa, Hawaii are still active while other ranges such as Kaho'olawe have now been closed. Results from the analysis of soil samples and comparison to pristine areas will be presented.

Contaminant Accumulation in Plants: Mechanisms, Models, and Potential Risk

337 Pressure chamber technique for evaluating the root to shoot transfer of organic compounds

W.J. Doucette, Utah State University / Utah Water Research Laboratory; B. Bugbee, Utah State University / Dept. of Plants, Soils and Biometeorolgy

Quantifying the root uptake of organic contaminants and their transfer into above ground plant tissue is critical for assessing the potential human, wildlife, and ecological health risks associated with the consumption of plants growing in contaminated environments. However, the lack of strong regulatory drivers and absence of standardized methods has resulted in relatively limited, highly variably set of plant uptake data appearing in the literature. The pressure chamber technique provides a relatively rapid and reproducible approach for generating transpiration concentration factors (TSCF) that describe the relative tendency of a chemical to move from root to shoot. This technique was used measure TSCF values for over 30 organic chemicals (ranging from -0.8 to 5 in log Kow) and show that neutral, water-soluble organics were most likely to transfer from roots to shoots and that the xylem composition of zucchini plays an important role in its unique uptake characteristics. Data comparing pressure chamber and whole plant hydrophonic methods will also be presented to illustrate the advantages and disadvantages of each approach.

338 Uptake and Translocation of Xenobiotics by Plants: Novel Measurements and Predictions

M. Limmer, Missouri University of Science and Technology / Environmental Engineering; G. Morrison, Missouri University of Science and Technology; J.G. Burken, Missouri University of Science and Technology / Civil, Architectural and Environmental Engineering

A wide variety of compounds can enter the plant root and translocate throughout the plant, presenting a potential exposure pathway via bioaccumulation and biomagnification. Historically, the ability of a compound to be translocated by plants has been measured using the transpiration stream concentration factor (TSCF). In this work, we have established a novel understanding of chemical physicochemical properties that predict efficacy of crossing the root membrane, using measures of hydrophobicity, molecular mass and hydrogen bonding. We find that most compounds able to enter the root have a log K less than 5, a molecular mass less than 300 Da, 3 or fewer hydrogen bond donors, and 7 or fewer hydrogen bond acceptors. A predictive, advection-diffusion model was developed from these findings and considers fluxes developed from plant physiology and molecular properties such as hydrophobicity, molecular mass and polar surface area. Chemical transport is considered to occur radially across the fine root, limited by cell membrane, partitioning to root tissue and the tortuous path to the xylem vessels. Collectively, the models provide valuable guidance on predicting uptake of compounds by plants and also highlight limitations of and variability in the TSCF, particularly under varying environmental conditions such as water flux. To improve measurements of the TSCF, a standard method is proposed and demonstrated using 1,4-dioxane as a test compound.

339 Effects of Transpiration on Accumulation of PPCP/EDCs in Plant Tissue

L. Dodgen, University of California Riverside / Environmental Science; J. Gan, University of California Riverside / Environmental Sciences / Department of Environmental Science

The United States reuses 9.5×10^{11} gallons (3.6×10^{12} liters) of treated wastewater each year for agricultural, landscape, industrial, and other applications. However, treated wastewater often contains unregulated organic contaminants, such as pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs). When agricultural fields are irrigated with reclaimed water, PPCP/EDCs have shown the potential to accumulate in plant tissues, which may present a human health risk through ingestion. Published literature has suggested that plant uptake of PPCP/EDCs is controlled by mass flow driven by transpiration; however, no studies have quantitatively examined this relationship. The effect of transpiration on accumulation of PPCP/EDCs was examined using four plant species, strawberries, lettuce, carrots, and tomatoes, which are economically important in California, an area that uses reclaimed water for agricultural irrigation. Plants were grown hydroponically for 21 d in nutrient solution spiked with 1 μg/L of each of a suite of PPCP/EDCs. During the experiment, one treatment group was located in a growth chamber with a warm, dry environment (50% relative humidity (RH), 30/20 °C, 500 μmol/m^{2}s photosynthetic photon flux density (PPFD)) and a second group was located in a growth chamber with a cool, humid environment (80% RH, 15/10 °C, 250 μmol/m^{2}s PPFD). Transpiration rate for each plant was calculated using gravimetric measurements of solution loss. After 21 d, plants were separated into roots, stems, original leaves, and new leaves. Tissue was extracted using solvent extraction with sonication, cleaned-up with solid phase extraction, and analyzed using ultra performance liquid chromatography tandem mass spectrometry. Statistical analysis was used to determine relationships between transpiration rates and accumulation of individual PPCP/EDCs.

340 Accumulation of Emerging Contaminants in Edible Crops Irrigated with Reclaimed Water

K.C. Hyland, Colorado School of Mines / Civil and Environmental Engineering; A.C. Blaine, Colorado School of Mines / Civil & Environmental Engineering; C. Rich; C.P. Higgins, Colorado School of Mines / Civil & Environmental Engineering

Anthropogenic organic contaminants present in wastewater which may persist through treatment processes pose a complex and relevant issue when treated wastewater, or reclaimed water, becomes a viable resource for agricultural irrigation. Contaminants of emerging concern (CECs) reach wastewater streams following human use and include pharmaceuticals, flame retardants, corrosion inhibitors, surfactants, plasticizers, and many others. Controlled greenhouse experiments designed to examine the potential bioaccumulation and dose- dependent uptake of trace organic contaminants into selected edible crops from reclaimed water were performed. Strawberry and lettuce plants were grown with tap water, reclaimed water, and reclaimed water fortified at eight levels with increasing contaminant doses. Analysis of various plant tissues for the suite of target chemicals allowed for the calculation of bioaccumulation factor (BAF) values for each chemical in each edible plant tissue. Additionally, root concentration factor (RCF) and translocation factor (TF) values were calculated to improve understanding and possible predictive capability of how CECs will be transported and accumulated in various plant tissues. Results from this study have important implications with respect to the potential exposure of humans to contaminants in fresh produce. The ultimate goal of this ongoing project is to improve mechanistic understanding of plant uptake of CECs, thereby allowing for advancement of models intended to predict human exposure.

341 Uptake of pharmaceuticals by Red Pepper, Chinese Cabbage and Cucumber plants -interactions and in-plant distribution

M. Shenker, B. Chefetz, R. Yakir, E. Colon de Mello, The Hebrew University of Jerusalem

Worldwide use of reclaimed wastewater (RW) as a water source for agricultural irrigation is expected to increase in semi-arid regions. Pharmaceuticals compounds (PCs), are not all removed in the water treating process; some are found in the RW used for crop irrigation at the μg/L concentration range. In a lysimeter setup located in the field and irrigated with secondary or tertiary RW spiked with a cocktail of 11 PCs (sulfapyridine, sulfamethoxazole, carbamazepine, metoprolol, clofibric acid, gemfibrozil, bezafibrate, naproxen, diclofenac, ibuprofen, and ketoprofen) at a concentration of 0.4-1.4 μg/L, only carbamazepine was found in all the tested organs of the raised Red pepper (*Capsicum Annuum*) and Chinese cabbage (*Brassica Pekinensis*). Its concentration in the edible plant portions was 2-5 μg/kg DW in the pepper fruits and 15-50 μg/kg DW in the outer leaves of the Chinese cabbage. The effect of mutual interactions between three PCs – carbamazepine, sulfapyridine, and carboplatin on the uptake and in-plant distribution were tested in cucumber (*Cucumis sativus*) plants grown in a hydroponic system. Analyses were carried separately in the roots, stems, and leaves, as well as in the nutrient solution and in the sap flow. The sap was differentiated into symplastic and apoplastic-enriched streams. The average concentrations of carbamazepine sulfapyridine, and platin (the analyte for carboplatin) in the sap solutions were 0.8, 0.3, and < 0.06 of the corresponding concentrations in the nutrient solutions, usually with no clear difference between the symplastic and apoplastic-enriched streams. For the most labile of the tested PCs, carbamazepine, an effective transport with the transpiration stream was manifested by its accumulation in the leaves, in which its concentration was 2 times larger than in the roots and 3-4 times larger than in the stems, with no significant effect of the presence of any of the other PCs. Different

from this pattern, sulfapyridine and carboplatin were not mobile and were largely retained in the roots. The overall recovery of the carbamazepine was 8.7±2.3%, suggesting in-plant metabolism. Indeed two metabolites of carbamazepine, 10,11-dihydroxy and 10,11-epoxy-carbamazepine were found in the cucumber roots, stems, and leaves. This study emphasizes that PCs analyses in plant material may largely underestimate the real PCs uptake if metabolites are overlooked.

342 Plant uptake and processing of trace organic chemicals in reclaimed water: In-planta degradation of benzotriazole

G.H. LeFevre, Civil & Environmental Engineering; C.E. Mueller, Stanford University / Civil & Environmental Engineering; A.E. Timotfe, ETH Zürich / Civil & Environmental Engineering; E.S. Sattely, Stanford University / Chemical Engineering; R.G. Luthy, Stanford University / Civil & Environmental Engineering

As a result of continued population growth in water-stressed regions, climate-induced changes in precipitation patterns and increasing ecological concerns, water utilities in many regions are turning attention to alternative sources of water, including reclaimed wastewater and captured stormwater. For example, highly treated wastewater is used for irrigation of food crops and stormwater is captured for infiltration into drinking water aquifers. Nevertheless, trace organic contaminants are present in these water sources and currently there is little knowledge of their environmental fate in vegetated systems, such as agricultural fields, bioretention areas, and constructed treatment wetlands. The objective of this work is to establish a greater mechanistic understanding of the fundamental uptake and fate of chemicals of emerging concern relevant to reclaimed water in vegetative systems. Contaminants of interest for this study include benzotriazoles (anticorrosive agents found in reclaimed wastewater and stormwater), nitrosamines (disinfection byproducts), and perfluorinated compounds (ubiquitous persistent environmental pollutants). These compounds are all polar trace organic pollutants chosen to represent a range of relative stability in the environment. The hydroponic uptake and transport of these selected contaminants at environmentally relevant concentrations was studied within the model plant *Arabidopsis thaliana*. We found that all compounds are taken up into the plant to varied degrees. In addition, we have strong evidence that benzotriazole is taken up and rapidly metabolized *in-planta*, with over 90% mass loss of parent compound within the planted systems in less than three days. This discovery is surprising because benzotriazole is not successfully removed via conventional wastewater treatment systems and is known to be extremely recalcitrant to microbial enzymes (aerobic microbial half-life: 114 d). Currently, we are conducting metabolomics work to determine benzotriazole degradation products in the vegetation tissue and determine enzymatic pathways; these results will be presented in full.

343 Bioconcentration of energetic compounds – munitions constituents – in grasses (barley)

T.L. Torralba-Sanchez, University of Delaware / Department of Civil & Environmental Engineering; D.T. Kuo, University of Delaware / Department of Civil and Environmental Engineering; D.M. Di Toro, University of Delaware / Dept. of Civil & Environmental Engineering; H.E. Allen, University of Delaware / Dept. Civil & Environmental Engineering

Elevated levels of explosives and related materials are often found in soil at military installations that involve munitions manufacturing, disposal, testing, and training. As a result, organisms growing in the soils at military ranges are exposed and potentially able to accumulate such organic pollutants. Therefore, assessment of the environmental fate of energetic compounds (munitions) and chemicals with similar structures (munitions-like) must include their bioaccumulation in plants. 'Eve' winter hulless barley (*Hordeum vulgare* L.) grass was used to study the uptake of munitions and munitions-like compounds [i.a. Nitroguanidine (NQ); 4-Nitroanisole (4-NAN); 2,4-Dinitroanisole (2,4-DNAN)]. The conventional experimental approach towards measuring bioconcentration in plants often involves conducting uptake experiments with natural or synthetic soils. However, soils contain multiple components/phases, and thus using it as exposure medium inadvertently increases experimental variation. To circumvent the problem, a new experimental protocol using fine quartz as an alternative supporting medium has been developed. The new protocol produced stable biomass profiles, constant plant-exposure concentrations and reproducible steady state bioconcentration factors – BCF – (L water/Kg plant dry wt.) within a one-month period. Initial results suggest that BCFs are not directly correlated with octanol-water partition coefficient for the studied compounds. NQ (log K_{OW} = – 0.89 L/Kg) reports a BCF at least three times bigger than that of 2,4-DNAN (log K_{OW} = 1.71 L/Kg), while 4-NAN (log K_{OW} = 2.03 L/Kg) BCF is thrice smaller than such value for 2,4-DNAN. This study has implications to asses and be used as an experimental reference for modeling the bioconcentration potential of hazardous chemicals in the environment.

344 Foliar uptake of CeO2 Nanoparticles by Cucumber Plants (Cucumis Sativus) and the physiological impacts

J. Hong, Environmental Science and Engineering Program; S. Sahi, Western Kentucky University / Department of Biology; C. Rico, L. Zhao, J. Peralta-Videa, J. Gardea-Torresdey, University of Texas at El Paso / Chemistry Department

Recent literatures have shown an increase of engineered nanoparticles (ENPs) in the environment whose consequences for ecosystem components are not well understood yet. Studies have shown that ENPs cause toxicity in some plant species when interacting through roots. However, very few studies have been focused on the exposure of ENPs with plant leaves. In this study, hydroponically grown cucumber (*Cucumis sativus*) plants were treated with CeO_2 NPs as powders (0.7 and 2.10 g/m^3) or suspensions (20, 40, 80, 160, 320 mg/L). Fifteen days after treatment application, plant samples were analyzed by using ICP-OES and TEM-EDS, and the activity of some stress enzymes were also measured. The ICP-OES showed cerium in leaves, stems, and roots, of CeO_2 ENP treated plants. The TEM-EDS results corroborated the translocation of the CeO_2 NPs to the cucumber roots. In addition, the biochemical assays showed that the NPs increased the activity of catalase and decreased ascorbate peroxidase activity in cucumber leaves and roots. These findings have shown, for the first time that the CeO_2 ENPs taken up through the leaves can be distributed within cucumber plant tissues, which could represent a threat for the food chain and human health.

Advances in Environmental Metabolomics

345 Defining the physiological niche and polluted exposed phenotype of a sentinel earthworm through metabolic profiling

D. Spurgeon, Centre for Ecology & Hydrology; M. Liebeke, Imperial College London; E. Lahive, Centre for Ecology & Hydrology (NERC); C. Svendsen, CEH, Wallingford / Pollution and Ecotoxicology, Centre for Ecology & Hydrology / Microbial Ecology and Ecotoxicology, NERC Centre for Ecology & Hydrology / Ecotoxicology; N. Norhave, Centre for Ecology and Hydrology; P. Kille, Cardiff University; J.G. Bundy, Imperial College London

For soil dwelling organisms, prevailing environmental conditions can vary in space and time as a result of diurnal and seasonal climatic variation as well as geological, biological and anthropogenic influences on soil physical and chemical properties including pollutant levels. To maintain viable populations in the face of such variation, sedentary species (such as earthworms) must adapt their physiology (and behaviour); a process that will ultimately result in changes in the concentrations of endogenous metabolites. Here using a ^{1}H-NMR and a direct infusion mass spectrometry metabolomic approach, we have first sought to characterise earthworm (*Lumbricus rubellus*) physiological phenotypes associated with acclimation to four commonly encountered environmental conditions gradients, namely food availability, temperature, soil moisture and pH, in separate laboratory studies. Our analyses identify key components of the earthworm metabolome and also established that only relatively small scale changes in metabolite concentrations occur even when earthworm are exposed to conditions outside their optimum physiological niche. An understanding that stressful environmental variations may only have relatively minor metabolic consequences, suggested that when deployed for pollution monitoring in the field, the additional effects of non-chemical stressors on the metabolome would not preclude the identification of a chemical stress effect. To test this hypothesis, we next used a similar metabolomic approach to compare phenotypic responses of field collected earthworms living at sites characterised by the presence of complexly polluted soils with those of individuals from adjacent uncontaminated locations. These analyses highlighted the full complexity of exposures under real natural conditions, since additional factors that complicate metabolomic interpretations were identified. These included genotypic effects associated with the presence of crypsis in the test species, as well as the multifaceted variation between control and polluted sites. The consequence of this was that while paired polluted and control site populations could be clearly identified, no common chemical stress associated phenotype was evident when that separated the pollutant and control sites. This suggests that while a metabolic approach may be useful for site specific

investigations, identification of a conserved pollutant stressor signature may be challenging to establish in wild populations.

346 Multi-organ analysis of starvation response in Sciaenops ocellatus using NMR-based metabolomics

M. Watanabe; T. Schock, NOAA / Analytical Chemistry Division; D.W. Bearden, National Institute of Standards and Technology / Analytical Chemistry Division; P.A. Sandifer, National Ocean Service, NOAA; M.R. Denson, South Carolina Department of Natural Resources; J. Leffler, South Carolina Dept of Natural Resources; J. Yost, South Carolina Department of Natural Resources; J.M. Monserrat, Universidade Federal do Rio Grande – FURG

In natural habitats, fish experience periods with lower food availability, and depending on the duration of these periods, significant biochemical changes occur in response to these near-starvation events. Similarly, in recirculating aquaculture systems, if diets are not optimal, metabolic responses mimicking starvation are likely to occur. Therefore, the biochemical effects of starvation have implications for wild animals as well as commercial aquaculture species. Here, the metabolic response of captive red drum, *Sciaenops ocellatus,* subjected to three weeks of feed deprivation was investigated using NMR-based metabolomics to model metabolic changes in response to starvation. Nine tissues: plasma, liver, heart, muscle, spleen, intestine, stomach, kidney and brain were collected from individual animals. The polar extract from each tissue was profiled and compared between control (n=6) and starved fish (n=6). Results from principal component analysis (PCA) indicated the presence of metabolic differences between starved and fed animals in all tissues, although the magnitudes of alterations in metabolites were organ dependent. Supervised extension of PCA and Welch's T-test were employed to identify the two principal components that best distinguish the two groups for each tissue. The impact of this 3-week starvation on the metabolome was most significant in liver, muscle, and intestine, with plasma, spleen and stomach showing the least impact. During starvation, alterations to several metabolites involved in protein metabolism such as alanine, glutamine, and glutamate were identified. In addition, organ-dependent responses to starvation were observed: e.g., glucose in liver, taurine in heart, valine and betaine in muscle, glycocholic acid in intestine, and carnosine and 4-aminobutyrate in brain. The results presented here map metabolic profiles between organs in control and starved red drum while highlighting the benefits of multi-organ analysis for experimental design in starvation models of marine species.

347 Toxicity evaluations of sediment-exposed to Japanese medaka (Oryzias latipes) larvae using metabolomics approach

S. Uno, Education and Research Center for Marine Resources and Environment, Faculty of Fisheries; K. Nakayama, Ehime University / Center for Marine Env. Studies; E. KOKUSHI, J. Koyama, Kagoshima University / Faculty of Fisheries

Ultimately sea sediments are final sink for chemicals, because discharged chemicals are transferred to coastal area by rainfall, river water, and atmospheric flow adsorbing to particle in sea water and finally settling. Coastal sediments especially near to big city have been continuously polluted by many kinds of chemicals. It is strongly required that appropriate method of toxicity evaluation for aquatic organisms in sediments is established, however, these methods are not well established. Recently, we have continuously evaluated negative impacts in fish with metabolomics approach especially aiming on establishing evaluation method with combined stressors. In this study, the toxicity of sea sediment extracts to Japanese medaka (*Oryzias latipes*) were examined using metabolomics method with GC/MS. Sediments were collected from possible polluted area in Subic bay, near Manila, Philippines, and Osaka bay, near Osaka and Kobe, Japan. The sediment-extracts obtained were spiked into test water with the following equivalent extract concentration in 1 g sediments: 100%, 20%, 4%, and 0.8%. Mortality of 75% in 2.5-week-old medaka of 100% test water group from Subic bay were observed within 72hr, while no mortality was observed from Osaka bay. Following, medaka larvae was reared in 20% and 4% test water from Subic or Osaka bay for 24hr. Then, all metabolome profiles detectable in GC/MS analysis were collected, because all metabolites variations were considered to reflect the magnitude of toxicological effects in medaka. Metabolome profiles with significantly different levels between exposed and control groups were screened by one-way analysis of variance (ANOVA) and principal component analysis (PCA) was then applied the screened data. As a result, 20% test water group from Subic was clustered the farthest position from control, suggesting that this group are mostly affected, although all exposure groups except for 4% water of Osaka were separated from control along with principal components 1 (PC1) on the PCA score plot. In addition, the magnitudes of effect to medaka larvae in 4% of Subic and 25% of Osaka were similar. Effects in 4% of Osaka could be tiny. These results were at least 25-fold or more sensitivity compared with toxicity test based on the mortality. This study showed that developed metabolomics approach with ANOVA and PCA is potentially a useful tool for toxicity evaluation of real sediments and possibly other sample.

348 Field-deployed Metabolomics for Assessing Waters Impacted by Point and Non-Point Sources of Contamination

T.W. Collette, USEPA / National Exposure Research Laboratory; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; J.E. Cavallin, USEPA, USEPA, ORISE / National Health and Environmental Effects Research Laboratory; E.J. Durhan, USEPA / Mid-Continent Ecology Division; K.M. Jensen, M. Kahl, USEPA / National Health and Environmental Effects Research Laboratory; E. Makynen, D. Skelton, Q. Teng, USEPA; D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; D.R. Ekman, USEPA / National Exposure Research Laboratory

Metabolomics is becoming well-established for studying chemical contaminant-induced alterations to normal biological function. For example, the literature contains a wealth of laboratory-based studies involving analysis of samples from organisms exposed to individual chemical toxicants. These lab studies have demonstrated the ability to rapidly screen and prioritize individual chemicals for adverse effects, and also to inform with regard to toxic modes-of-action. However, metabolomics has rarely been used for characterizing the impacts of exposure to complex "real-world" chemical mixtures, or for biomonitoring in the natural environment. This is unfortunate, because metabolomics is well suited for these applications as well. For example, metabolomics is applicable to virtually any species because a sequenced genome is not required. Also, it can be applied with relatively low per-sample cost, is "open-ended" (requiring no pre-selection of targets), and highly reproducible with modern NMR and MS instruments. Recognizing that these are considerable advantages for *in situ* effects-based monitoring, we have been conducting numerous biomonitoring studies with metabolomics in, and around, the Great Lakes. Most of this work involves caged fathead minnows *(Pimephales promelas),* which are strategically deployed at various sites in relation to point and non-point sources of contamination (e.g., WWTPs, agricultural operations, etc.) For much of this work, the metabolomic data is linked with other 'omic measurements, classical whole-animal outcomes, and site characterization and chemical monitoring data. These studies – which will be discussed here – clearly indicate the unique potential for effects-based monitoring with metabolomics.

349 The Importance of Genotypic- and Phenotypic-Anchoring on Interpretation of Metabolomics Data in Wildlife

J.P. Benskin, Axys Analytical Services Ltd.; M.G. Ikonomou, Institute of Ocean Sciences; N. Veldhoen, University of Victoria; C. Dubetz, Institute of Ocean Sciences; C. Helbing, University of Victoria; J.R. Cosgrove, AXYS Analytical Services Ltd

Normal variation in the metabolome of wildlife presents a considerable challenge for interpretation of metabolomics data. This variability can arise due to differences in genotype, phenotype (representing genotype-environment interactions), or circadian fluctuations. In the present work, we applied phenotypic- and genotypic-anchoring to classify Sockeye salmon from the Skeena River system prior to interpretation of metabolomics data. Adult salmon were collected at the mouth of the Skeena River(n=76) and at the spawning grounds of two tributaries:Fulton River(n=43) and Pinkut Creek (n=50). Genotypic anchoring was based on DNA fingerprinting-based stock assessment and genetic sex, while phenotypic anchoring relied on gametic sex (through visual inspection for the presence of milt or roe) and gender-specific hepatic mRNA abundance profiles. Quantification of 186 metabolites in salmon liver, including acylcarnitines (n=40), amino acids (n=21), glycerophospholipids (n=90), ∑hexose, sphingolipids (n=15), and biogenic amines (n=19) was accomplished using a kit-based approach with analysis by flow injection- or liquid chromatography-tandem mass spectrometry (FI-MS/MS or LC-MS/MS, respectively). Reference human plasma (with known concentrations of selected metabolites), spiked salmon liver, and blanks were included to assess overall and inter-assay method performance. Statistical and pathway analyses were carried out using MetaboAnalyst 2.0. Pre-classification of fish based on genotype and phenotype permitted meaningful interpretation of metabolomics data. Among the

metabolites significantly associated with gender were ∑hexose, aspargine, phospholipids and sphingomyelines. In the Fulton stock, carnitine was the major metabolite associated with migration in both sexes. A number of sphingomyelins, phospholipids and amino acids were associated with aberrant hepatic transcriptome profiles. Overall these data demonstrate the utility of omics in assessing the health of wild Sockeye Salmon.

350 Urine-based Metabolomics with Fish: Use of Repeat Sampling (of an individual) to Non-lethally Assess Temporal Effects of Contaminants

D. Skelton, USEPA; T.W. Collette, USEPA / National Exposure Research Laboratory; C.A. LaLone, USEPA; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; D.R. Ekman, USEPA / National Exposure Research Laboratory

Environmental metabolomics is a rapidly developing field for assessing the global metabolite profiles of tissues and/or biofluids from ecologically relevant organisms to identify biomarkers of exposure to various stressors, elucidate a chemical's modes-of-action, and decipher the pathogenic pathways of diseases. Urine is often an ideal biomedium for metabolomics because, compared to other tissues and biofluids, it can be easily and non-invasively collected, requires less complex processing, and allows for repeat sampling of an individual over time to monitor temporal responses. Indeed, urine metabolomics has been utilized in numerous human and rodent studies to distinguish between healthy and disease status, identify novel biomarkers of disease, and map toxicity pathways. Despite the wealth of information that can be obtained from urine metabolomics, it has been largely under-utilized in environmental studies using fish as a model organism. Urine collection in fish poses the potential challenges of low urine volume and low abundances of metabolites. Furthermore, it is unknown how effects from handling the fish (to repeatedly collect urine) might manifest in the urinary metabolome. To address these issues, we investigated the efficacy of using repeat sampling of urine as a biofluid for environmental metabolomics studies. Fathead minnows (*Pimephales promelas*) were exposed to the model anti-androgen vinclozolin for 14 days with serial urine sampling of each individual every 48 hours. Repeat urine sampling from control fish was also conducted in this fashion. Urine samples were analyzed via gas chromatography-mass spectrometry. We found that the urine metabolite profile of control fish did not vary significantly over the course of the study, indicating that the repeated handling of an individual fish for collecting urine did not significantly impact the urinary metabolome. Additionally, we were able to detect temporal effects in the vinclozolin-exposed fish and identify the corresponding key metabolites that varied over time. The results of our study showed the efficacy of repeat sampling of urine as a biofluid for environmental metabolomics using fish, and provide validation for future studies of this type.

351 Development of an in vitro metabolomic approach for enhanced neurotoxic effects by contaminants, with emphasis on neurotransmitter pathways

P. Leonards, VU University, Institute for Environmental Studies / Chemistry & Biology; S. Tufi, J. Kamstra, R. van Dijk, R. van Kesteren, T. Hamers, VU University, Institute for Environmental Studies

Worldwide, serious concern has arisen about the increased incidence of learning and developmental disorders in children. From a scientific point of view, there is no doubt that exposure to neurotoxic chemicals during early brain development can adversely affect learning and development. Various recent epidemiological studies have indicated that exposure to low doses of environmental biologically active contaminants during human development can have deleterious effects on cognitive development in childhood. The European commission-funded project DENAMIC "Developmental Neurotoxicity Assessment of Mixtures in Children" investigates neurotoxic effects (e.g., learning and developmental disorders) of low-concentration mixtures of pesticides and a number of common environmental pollutants in children. One of the aims is to develop better and sophisticated tools, procedures and testing methods to screen compounds for (developmental) neurotoxicity and to improve assessment of exposures and effects (www.denamic-project.eu). As part of the project, a new alternative assessment strategy based on a combination of in vitro and in vivo assays is under development in order to prioritize compounds for further in vivo testing. To this end, hazard characterization of pesticides and environmental pollutants on a molecular and cellular level is carried out, with emphasis on adverse effects during neuronal development. An array of in vitro assays is used to investigate (developmental) neurotoxic effects, including neuron differentiation in the SH-SY5Y human neuroblastoma cell line, acetylcholinesterase (AChE) inhibition, and transthyretin (TTR) binding. An important aspect is the development of biomarkers for (developmental) neurotoxicity in animal models using (epi-)genomics, proteomics and metabolomics. This paper present the development of a metabolomic and neurotoxicity approach for the SH-SY5Y cell line. The focus is on four neurotransmitter pathways: Dopamine, Serotonin, Gaba, and Acetylcholine. Analytical methods were developed to detect and quantify the precursors, neurotransmitters and metabolites in the SH-SY5Y cells using a 12 well based system and LC-MS/MS. An LC-HRTOF system was used for untargeted analysis. SH-SY5Y cells were exposed to various pesticides, their metabolites and MeHg to investigate the effects on the neurotransmitter pathways.

What Worked and Why?

352 Kesterson Reservoir: 30 Years of Selenium Risk Assessment and Management

H.M. Ohlendorf, G.M. Santolo, E.R. Byron, M.A. Eisert, CH2M Hill

In 1983-1985, selenium (Se) was identified as the cause of severe reproductive impairment in aquatic-dependent birds feeding and nesting at Kesterson Reservoir, located within the Kesterson National Wildlife Refuge in the San Joaquin Valley of California. The Reservoir was used for disposal of subsurface saline drainage waters from agricultural fields and was intended to provide beneficial habitat for wildlife, particularly waterfowl and other aquatic birds. Because of bird mortalities, the Reservoir was closed to further discharge of agricultural drainage, and alternatives for closure and remediation were evaluated. In 1988, the Reservoir was drained and filled with soil to at least 15 cm above the expected seasonal (winter) elevation of high-Se groundwater (which had become contaminated from surface water). These actions effectively transformed the Reservoir into terrestrial habitats. To mitigate for the loss of wetlands at the Reservoir, additional fresh water was provided to the surrounding Grassland Water District as alternative habitat and for nuisance abatement. A very comprehensive annual monitoring program (including Se in soil [and water-extractable Se from soil], organic detritus, mushrooms, diverse plant types, invertebrates, birds [eggs and blood], and mammals from terrestrial habitats as well as surface water, invertebrates, and algae from rainwater pools [when present]) was established to assess the impact of Kesterson Reservoir on local and migratory wildlife, provide a basis for adjusting Kesterson Reservoir management, verify the effectiveness of cleanup actions at Kesterson Reservoir, and provide a basis for modifying future biological monitoring. Ecological risk assessments (ERAs) in 1993 and 2000 evaluated the results of the monitoring program and provided a basis for reducing the scope and frequency of the biological monitoring program, thus greatly reducing costs while ensuring management actions were effectively reducing risk. For example, frequency of monitoring was progressively reduced from annual to every 3 years (based on the 1993 ERA) and subsequently to every 5 years (based on the 2000 ERA). Selenium concentrations in environmental media have been stable at relatively low levels over the recent monitoring periods, and the monitoring that is occurring in 2013 is expected to be the final effort unless unanticipated risk is documented.

353 Impacted Watershed Recovery in the Copper Basin of Tennessee

M.E. Bowers, Secaps Environmental Inc.; B. Faulkner, Bratton Farm

The Lower North Potato Creek (LNPC) watershed in the Copper Basin of southeast Tennessee was the center of extensive copper mining and allied chemical production activities beginning in the mid-19th century. Open roasting of ore resulted in emissions of metals and sulfur dioxide, which adversely impacted vegetation in and around the Copper Basin leaving the LNPC watershed denuded and severely impaired. The original stream channels in the watershed were disturbed by mining and processing operations with stream paths deliberately modified to control water movement to facilitate those activities. In addition, the absence of vegetation left the watershed susceptible to severe erosion and high peak flows during storms. Sediments and waste materials were deposited in and along streams further modifying hydrologic conditions and leading to severe degradation of habitat and water quality. All of these factors led to extirpation of all but the most tolerant macroinvertebrate species from much of the watershed. Starting in 2001, Glenn Springs Holdings, Inc. (GSHI) in cooperation with federal and state

agencies embarked on a multi-faceted voluntary remedial effort employing adaptive management in the LNPC watershed. Activities have included waste disposal and relocation, water collection and treatment, clean water diversion, stream reconstruction and enhancement, and riparian and upland revegetation. The sum effect of these actions has been dramatic improvement in-stream habitat and water quality in LNPC and its tributaries. Comprehensive annual watershed monitoring, initiated in 2001, has chronicled the stream biological response to these improvements. The macroinvertebrate community will serve as the ultimate measure of remedial success under a novel framework developed by GSHI in cooperation with state and federal agencies.

354 The Blackbird Mine cleanup: successful outcomes in spite of it all

C. Mebane, Member Commentary without Affiliation

The Blackbird Mine, near Cobalt, Idaho, USA, operated from the early 1900s to the late 1960s. Acid mine drainage from underground workings, leaching of metals and sedimentation from waste rock piles, and uncontained mill tailings all combined to extirpate almost all aquatic life from about 60 km of downstream waters. Concerted remediation efforts began in 1995, and metals concentrations dropped by about 90% by 2002, with incremental reductions since. The affected streams were quickly recolonized by invertebrates and fish as conditions became habitable, and as of 2012 fish abundances and diversity were similar to that of reference conditions. Reestablished benthic invertebrate communities have become abundant and diverse. While any views on "what went right and why" at a Superfund site are clearly matters of perspective, from my viewpoint, several factors that influenced the successful outcomes to date included: (1) the meso-site physical scale; (2) early resolution of environmental and natural resource damage liabilities; (3) the responsible parties undertook major "early actions" in advance of a formal record of decision (4) agreement on ambitious but achievable remedial goals, (5) regulatory harmonization with the Clean Water Act's "use attainability analysis" for waters that seemed beyond hope of full restoration; (6) stability and deep institutional knowledge by key players, (7) adaptive strategies informed by robust chemical and biological monitoring; and (8) sincere personal commitments by all of the major protagonists to achieve and sustain restoration. (This presentation is based on observations shaped over 20 years through work in four positions, and thus represents a purely personal viewpoint. It is not intended to, and cannot, represent views or findings from any institution).

355 Ecological Risk Management in Coastal Dune mining in South Africa: Role of collaboration and persistence

T. Ott, M. Boshoff, Richards Bay Minerals/Rio Tinto; S.N. Luoma, University of California, Davis / John Muir Institute of the Environment; E.J. Dorward-King, Newmont Mining / HSE

Richards Bay Minerals has been mining and smelting minerals sands from the coastal dunes on the east coast of South Africa for 36 years and is one of world's leading producers of titanium dioxide and zirconium. Thirty years ago the company committed to a program of ecosystem restoration as mining moved through the dunes, wherein two-thirds of the 24 km mined area was is planted into plantation trees as a source of income and jobs for surrounding communities and one-third is restored to native coastal dune forest. Coastal forest was restored on minded sand by "kick-starting" natural succession process. Dunes were re-shaped after mining, top soil was added from pre-mining areas, and forest succession was allowed to proceed, facilitated by natural seeds already in the soil and migration corridors from local patches of forest. Nearly twenty years ago monitoring and scientific study of the coastal dune forest restoration was initiated via a partnership with an independent academic unit, the Conservation Ecology Research Unit at the University of Pretoria. Via numerous diagnostics, published in peer reviewed papers, the CERU program showed how and why the new growth forest follows patterns predicted by ecological theory. As patches of restored forest age, increases in the ecological complelxity of the forest and the species diversity/richness of millipeds, rodents and birds are documented. Thirty year old patches of restored forest are quite similar to undisturbed forest, although the baseline provided by the "natural forest is constantly changing due to regional influences. Social and ecological challenges to sustaining the success are also becoming evident. The RBM case study will show the benefits of a long-term corporate commitment to risk management in collaboration with long-term, independent scientific study; and the equal importance to sustainable restoration of understanding ecological processes and sociological challenges.

356 Focused Study to Reduce Remediation Footprint and Facilitate Restoration of a Forested Floodplain Wetland

R. Davis, Anchor QEA LLC; S. Blauvelt, Parsons

Ninemile Creek is a major tributary to Onondaga Lake in Syracuse, New York. The creek was historically impacted by upstream industrial activities that discharged mercury and other contaminants into the local water body that were then transported through Ninemile Creek and into the lake. SYW-10 is a large forested wetland situated near the mouth of Ninemile Creek and along the Onondaga Lake shore. Based on field investigations, the soil/sediment within SYW-10 contains mercury concentrations at levels that warrant remedial action. However, because SYW-10 is one of the few remaining forested wetlands contiguous to the lake, Honeywell and the regulatory agencies agreed to conduct a focused pre-design study to determine if portions of the forested wetland could be preserved. The agreement was included in the Record of Decision. The study involved additional detailed soil/sediment sampling and biota monitoring (including call surveys and pit traps) at SYW-10 and a paired reference forested wetland on a nearby lake, including tissue analysis for total and methymercury. The results of the focused study were used to identify the specific portions of SYW-10 requiring remediation and an area that will remain intact. The remediation will result in the removal of approximately 65% of the mercury mass in the SYW-10 forested wetland, and approximately 86% of the mercury mass in the collective area that includes the forested wetland itself, the adjacent forested uplands, and adjacent emergent wetlands. It is expected that remediation of the indicated area, in conjunction with remediation of those immediately adjacent areas, will result in lower mercury concentrations in the soil / sediment on a site-wide average basis, reduced mercury concentrations in biota from remediated areas, and reduced exposure to higher level receptors. The preserved portion of the forested wetland will continue to provide certain habitat functions while the remainder of the site is remediated and restored; trees from the remediated portion are being salvaged for reuse in the restoration. The restoration plan includes substantial planting and seeding of a wide variety of native wetland species.

357 Green City, Clean Waters – Restoring Urban Streams in Philadelphia with Green Infrastructure

C. Crockett, Philadelphia Water Department

The City of Philadelphia is embarking on a one of a kind national model program to incorporate green stormwater infrastructure into the fabric of urban life to manage stormwater to prevent combined sewer overflows, meet stormwater permit needs, reduce flooding, and provide resilience for climate change. This comprehensive program looks to move the "needle" forward on making investments to achieve measurable and noticeable environmental improvements to the City's streams, rivers, and neighborhoods. This presentation will focus on describing the overall approach, strategy, science, and triple bottom line benefits expected from the program as well as examples of how the program has been working to daylight streams, restore streams, improve fish passage, and reduce stormwater runoff. The green infrastructure examples will include projects built in urban streetscapes and on private properties including techniques using tree trenches, porous pavement, and green roofs. Attendees will learn about the policies, approaches, and standards required at a City and county level to make significant and lasting environmental restoration occur. Attendees will also learn what has been working and lessons learned restoring urban waterways.

358 Reframing the Problem – Urban Waterway Redevelopment Projects as a Model for Improved Sediment Management

T. Dekker, LimnoTech

Waterways in urban settings throughout the US have a set of problems that stand in the way of real and lasting change. A history of sanitary and CSO discharges, industrial contamination, and legacy pollutants that reside in sediments have created risks to human health and the environment, and have engendered a perception of the urban waterway as a problem to be managed. More recently, the disciplines of urban planning and landscape architecture have pointed to the possibility of a better urban waterway, that becomes a focus of the urban environment and a center of development and recreational use. This talk describes the outcomes of several urban design

competitions conducted across North America over the last 5 years in which waterways were the focus of a revitalized urban setting. These projects address legacy contamination issues by assigning their importance as secondary to the value created by the broader development goals of the projects. A successful waterfront development project creates a value trajectory that is very different from the typical sediment remedy, in which remediation to a condition of neutral value is the goal.

359 How the Cost-Sharing and Innovative Approach under the Great Lakes Legacy Act Can Accelerate Sediment Remediation Projects -Sheboygan WI as an example

H. Williams, USEPA Great Lakes Program Office Region 5; M. Tuchman, USEPA / Great Lakes National Program Office; M. Loomis, USEPA GLNPO; A.P. Mucha, USEPA / Great Lakes National Program Office

Delisting and restoring Great Lakes Areas of Concern (AOCs) is a high priority for USEPA and a significant component of the Great Lakes Restoration Initiative. In late 2010, the Sheboygan River AOC was targeted to complete all management actions (including sediment remediation and needed habitat projects) by September 2012. While more than one mechanism was utilized to plan and implement these actions, a critical component was the sediment remediation of 148,000 yds^3 at the Sheboygan AOC under the Great Lakes Legacy Act (GLLA) program. The remediation efforts at Sheboygan highlights how a voluntary, cost-sharing program created an expedited path to implementation that achieved substantial environmental results. At Sheboygan, the sediments were contaminated with PCBs and PAHs , and several habitat projects identified as well as an active Superfund project adjacent to the area targeted for the GLLA remediation. Substantial hurdles that needed to be overcome included: coordinating with the Superfund program and PRP to effectively and holistically achieve all needed sediment remediation (bureaucratic and technical), working closely with stakeholders at multiple levels to develop the necessary non-Federal cost share (flexibility) and communicate successfully with the community as to the large amount of work that would take place in a short amount of time. The key factors in overcoming these hurdles will be presented as well as the environmental results of both the habitat and sediment remediation work will be shared.

Advances in the Estimation and Assessment of Terrestrial Bioaccumulation

360 Terrestrial Bioaccumulation Models for Bioaccumulation Screening and Exposure Assessment: Workshop Summary

F.A. Gobas, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment); L.P. Burkhard, USEPA; W.J. Doucette, Utah State University / Utah Water Research Laboratory; K.G. Sappington, Office of Pesticide Programs, Environmental Fate & Effects Div.; E. Verbruggen, RIVM Expertise Centre for Substance / Centre for Safety of Substances and Products; B.K. Hope, CH2M Hill; M.A. Bonnell, Environment Canada / Ecological Assessment Division; J.A. Arnot, ARC Arnot Research & Consulting / University of Toronto Scarborough / Department of Physical and Environmental Sciences; J.V. Tarazona, ECHA

In most countries, the bioaccumulation behaviour of chemicals is assessed by using the chemical's Bioconcentration Factor (BCF), Bioaccumulation Factor (BAF) or Octanol-water partition coefficient (Kow). These methods are especially applicable to water breathing organisms. There is now substantial and mounting evidence that methods for bioaccumulation assessment in water breathing organisms are not always appropriate for assessing bioaccumulation in terrestrial air-breathing organisms. Terrestrial bioaccumulation modeling is one of several approaches that can be used to improve bioaccumulation assessment of chemicals in terrestrial food-webs. The application of modeling to improve chemical bioaccumulation assessment was recently studied by an interdisciplinary group of regulators, academics and industry scientists in an ILSI Health and Environmental Sciences Institute (HESI) sponsored workshop on terrestrial bioaccumulation held in January 2013 in Miami, FL. The main objectives of the study was (i) to review the current state of the science on modeling the terrestrial bioaccumulation of chemical substances and (ii) to make recommendations on the use of bioaccumulation models for the purpose of screening chemicals for their bioaccumulation behaviour in terrestrial food-chains and for exposure assessment in terrestrial organisms. The ultimate goal of the workshop is to contribute to the development of a framework for the assessment of bioaccumulation in terrestrial food-chains. The workshop, outcomes summarized and discussed in this presentation, include definition(s) and metrics of bioaccumulation that encompass terrestrial food-webs; a discussion of models and QSARs that can be used to assess the bioaccumulation behaviour of chemicals in terrestrial ecosystems; and issues related to spatial and temporal scale in bioaccumulation assessment. The presentation will conclude with a discussion of research gaps and priorities; integration of modeling with results from laboratory and field studies; and a path forward to the development of a general framework for the assessment of chemicals for bioaccumulation in both aquatic and terrestrial food-webs. This abstract does not necessarily reflect USEPA policy.

361 Use of terrestrial field data to address chemical bioaccumulation potential: Advice and research needs

J.M. Conder, ENVIRON International Corp; N.W. Van den Brink, Alterra / Dept. of Ecology & Environment; D.C. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division; J. Arblaster, ENVIRON International Corp; S.R. Bowman, The Ohio State University / Evolution, Ecology, and Organismal Biology; W. Drost, Umweltbundesamt; J.E. Elliott, Environment Canada; M.S. Johnson, US Army Center for Health Promotion and Preventive Medicine, US Army Public Health Command / Health Effects Research Program; T. Natal da Luz, University of Coimbra / Department of Life Sciences; B.A. Rattner, USGS-Patuxent Wildlife Research Center; B.E. Sample, Ecological Risk, Inc; R.F. Shore, CEH Lancaster

Explicit consideration of chemical bioaccumulation potential in terrestrial food webs has only recently been a focus of chemical and product registration programs , and was the subject of a January 2013 Health and Environmental Sciences Institute (HESI)-sponsored workshop attended by scientists from government, academic, and industry organizations. Workshop attendees agreed that bioaccumulation potential information derived from the measurement of chemicals in field (wild free-ranging) organisms provides critical evidence for understanding bioaccumulation potential of chemicals (retrospective assessments), and is useful in developing and augmenting prospective modeling and laboratory approaches. However, most available field datasets for addressing bioaccumulation in terrestrial ecosystems are collected to address other needs (long-term biomonitoring, site-specific and pesticide risk assessments, poisoning incidents, etc.) such that the interpretation of the data to quantify bioaccumulation potential is very challenging. It is clear that an explicit evaluation of terrestrial systems is required to provide a holistic assessment of chemical bioaccumulation potential – aquatic field data cannot be used as a surrogate or substitute for terrestrial systems. Additionally, many of the metrics used to evaluate bioaccumulation in aquatic systems (e.g., bioconcentration factors (BCFs), Trophic Magnification Factors (TMFs)) may not be applicable to terrestrial systems due to limitations in applicability or uncertainty currently associated with lack of proof-of-concept evaluations. Presently, Biota-soil Accumulation Factors (BSAFs) and Biomagnification Factors (BMFs) are the most robust metrics for evaluating bioaccumulation potential in terrestrial food webs. There is a strong desire among the scientific community to promote non-lethal sampling techniques for vertebrate species in terrestrial field studies. Example field-derived BMFs reviewed in this study indicate that samples obtained via non-lethal methods are appropriate for use in assessing bioaccumulation potential in terrestrial species. Additional discussion is included on various aspects of terrestrial field study design and data interpretation, including the selection of target terrestrial species and food webs, consideration of spatial and temporal issues, recommended statistical analysis considerations, and the potential for mesocosm studies to bridge the gap between field and laboratory efforts.

362 Terrestrial bioavailability and fate of lead in metal-rich granules produced by the earthworm Eisenia fetida

R.P. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology; S.R. Bowman, The Ohio State University / Evolution, Ecology, and Organismal Biology; M. Vasko-Bennett, B. Little, Ohio State University; S.M. Brasfield, US Army Engineer Research and Development Center; J.G. Coleman, US Army Engineer Reseach and Development Center, US Research and Development Center / Environmental Laboratory; J. Seiter, US Army Corps of Engineers / Research Physical Scientist; R.P. Jones, US Army Engineer Research & Development Center; M. Ansley, US Army Engineer Research and Development Center

Lead (Pb) is the most common metal contaminant found in the soils of small-arms ranges of US military installations. Risk assessors often assume that Pb is 100% bioavailable for ecological receptors, even though many

studies have shown the modification of Pb bioavailability and toxicity by soil physicochemical characteristics, aging, and metabolism by organisms. Many terrestrial invertebrates are capable of rendering Pb toxicologically inactive through the formation of metal-rich granules (MRGs). While Pb in MRGs is not considered bioavailable via trophic transfer, little is known regarding the fate of MRGs in the terrestrial environment once released from exposed organisms. The current research characterizes the microstructure of MRGs formed by the earthworm, *Eisenia fetida* and determines the potential for the microbial degradation and remobilization of Pb previously sequestered in MRGs. If liberated by bacterial degradation, sequestered Pb may become bioavailable to invertebrates and their predators. The direct bioavailability of Pb in MRGs to oligochaetes in soil was also examined. Earthworms were exposed to soils spiked with 4,000 mg/kg Pb for a six-week period and fractionated utilizing differential centrifugation and digestion to separate whole-animal tissue from MRGs to obtain sufficient MRGs for amending soils for microbe and oligochaete exposures. Once extracted, MRG composition and concentration was analyzed utilizing synchrotron based analysis at Argonne National Laboratories (Argonne, IL). X-ray diffraction analysis of MRGs isolated from the worm show crystalline Pb-carbonate minerals with Plumbonacrite and Shannonite (Pb_2OCO_3) as potential Pb minerals. Synchrotron imaging provided multi-channel analyzer (MCA) plots which revealed localized MRG hotspots within the samples, not only confirming the presence of Pb, but also the binding of additional metals such as zinc and iron. MCA plots of cross sections revealed a wide dispersion of Pb inside the gut wall, suggesting the presence of unbound Pb in addition to MRG formation. Respiration by soil microbes exposed to Pb in MRGs (39 mg Pb/kg, total) was elevated compared to treatments receiving Pb as $PbNO_3$. The results of the exposure of oligochaetes to Pb in MRGs will also be discussed. Results confirm that Pb present in MRG is not bioavailalbe to terrestrial organisms.

363 Current use pesticide and polybrominated diphenyl ether bioaccumulation in a Canadian Arctic vegetation-caribou-wolf food chain

A.D. Morris, University of Guelph / Environment Canada / Environmental Biology / University of Guelph / School of Environmental Sciences; D.C. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division; K.R. Solomon, University of Guelph / School of Environmental Sciences; C. Teixeira, M. Duric, Environment Canada

Organic contaminant studies in Arctic terrestrial food chains have previously focused on highly persistent, bioaccumulative contaminants such as legacy organochlorines (OCs) and perfluorinated compounds (PFCs). The bioaccumulation potential (BAP) of less hydrophobic organic contaminants with high octanol-air partition coefficients (K_{OA}) may increase in food chains containing air-breathers, however there are few field studies that test this hypothesis with current/recent use contaminants. Therefore, we chose to investigate the bioaccumulation of current use pesticides (CUPs) and PBDEs (tri-deca BDEs) in the Bathurst region vegetation-caribou-wolf food chain in the Canadian Arctic. Carbon and nitrogen stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) in vegetation, and enrichment factors ($\Delta^{15}N$) between dietary sources and caribou, were highly variable ($\Delta^{15}N$ = 1.3 – 10.7). Ecological interactions between vegetation, efficient digestion and long turnover times in ruminants likely contributed to this variation. CUPs volumetric bioconcentration factors (BCF_v) for vegetation were generally greatest in lichens ≥ mosses > willow > grasses > mushrooms. BCF_vs (mean vegetation) were greatest for endosulfans (8.7 – 10) > dacthal (9.6) > pentachloronitrobenzene (9.2) > chlorothalonil (8.2) > chlorpyrifos (7.8). PBDE BCF_vs were not different between vegetation types, and were greatest for BDE-47 (11.1) > BDE-99 (10.8) > BDE-154 (10.7) ≥ BDE-209 (10.7). Total body burden (TBB) caribou:vegetation BMFs were generally < 1 for both groups of contaminants, however dietary comparisons affected some BMFs substantially. For example, chlorpyrifos BMFs ranged from 0.39 – 5.1 when compared with mushrooms or lichens respectively and BDE-66 ranged from 0.60 – 8.1 when compared with winter diet (70% lichens, 30% moss) or lichens alone. The greatest $wolf_{TBB}$:$caribou_{TBB}$ CUP BMF was β-endosulfan (6.3); all other CUPs BMFs were < 1. In contrast, $wolf_{TBB}$:$caribou_{TBB}$ BMFs were > 1 for BDE-28 (9.1), 47 (1.3), 154 (2.8), 153 (2.9) and 209 (3.6). Despite individual BMFs that indicate some predator-prey biomagnification, lichen-caribou-wolf trophic magnification factors (TMFs) were all < 1 (for significant relationships), indicating trophic dilution across the food chain. Since PBDE and CUPs BCF_vs were high, it is likely that metabolic biotransformation in mammals negates any increased contaminant retention due to high K_{OA}s.

364 Laboratory Assessment of Terrestrial Bioaccumulation: current status and future possibilities

D.B. Huggett, University of North Texas / Department of Biological Sciences; R. Hoke, E.I. DuPont de Nemours and Company; S.M. Brasfield, US Army Engineer Research and Development Center; B. Brown, AstraZeneca, Brixham Environmental Laboratory; M.R. Embry, ILSI Health & Environmental Sciences Institute (HESI); A. Fairbrother, Exponent, Inc. / EcoSciences; M. Kivi, Pest Management Regulatory Agency, Health Canada; M. Leon Paumen, ExxonMobil Biomedical Sciences / T&ES Division; R.S. Prosser, School of Environmental Sciences; D.T. Salvito, Research Institute for Fragrance Materials, Inc. / Department of Environmental Science; R.P. Scroggins, Environment Canada / Ecotoxicology and Wildlife Health Division

In January of 2013, scientists from regulatory, academic, and industry organizations participated in an ILSI Health and Environmental Sciences Institute (HESI) sponsored workshop on the terrestrial bioaccumulation potential of organic chemicals. Participants agreed that current approaches for the assessment of terrestrial bioaccumulation are inadequate. The focus of the Laboratory Workgroup was to: 1) evaluate existing knowledge and data gaps in the laboratory assessment of bioaccumulation in terrestrial organisms, including plants, invertebrates and vertebrates. The working group discussion was broken down in the following sections: Identification of existing terrestrial bioaccumulation study designs, protocols and methods; Identification of study designs, methods, and protocols that do not assess terrestrial bioaccumulation *per se*, but have the potential to be adapted and/or used as a starting point for developing new protocols, methods or guidelines; Identify areas were where new laboratory approaches and/or test methods to address terrestrial bioaccumulation could be developed; Evaluate the potential utility of leveraging traditionally "non-ecotox" data (e.g., mammalian laboratory data used in human health risk assessments) into terrestrial bioaccumulation assessments, and Identify short-term, mid-term, and long-term opportunities to improve laboratory methodologies to assess bioaccumulation in terrestrial organisms. The highlights of our discussions are presented along with potential adjustments in the laboratory approaches that could be used for assessing terrestrial bioaccumulation potential.

365 Use of biomagnification factors derived from field datasets to address chemical bioaccumulation potential in terrestrial food webs

J. Arblaster, ENVIRON International Corp; J.M. Conder, ENVIRON International Corp; D.C. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division; N.W. Van den Brink, Alterra / Dept. of Ecology & Environment; B.E. Sample, Ecological Risk, Inc; B.A. Rattner, USGS-Patuxent Wildlife Research Ctr / USGS; M.S. Johnson, US Army Ctr for Health Promotion & Preventive Med., Public Health Command / Health Effects Research Program, US Army Institute of Public Health

Current approaches for the assessment of bioaccumulation potential for chemicals focus on aquatic environments and are inadequate to assess the potential of a chemical to bioaccumulate in terrestrial systems. As with aquatic field data, Biomagnification Factors (BMFs) are a robust metric for interpreting bioaccumulation potential in terrestrial systems. To illustrate key challenges and approaches with the use of terrestrial BMF data, published field study BMF values for compounds typically considered to exhibit bioaccumulation (i.e., PCBs, p,p-DDE, PFOS, and PBDEs) and an easily metabolized PAH (pyrene) were compiled. A limited data analysis has led to four main conclusions. First, aquatic BMFs are not a substitute for terrestrial BMFs; bioaccumulation potential must be addressed explicitly for terrestrial food webs. Second, non-lethal sampling of terrestrial organisms is a viable and preferable option for field studies (e.g., BMFs for lipophilic chemicals calculated from lipid-normalized liver and muscle concentrations show good agreement (r = 0.88), confirming general theory that lipid normalization can resolve the differences in concentrations between tissues to provide information about bioaccumulation potential. Third, statistical analysis is crucial to account for variation in BMF estimates from field data. Of the 32 BMF values compiled in this effort, only 38% of BMFs > 1 were significantly (α = 0.05) > 1, indicating that a single BMF value should not be taken at face value without accounting for measurement variation. Lastly, BMFs vary greatly between species and trophic guilds. BMFs for predatory birds were generally more statistically robust than BMFs for mammalian carnivores, herbivores and insectivores (e.g., 95% of avian BMFs were significantly > 1 for the bioaccumulative chemicals, compared to only 23% of BMFs for other species). Targeting predatory bird and their prey in field studies for bioaccumulation assessment may result in the most definitive assessment with limited sampling resources. In conclusion, BMF values developed from

terrestrial ecosystems are a robust metric for informing on chemical bioaccumulation potential, but data should be interpreted carefully and additional research is needed to optimize field study and analytical approaches.

366 Application of complexation models in predicting metal bioaccumulation in plants

Y.T. Le, Radboud University Nijmegen / Environmental Science, Institute for Water and Wetland Research; J.A. Hendriks, Radboud University Nijmegen / Department of Environmental Sciences; F. Swartjes, National Institute for Public Health and the Environment, the Netherlands; P. Romkens, B. Groenenberg, Wageningen University and Research Centre, The Netherlands

It is of importance to predict metal bioaccumulation in vegetables as crop assumption is the dominant uptake route in humans for a number of metals. In a number of risk assessment frameworks, e.g., CSOIL, metal concentrations in crops are related to total metal concentrations in soil, limiting the extrapolation potential and leading to high uncertainties in human exposure assessment. More advanced modelling approaches are therefore required to improve these frameworks. In our study, different processes were separated in order to predict metal bioaccumulation, i.e., predicting metal concentrations/activities in the dissolved phase from metal concentrations in soil and predicting uptake of metals by roots from the metal concentrations in soil solution. Multi-surface complexation models have been increasingly used to estimate solid-solution partition for metals. A number of parameters of soil properties, e.g., contents of soil organic matter (SOM), dissolved organic carbon (DOC), clay, and oxides, are required in the assessment by the model. However, these parameters are not always available. Uncertainties from the lack of these parameters were assessed in different scenarios using varying generic values for the above properties. For a number of metals, e.g., Cd, Cu, Zn, Cr, and V, estimates of their concentrations in soil solution in different scenarios were within an order of magnitude of the measurements. The performance was worse for some other metals such as Se, Co and Mo. In general, the highest variability in the predicted metal concentration in the dissolved phase was observed with varying generic values describing sorption to organic carbon. Metal sorption to roots is affected by both chemical heterogeneity of roots and electrostatic interactions. However, these two factors are usually not simultaneously taken into account in modelling root uptake. Humic acids and roots are consisted of functional groups like carboxyl and phenolic with similar ratios between these groups. Moreover, the both chemical heterogeneity and electrostatic effects, which are of importance in root uptake, have been seen in humic acids. Therefore, humic acid of the WHAM (Windermere Humic Aqueous Model) was used as a surrogate of root, i.e., WHAM-HA approach. In our study, this method was applied for different species. Results show that metal bioaccumulation by roots could be simulated well by the sorption to humic acid.

367 Bioaccumulation Behavior of Organic Contaminants in Coastal Mangrove Food Webs

B.C. Kelly, National University of Singapore; S. Bayen, National University of Singapore / Singapore-Delft Water Alliance; H. Zhang, Faculty of Engineering, NUS / Department of Civil and Environmental Engineering, National University of Singapore

Tropical mangroves are unique ecosystems, providing essential habitat for numerous species of aquatic and terrestrial organisms. The present study involves a comprehensive investigation of the occurrence, distribution and bioaccumulation behavior of various organic contaminants in Singapore's coastal mangrove ecosystems. The study involves (i) a field survey to generate chemical concentration data in water, sediments and mangrove biota and (ii) development and evaluation of a mechanistic model representing chemical bioaccumulation in aquatic and terrestrial organisms of the mangrove food web. Concentrations were determined in environmental and biota samples using gas chromatography triple-quadrupole tandem mass spectrometry (GC-QqQ-MS/MS). Target analytes included several classes of hydrophobic organic contaminants, including of polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), brominated flame retardants (BFRs), synthetic musks and Triclosan. The studied chemicals span a wide-range of physical-chemical properties. Octanol-water partition coefficients (log K_{OW}) ranged between 3 and 10, while octanol-air partition coefficients (log K_{OA}) range between 6 and 13. Field samples included environmental media (air, water, bottom and suspended sediments) and biota (seagrass, phytoplankton, zooplankton, benthic worms, mollusks, crabs and fish, birds and monitor lizards), collected from two local mangrove sites. Measured concentrations of individual chemicals ranged from 0.1 to 1 ng/L in water, 0.1 to 50 ng/g organic carbon weight in sediments and 0.5 to > 2,000 ng/g lipid in biota. The food web bioaccumulation model was parameterized and used to predict steady-state chemical concentrations and bioaccumulation metrics (BSAFs, BAFs, TMFs), based on inputs of ambient chemical concentration (air, water, sediments) and physical-chemical properties. Model predicted concentrations and bioaccumulation metrics were compared to those generated from the field survey. The results suggest chemical K_{OW} greatly influences the chemical bioaccumulation in aquatic, water-respiring organisms, while both $K_{OW}$and K_{OA} are important for chemical biomagnification in air-breathing organisms such as tree-climbing crabs, birds and monitor lizards inhabiting the mudflats and terrestrial zones of the mangrove. The role of K_{OW}, K_{OA} and other key factors influencing chemical bioaccumulation potential in aquatic and terrestrial mangrove organisms will be discussed.

Ecotoxicology and Risk Assessment of Soils: Part A

368 Trace Elements in Agricultural Soils of the Saanich Peninsula, Vancouver Island, B.C., Canada

L. Ghimire, Royal Roads University / School of Environment and Sustainability; M. Dodd, Royal Roads University / School of Environment & Sustainability

The Saanich Peninsula is located on the southern end of Vancouver Island, north of the city of Victoria. With arable land and one of the mildest climates in Canada various fruits, vegetables, and corn farms in the peninsula offer fresh local produce to Vancouver Islanders. Most of the farms are more than 100 years old and hence the need to assess potential metal accumulation and their availability in soils. Total metal concentrations in 30 soil samples collected from 10 farms in the peninsula indicated that As, Cu, Mo, Sb, Se and Sn concentrations had increased whereas the concentrations of Ba, Be, Cd, Cr, Co, Mn, Ni, Pb, V and Zn had decreased compared to historical data from the B.C. Ministry of Environment database. The average soil pH had also increased by 1.15 pH units. Based on interviews with farmers changes in metal concentrations were attributed to fertilizer and manure application, while weathering and atmospheric deposition also played a minor role. Studies on the correlation between total metal concentrations and soil pH, texture and organic matter indicated some relationship between the parameters. The soil solid/liquid partition coefficients (Kd) indicated metal mobility in the soils was mainly controlled by clay content. Based on the Kd values Be, Hg, Ni, Pb, Sb and Sn were relatively immobile. Arsenic mobility in the soils was also relatively low and therefore environmental risks associated with the noted increase in As concentration was considered minor. Cadmium was highly mobile with Kd values ranging from 0.5 to 6.0 L/kg, however Cd concentrations in the soils were very low (0.2 – 0.6 mg/kg) and as such the risk associated with Cd was also considered minimal. Selenium speciation studies were conducted on samples that contained concentrations in excess of the B.C. Contaminated Sites regulation soil standards for agricultural land use. The percentages of the water extractable Se species (Se^{2-}, Se^{4+} and Se^{6+}) compared to the total Se in the soils were between 1.0 – 3.3% suggesting that the mobility (and subsequent phytoavailability) of Se was relatively low. Elevated concentrations of B (25 – 39 mg/kg) with high mobility (Kd values 2.0 – 5.2 L/kg) were detected. Best management practices are required to reduce boron concentrations arising from the use of borate and borax fertilizers by farmers.

369 Effects and risk assessment of lead in soils: implementation of bioavailability

K. Oorts, ARCHE; E.E. Smolders, Katholieke Universiteit Leuven; R.P. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology; M.J. Chowdhury, International Lead Zinc Research Organization / Assistant Manager – Environment

At present, the risk assessment of lead (Pb) for soil in the European REACH registration file is based on a fixed generic predicted no effect concentration ($PNEC_{soil}$) for all soils. Although bioavailability and toxicity of metals can be strongly affected by the variation in soil physicochemical properties, the available Pb data did not allow establishing a relationship between soil properties and Pb toxicity to terrestrial organisms. This issue was addressed by measuring toxicity of Pb for 7 endpoints (2 plants, 2 invertebrates and 3 microbial endpoints) in a set of soils covering a representative range of soil properties (pH, organic matter content, texture and eCEC). The effect of soil properties on bioavailability and toxicity of Pb was less pronounced

compared to other metals (Cu, Zn, Ni, Co, Mo). Variation in soil properties only significantly explained variation in Pb toxicity for some endpoints (e.g., nitrification), but not for other endpoints (e.g., microbial respiration, *Folsomia candida* reproduction). Lead toxicity in field soils is generally significantly lower than in corresponding soils freshly spiked with soluble metal salts. This discrepancy was quantified by studying toxicity of Pb after 3 different contamination conditions: i) freshly spiked with $PbCl_2$, ii) freshly spiked, leached and pH corrected in order to remove salinity and pH stress, and iii) freshly spiked and aged for 5 years. Leaching and long-term aging of soils attenuated Pb toxicity significantly, confirming higher toxicity in freshly spiked soils compared to soils equilibrated under field conditions. Soils spiked with a lead salt to perform toxicity testing are therefore poor indicators for toxicity of Pb in soils gradually contaminated in the field and a correction factor of 4.0 was derived to correct for this discrepancy. A generic $PNEC_{soil}$ of 228 mg Pb/kg soil was derived as the 5th percentile of the log-normal distribution of 104 leached and aged chronic NOEC and EC10 values, covering 21 terrestrial species or microbial processes. Bioavailability normalization of toxicity data to a relevant range (10th-90th percentile) in soil properties across European soils yielded $PNEC_{soil}$ values between 179 and 466 mg Pb/kg. Overall, the study provides comprehensive information to implement bioavailability factors in the risk assessment of lead for soil.

370 Integrating bioaccessibility, tissue residues, and invertebrate community analyses into ecological risk assessment of a shooting range

S.R. Bowman, The Ohio State University / Evolution, Ecology, and Organismal Biology; R.P. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology; J.L. Bryant, The Ohio State University / Department of Entomology

Since metal bioavailability varies with soil modifying characteristics and species-specific uptake and elimination, it is sometimes necessary to use site-specific measures such as bioaccessibility estimates and analysis of biotic communities to evaluate contaminated sites. Our study sites include a private shooting range and an off-site reference area (local park) located in central Ohio, USA. Study areas include the main shotfall zone of a trap and skeet range (2899 ±1228 mg/kg soil Pb), an area where Pb pellets were excavated (Fall 2009; 628 ±44 mg/kg soil Pb), and a reference area (1356 ±86 mg/kg soil Pb) that does not directly receive spent shot. Our first objective was to determine how ground beetle (Carabidae) community structure (i.e., diversity and abundance) changes along a Pb gradient. Ground beetles were collected from 2008 to 2013 using pitfall traps in shotfall, reference, and Pb extracted areas. Thirty-five species were collected during the first two years. Overall species richness did not differ between sites; however, an indicator species analysis suggested that six species were indicators of the reference site. With additional years of sampling (and a more robust dataset), we expect that we will be able to understand temporal and spatial trends in ground beetle communities at our study site. Our second objective was to adapt bioaccessibility assays to small mammal physiology so that we can better estimate exposure to Pb from ingestion of soil and food. Early results suggest that soil bioaccessibility is 5-10% of the total Pb in the shooting range soils. For earthworms (*Eisenia fetida*) exposed to field soils for 30 days, bioaccessible Pb ranged from < 1% – 4% of the total earthworm Pb. We will also present tissue residue data for field-caught shrews, voles and invertebrates. Our next step is to analyze gut content and incidental soil ingestion of shrews and voles from the shooting range and reference site. We will use diet composition, incidental soil ingestion and bioaccessibility estimates to adjust exposure estimates at our field site. We anticipate that results from our study will provide evidence for the usefulness of bioaccessibility and invertebrate community analyses in site-specific ecological risk assessment of metals.

371 Land use and mixture effects of zinc and copper toxicity to soil microbial processes

P.M. Garvey, University of Saskatchewan; S.D. Siciliano, University of Saskatchewan / Toxicology Group and Department of Soil Science

Soil ecosystems are largely thought to be resilient to metal toxicity. Typically, exposure to a single metal decreases soil processes for a relatively short period followed by a recover to pre-exposure functioning. This recovery is largely thought to be a result of biological adaptation to the metal exposure and not due to decreases in metal bioavailability. However, despite these single metal studies, many ecosystems impacted by metal mixtures do not recover from the metal impact and have impaired functioning over long terms. Here, we investigated if the reason for this discrepancy between single metal studies and field results was due to differences in land use and/or metal mixture. Three different soils from six different land uses (boreal, mining industrial, urban industrial, native grassland, agriculture and urban residential) were exposed to zinc and/or copper for upto nine months. The nitrification activity was assessed, using the solid state assay used in the EU-REACH protocols, to evaluate metal toxicity and assess recovery.

372 Ion competition and speciation of metal mixtures in soils

D.M. Schwertfeger, McGill University / Dept. of Natural Resource Sciences; M. Boudreau, McGill University / Natural Resource Sciences; W.H. Hendershot, McGill University / Dept. of Natural Resource Sciences

While most predictive soil risk assessment models are based on single metal experiments, field sites are often affected by large concentrations of multiple metals. The ability to predict soil metal speciation and metal competition at the soil surface is paramount for assessing bioavailability and toxicity of metal mixtures in the terrestrial environment. While a number of chemical equilibrium speciation models (e.g., Visual MINTEQ, WHAM) are able to predict metal speciation when solution data are used as input, predictions tend to worsen when the more readily available, solid-phase soil data are used. In addition, there is a lack of evidence validating such models in mixture scenarios. This research project compares the ability of Visual MINTEQ v.3.0 to predict metal free ion activity of Cu, Ni and Zn in soils with measured values obtained by Ion Exchange Technique (IET) as well as ion selective electrode (ISE) for Cu. Three test soils exhibiting contrasting soil properties (pH 5.8 to 7.2) were used in a soil column experiment. Sixty gram samples were spiked with one level of metal A (~ ½ EC50 for barley root elongation) and one of three levels of metal B (0, ½EC50, EC50). A tertiary mixture for which each metal was added at one level (½ EC50) was also included. Spiked samples were leached with a low-ionic strength solution until leachate electrical conductivity values stabilized, to ensure the "salt-effect" had been minimized. Soil metal concentrations were determined by ICP-MS in concentrated nitric acid digests and 0.4 M HNO3 soil extracts. Final leachate extracts were analyzed for pH, DOC, total dissolved metals and selected anions. Free ion activities of Cu2+, Ni2+ and Zn2+ in soil solutions were determined using IET, as well as ISE for Cu2+. We then investigated the ability of Visual MINTEQ v.3.0 to predict metal soil/solution partitioning and free ion activities, using solution parameter inputs, as well as solid-phase parameter inputs.

373 Site transformation model of adsorption-desorption hysteresis: Modeling cycles of adsorption and desorption

D.M. Di Toro, University of Delaware / Dept. of Civil & Environmental Engineering

The non-reversibility of adsorption and desorption, i.e. the fact that desorption isotherms do not conform to the adsorption isotherm is commonly observed. This hysteresis has been variously attributed to kinetic effects, i.e. the hysteresis would disappear if the desorption time were long enough, or to the existence of a metastable equilibrium where the hysteresis persists long enough and is stable enough so that it can be modeled as though it were at equilibrium. The site transformation model of sorption hysteresis is based on the idea that at adsorption equilibrium a fraction of the weak sites with adsorbed chemical are transformed into strong sites that reversibly bind the chemical more strongly than the untransformed weak sites. Both the untransformed and transformed sites are reversible. The model equations make explicit the transformation step and predicts the consecutive desorption isotherms as a function of the initial adsorption concentration. The consecutive desorption isotherm is not chosen to fit the data but rather follows from the adsorption isotherm and the equation that describes the site transformation. This isolates the hypothesis that produces the hysteresis so that experiments can be designed to test various site transformation mechanisms. In particular data from cycles of adsorption/desorption can be predicted within the same framework with no additional parameters.

374 Multilinear Model of Sorption of Munitions Constituents using Clay, Iron and Organic Carbon Sites in a Wide Range of Soil Characteristics

R. Gonzalez, University of Delaware / Department of Civil and Environmental Engineering; A. Miglino, Dept. of Civil & Environmental Engineering; D.M. Di Toro, University of Delaware / Dept. of Civil & Environmental Engineering; H.E. Allen, University of Delaware / Dept. Civil & Environmental Engineering

Organic matter contained in soil is generally the most important soil constituent responsible for the sorption of organic compounds. This has led to the use of the organic carbon normalized partition coefficient, K_{oc} (L/kg) and fraction of organic carbon in the soil, f_{oc} to describe the partitioning. This approach successfully describes the partitioning of many hydrophobic organic chemicals such as PCBs and PAHs, but their application to more hydrophilic compounds such as Munitions Constituents (MC) is less predictive. Literature indicates that clay mineral exchange sites influence the adsorption for MC, and that K_{oc} is not constant. Clay is usually reported based on particle size, not mineralogy, which is rarely. We conducted batch experiments near 1:1 soil to solution ratios reflecting field conditions using a mix of HMX, RDX, nitroglycerine (NG), nitroguanidine (NQ), TNT and 2,4-dinitrotoluene as MC. We used seven soils that varied from 5 to 40% clay and 0.04 to 18% total carbon in an experiment that involved 2 days of adsorption followed by four consecutive desorption steps. We applied a method developed by Anderson and Sposito in 1991 to determine the variable and permanent charge sites in our soils and joined this information to the organic carbon species of the soils to model the sorption of the MC studied. This method is based on the strong preference of permanent-charge sites and low affinity of most variable-charge sites for cesium. We also used the cation exchange capacity (CEC) and particle size data as surrogates for the clay content. Each of the 3 measures of clay content was able to similarly improve the partition coefficients by a factor of 2 in accuracy compared with the carbon normalization traditional approach. The importance of including clay in the partitioning equation increased for soils with lower organic carbon content. Further improvement was achieved by including the iron oxide content of the soils.

375 Changes in toxicity of the As-contaminated former Janghang smelter site soils in Korea after phosphate-aided soil washing treatment

E. Jho, K. Yang, Seoul National University / Department of Civil and Environmental Engineering; J. Im, Seoul National University; K. Nam, Seoul National University / Dept. of Civil and Environmental Engineering

The As contamination is often the major concern for smelting sites as it imposes toxic effects on the surrounding ecosystems. Soil washing is often used to remove As and phosphate can be used as a washing solution as a more environmentally-friendly and cost-effective alternative. According to the classification of As-bound soil fractions described by Wenzel et al., a large portion of As associated with oxides of Fe/Al and phosphate-extractable As can be removed using phosphate, which represent bioaccessible As based on our preceding study. The As-contaminated site soils was collected from the former Janghang smelter site in Korea and the majority of As is associated with hydrous oxides of Fe/Al (59-81%, average of 70±6.4%) and phosphate-extractable (11±5%). Although phosphate can effectively remove As in soils, the residual As after soil washing or the soil washing procedure itself that could deteriorate the soil qualities could still impose toxic effects on the ecosystem when the treated soils are put back. Thus, this study aims at determining the toxic effects of the As-contaminated site soils before and after laboratory-scale phosphate-aided soil washing treatment. This study is a part of the study performed to determine the remediation strategy of the site. The contaminated soil samples were collected from two different locations (A and B) of the site. The soil samples from the location A were sand with 86±52 mg kg^{-1} of As and 6.5±2.4% of organic matter, while the soil samples from the location B were silt loam with 34±13 mg kg^{-1} As and 2.4±0.2% organic matter. For soil washing, 0.5 M ammonium phosphate is to be used at the soil to solution ratio of 1:5 for 4 h, which was determined based on the preceding study results. The Microtox assay using *Vibrio fischeri* was performed to determine the toxic effects of the soils before soil washing and the toxic effects after soil washing are to be determined. In addition to the soil toxicity test, the changes in the toxic effects of the soil solutions will be studied to investigate any changes in the bioavailable As. It is expected that the reduction in the phosphate-extractable As and As associated with oxides of Fe/Al can be correlated with the reduction in ecotoxicities after the soil washing treatment. The findings from this study will contribute towards determining the site-specific As remediation strategy together with the results of the preceding studies.

Fate and Effects of Metals: Aquatic Biological Perspective: Toxicity Mechanisms

376 Developing a site specific understanding of the toxicity of cerium to Hyalella azteca

O. Vukov, Wilfrid Laurier University / Biology; J. McGeer, Wilfrid Laurier University / Department of Biology

The toxicological understanding of Ce and other rare earth elements (REEs) in the aquatic environment is very limited but there is increasing concern as the use of these elements continues to grow. There are no water quality guidelines or criteria for REEs. The overall objective of this research is to contribute towards the understanding of the potential impacts of REEs in aquatic environments. In this particular project the toxicity modifying influences of cationic competition (Ca, Mg and Na) and dissolved organic matter (DOM) on the toxicity of Ce to the aquatic invertebrate *Hyalella azteca* was assessed with the goal of determining if the biotic ligand model approach could be applied to this element. Standard methods (Environment Canada) were used for culture and testing which was done in soft water (pH 7.2, Ca 0.1 mM, Mg 0.03 mM and 21°C). Acute toxicity tests (96 h) were initiated with 2-9 d old offspring and atomic absorption certified stock solutions were used to create exposure solutions which were pH adjusted 24 h prior to test initiation. The protective effect of cationic competition was tested with Ca (0.1 to 2.0 mM), Mg (0.03 to 0.5 mM) and Na (0.1 to 2.0 mM). As well, the ability of DOM to complex Ce and reduce toxicity was tested at dissolved organic carbon (DOC) concentrations of 6 and 12 mg C/L. Ca provide a strong protection against Ce toxicity and EC50 values increased nearly 8 fold over the range of concentrations tested. Na and Mg results were less clear as intermediate concentrations (up to 1 mM) provided protection but higher concentrations did not. Complexation of Ce by DOM resulted in significant reductions in toxicity but these were not DOC concentration dependent. The applicability of geochemical equilibrium modeling approaches to understand site specific toxicity modifying factors is developed in the context of the BLM. This research is funded by NSERC through a Strategic grant in partnership with Environment Canada.

377 Assessing zinc and calcium interactive effects on mitochondrial bioenergetics in rainbow trout, Oncorhynchus mykiss

M. Sharaf, UPEI / Biomedical Sciences, Atlantic Veterinary College; D. Stevens, University of Prince Edward Island / Department of Biomedical Sciences, Atlantic Veterinary College; C. Kamunde, University of Prince Edward Island / Biomedical Sciences, Atlantic Veterinary College

Metals are among the major stressors threatening the integrity of the aquatic environment. For these pollutants, water quality guidelines and risk assessment are based on single stressors laboratory toxicity data which do not take into account the interactions that typically occur between co-occurring stressors in the natural environment. Zinc, an essential trace metal, is a common environmental pollutant whose toxicity in aquatic organisms is mitigated by calcium subsequent to antagonistic interactions of the two elements at the gill surface. To the best of our knowledge nothing is known about post-absorption zinc-calcium interactions at internal target sites including the mitochondria. Because mitochondria are the powerhouses of the cell responsible for generating 95% of an organism's energy, effects and interactions of stressors on these organelles can disturb cellular and organismal energy balance with implications for apical endpoints of toxicity such as survival, growth and reproduction. The present study therefore investigated the effect of zinc and calcium, singly and in combination, on mitochondrial bioenergetics *in vitro* with a view to revealing the mechanisms that may underlie cellular energy imbalance that can potentially be induced by these stressors. Mitochondria were isolated from rainbow trout (498 ± 105 g) livers and exposed to 0-100 µM zinc and calcium singly and in equimolar combinations. The effects on malate-glutamate driven oxidative phosphorylation (OXPHOS) and mitochondrial complex I (mtCI) enzyme activity were then assessed. The results show that although both zinc and calcium impaired OXPHOS, calcium was much less potent than zinc based on a 6-fold higher state 3 mitochondrial respiration and mtCI activity zinc EC_{50} values. Interestingly and contrary to our hypothesis that calcium would be

protective against zinc toxicity, the two elements acted synergistically to impair OXPHOS. Specifically, the state 3 mitochondrial respiration EC_{50} was 2.5 and 16.5 times lower in the combined relative to zinc and calcium alone exposures, respectively. These findings suggest that joint action of metals at internal target sites cannot necessarily be extrapolated from known interactive effects at external epithelial surfaces such as the gill. Future studies will assess if the observed mitochondrial effects occur *in vivo* at environmentally relevant levels of zinc and calcium.

378 Do metals (Cd, Zn) impair calcium influx in aquatic insects?

M.D. Poteat, North Carolina State University / Department of Environmental and Molecular Toxicology; D.B. Buchwalter, North Carolina State University / Department of Environmental and Molecular Toxicology

Osmoregulatory disturbance has been commonly described in aquatic species acutely sensitive to aqueous trace metal exposures. However, aquatic insects (the predominant invertebrate species found in freshwater) are generally not acutely sensitive to dissolved metals at ecologically relevant concentrations, but can be highly responsive to metals in nature. Here we used a radiotracer approach to ask whether exposure to environmentally relevant concentrations of either Zn (1, 10, 100 μg L^{-1}) or Cd (0.1, 1, 10 μg L^{-1}) would decrease the influx rate of 45-Ca in very soft water (500 μg L^{-1}). In *Ephemerella invaria* (family: Ephemerellidae) at exposure concentrations of 1 μg L^{-1} and 10 μg L^{-1} Zn, Ca uptake increased 37% and 42%, respectively. At 0.1 μg L^{-1} Cd, Ca uptake increased 38%. At other exposure concentrations, Ca uptake did not significantly change with the addition of metals. In both *Ephemerella catawba* and *Hydropsyche sparna* (Family: Hydropsychidae), no significant differences were revealed between treatments, however similar trends of increasing Ca-45 uptake with the addition of small concentrations of Zn and Cd still occurred. These counter-intuitive results coupled with our previous observation that Ca offers minimal protection against Zn and Cd uptake in a caddisfly suggest that the fundamental assumptions about dissolved metal toxicity derived from fish and crustaceans do not hold true for aquatic insects. We further compared Ca uptake rates in six species of mayflies (Order: Ephemeroptera, Family: Ephemerellidae) and three species of caddisflies (Order: Trichoptera, Family: Hydropsychidae). Across all nine species, hourly Ca uptake rates spanned one order of magnitude and varied 20-fold, ranging from 24.0 μg Ca-45 g^{-1} wet wt. $hr.^{-1}$ to 492.2 μg Ca-45 g^{-1} wet wt. $hr.^{-1}$. Ephemerellids had significantly faster Ca uptake rates than hydropsychids, with ephemerellids averaging 225 μg Ca-45 g^{-1} wet wt. $hr.^{-1}$ and hydropsychids averaging 77.4 μg Ca-45 g^{-1} wet wt. $hr.^{-1}$ ($p < 0.0001$). In sum, these results reveal drastic differences in the Ca uptake rates of aquatic insects, but show that more closely-related species tend to have similar uptake rates. Current work is comparing Ca uptake rates with Cd and Zn uptake in a comparative context.

379 Acute Cu Exposure Inhibits Ionoregulatory Transport at both the Skin and Gills of Early Life Stage Rainbow Trout (Oncorhynchus mykiss)

A.M. Zimmer, McMaster University / Department of Biology; C.J. Brauner, University of British Columbia / Department of Zoology; C.M. Wood, McMaster University / Department of Biology

Two of the most commonly observed effects of sub-lethal waterborne Cu exposure in fish are the impairment of sodium uptake and ammonia excretion. In larval fish, Cu toxicity generally increases with development as the gills develop. Immediately following hatch (the stage corresponding to the lowest Cu sensitivity) the skin represents the primary site for both sodium uptake and ammonia excretion. In later stages, these processes transfer to the gills as branchial surface area increases, which corresponds with increased sensitivity to Cu exposure. Our goal was to determine the relative sensitivities of cutaneous and branchial sodium uptake and ammonia excretion in response to Cu exposure at different stages of development in rainbow trout employing a divided chamber technique. We assessed the effects of 4 h exposures to 20 or 50 μg/l waterborne Cu at three developmental stages: early, mid, and late, which spanned the time period between hatching and yolk sac absorption. The biotic ligand model (BLM) predicts an acute LC50 of 145 μg/l for rainbow trout in Vancouver, BC tap water which translates to a 30 d LC20 value of 45 μg/l (using the acute-to-chronic ratio provided by the USEPA). We predicted that Cu would impair sodium uptake and ammonia excretion only in the late stage of development and only at the branchial surface. Consistent with this hypothesis, whole-body ammonia excretion was only inhibited (by 34% at 20 μg/l and 46% at 50 μg/l Cu) in the late stage of development through a decrease in anterior ammonia excretion. Impairment of whole-body sodium uptake, however, was observed in both the early and late life stages. In the early life stage, this inhibition (50% at 20 and 50 ug/l Cu) occurred via an inhibition of posterior excretion. In the late stage, only 50 μg/l Cu had a significant effect (43% decrease) on sodium uptake via an inhibition of anterior excretion. These results demonstrate that trout whole body sodium uptake is indeed sensitive to Cu exposure following hatch and that, depending on the time window of exposure, the gill may not be the primary biotic ligand for Cu binding in fish. This may have important implications for the biotic ligand model and for the design and interpretation of metal toxicity tests in early life stage fish. (NSERC Discovery Grants to CMW and CB).

380 Ionoregulatory and respiratory disturbances induced by copper in the osmoconforming clam Mesodesma mactroides

M.B. Jorge, Universidade Federal do Rio Grande; M.M. Lauer, Instituto de Ciências Biológicas; C.D. Martins, Universidade Federal do Rio Grande – FURG / Instituto de Ciências Biológicas; A. Bianchini, Fundação Universidade / Instituto de Ciências Biológicas, Universidade Federal do Rio Grande

Copper (Cu) is shown to induce both respiratory and ionoregulatory disturbances in osmoregulating invertebrates. However, the mechanism of copper toxicity in osmoconforming invertebrates is still unclear. In the present study, juveniles of the marine clam *Mesodesma mactroides* were in vivo exposed (96 h) to the Cu concentration (150 μg Cu L^{-1}) inducing 10% of clam mortality in sea water (30 ppt). Endpoints analyzed in living clams included tissue (hemolymph, gill and digestive gland) Cu accumulation, whole-body and tissue (gills and digestive gland) oxygen consumption, hemolymph ionic (Na^+, K^+, Mg^{2+} and Ca^{2+}) and osmotic concentration, as well as tissue (gill and digestive gland) enzyme (Na^+,K^+-ATPase and carbonic anhydrase) activity. Also, succinate dehydrogenase (respiratory Complex II) activity was evaluated in mitochondria isolated from gills and digestive gland of control clams (non-exposed to Cu) and in vitro exposed (1 h) to Cu (63.5, 635.0 and 6350.0 μg Cu L^{-1}). In vivo exposure to Cu induced tissue (hemolymph, gills and digestive gland) Cu accumulation, reduced whole-body and tissue (gill and digestive gland) oxygen consumption, increased hemolymph Mg^{2+} concentration, decreased hemolymph Ca^{2+} concentration; reduced tissue (gills and digestive gland) Mg^{2+} content, increased digestive gland Ca^{2+} content, increased tissue (gills and digestive gland) Na^+, K^+ -ATPase activity, and reduced gill carbonic anhydrase activity. Also, reduced succinate dehydrogenase activity was observed after in vitro exposure of isolated gill and digestive gland mitochondria to 6350.0 μg CuL^{-1}. As reported for osmoregulating invertebrates, findings from the present study show that Cu is accumulated in gills and digestive gland of *M. mactroides* inducing ionoregulatory (Mg^{2+} and Ca^{2+}) and respiratory disturbances. They also show that these effects are reflected at the whole-body level (reduced whole-body oxygen consumption and imbalance of hemolymph divalent cation regulation). Therefore, findings reported in the present study indicate that further studies on tissue regulation of divalent cations and respiration in Cu-exposed clams can help to identify the mechanism involved in the acute Cu toxicity in seawater osmoconforming invertebrates.

381 Combined effects of temperature and metal contamination in yellow perch (Perca flavescens): a physiological and genomic study

J. Grasset, INRS, ; E. Ollivier, B. Bougas, INRS – Centre Eau Terre Environnement; E. Normandeau, IBIS, Universite Laval; P.C. Campbell, Syngenta / INRS-ETE; L. Bernatchez, IBIS, Universite Laval; P. Couture, INRS / Centre Eau Terre Environnement

Metal contamination is known to impact biological communities in lake ecosystems and can be studied through the use of biomarkers, such as enzymatic activity and gene expression regulation. However, some biomarkers respond to both environmental conditions, such as temperature changes, and metal contamination, making it difficult to characterize the specific effects of metal pollutants in the wild. The objective of this study is to understand the effects of both metal contamination and temperature changes on fish in order to identify biomarkers that respond only to metal contamination and are not impacted by temperature. Using yellow perch (*Perca flavescens*) exposed to three different temperatures (9 °C, 20 °C, 28 °C), we have investigated the effects of nickel and cadmium contamination at the transcriptomic, enzymatic and biometric levels. The metabolic capacities and gene transcription levels linked to different biological pathways, such as oxidative stress, glucose and lipid metabolism, anaerobic capacities,

and apoptosis, were measured in both liver and muscle samples. Condition factors (Fulton and pyloric cæca) were negatively correlated with temperature. In addition, our results show an increased accumulation of both metals in the kidney at higher temperatures. This correlation of metal accumulation with temperature is more pronounced for nickel than cadmium, although a significant correlation between cadmium accumulation and temperature was also found. Antioxidant enzyme activity (superoxide dismutase) was also observed with an increase in temperature. The enzymes involved in metabolism, such as nucleoside diphosphate kinase and glucose-6-phosphate dehydrogenase, are also impacted by the combined action of metal contamination and temperature. The activity of these enzymes was greatly reduced in presence of cadmium at high temperatures. Globally, this study should help us to distinguish the effects of metal contamination from those of temperature variation and thus to better understand the specific gene expression and physiological signatures of the studied metals. The link between transcriptomic and physiological responses to heat stress combined to metal contamination, as well as the implications for the development of metal-specific biomarkers, will be discussed in the presentation.

382 Differential fitness response to chronic metal exposure in Daphnia pulex mutation accumulation lines

G. Harrison, McGill University; M.P. Celis, DESC-MOE; N. Yan, York University; M. Cristescu, McGill University

In the Sudbury region of Ontario, Canada, over 7,000 lakes within a 17,000 km^2 area have experienced pollution as a result of the mining and smelting industry. This includes contamination of the heavy metals copper and nickel. Because Cu and Ni are known to inhibit DNA repair systems, and produce reactive oxygen molecules, chronic exposure to these metals could cause an increase in mutation rates. To examine the fitness effects of chronic metal exposure on *Daphnia pulex*, four treatment groups of mutation accumulation lines were established which included soft water and metal solutions containing concentration of either 20 ug/L Cu, 40 ug/L Ni, a 20:40 ug/L solution of Cu and Ni, and an unexposed control group. To compare each treatment group's ability to cope with an environmental stressor a bio-assay utilizing toxic levels of Cu was run when lines were at a mean generation of 50. Percent mortality was quantified over the concentration range 160-175 ug/L, with a 2.5 ug/L increase between concentrations. The concentration range resulting in 100% survival and 100% mortality was between 165-170 ug/L for the Cu and Ni:Cu group, 162.5-175 ug/L for Ni, and between 170-180 ug/L for the control. Nickel had the lowest LC50 at 168.0 ug/L, followed by the Ni:Cu solution at 170.0 ug/L, then Cu at 170.5 ug/L. The LC50 for the control group was between 175-180 ug/L. A pariwise t-test revealed that all three metal exposed groups were significantly more sensitive to toxic Cu levels than the control, but there was no significant difference between any of the metal treatment groups. This work indicates that chronic exposure of Cu and Ni contributes to a decline in fitness in this species when natural and sexual selection pressures are removed.

383 Effects of maternal exposure to metals for offspring of the western-mosquitofish

A.M. Morales, Biology; P.L. Klerks, University of Louisiana at Lafayette / Department of Biology

The effects of maternal exposure to an essential (copper) and a non-essential metal (cadmium) were studied in the offspring of the live-bearing fish *Gambusia affinis.* The goal of this study was to determine if maternal metal exposure has an effect on the offspring's size at birth, survival rate, calcium levels, lipid peroxidation levels, and the frequency of abnormalities. Gravid females were exposed to 0 (control) or 0.15 µM of copper or cadmium for ten days, and then transferred to clean water. Females were then allowed to give birth. Each female's offspring were subdivided in three different groups – to allow for multiple analyses. Group one was kept alive and allowed to reach sexual maturity while being monitored for survival rate, sex ratio, and incidence of abnormalities. Groups two and three were euthanized shortly after birth. Group two was processed and analyzed for metal levels, including calcium, using flame or furnace furnace atomic spectrophotometry. Group three was analyzed for membrane damage by measuring the amount of lipid peroxidation. The metal analysis results showed that offspring born after the maternal exposure to cadmium or copper had elevated levels of these metals in their tissues. Results with respect to the effects of this maternal metal transfer are still being analyzed and will be presented at the conference.

Canadian Oil Sands Part I: Assessing Impacts on the Environment and the Advancement of Oil Sands Reclamation Strategies

384 Different Analytical Mass Spectrometric and Bioassay to Probe How Ozonation Process Degrades Naphthenic Acids in Oil Sands Process-Affected Water

N. Sun, L.A. Perez-Estrada, University of Alberta / Department of Civil and Environmental Engineering; P. Drzewicz, University of Alberta / Civil and Environmental Engineering; P. Chelme-Ayala, N. Klamerth, M. Islam, Y. Chen, S. Dong, University of Alberta / Department of Civil and Environmental Engineering; B.J. Blunt, M. Reichert, University of Alberta / Department of Biological Sciences; M.O. Hagen, K.B. Tierney, M. Belosevic, University of Alberta / Biological Sciences; M. Gamal El-Din, University of Alberta / Department of Cival and Environmental Engineering

Oil sands process-affected water (OSPW) refers to water that has been in contact with oil sands or released from tailings deposits. The toxicity of OSPW has been largely attributed to naphthenic acids (NAs), a complex mixture of naturally occurring aliphatic and (poly-) alicyclic carboxylic acids. Long-term management of stored OSPW is of environmental concern since a persistent fraction of NAs remains in OSPW for decades. Ozonation of OSPW has been shown to be an efficient treatment method to degrade NAs, oxy-NAs as well as S/N-related NAs. In this work, OSPW was treated using ozonation at intermediate and high-concentration levels, and various analytical and bio-analytical methods were used to probe the degradation process of NAs upon treatment. UPLC-IM-MS method was used as a quick and qualitative way to monitor the reduction in toxic NAs and related species in OSPW samples. Semi-quantitative analysis of NAs was done by ultra-high resolution FTICR-MS method and, furthermore, the concentration of NAs and oxy-NAs were estimated by UPLC-TOF-MS assay using the SYNAPT G2 high resolution mass spectrometer (HRMS). A good correlation between FTICR- and UPLC-TOF-MS was established. To address the biological impact of acute and sub-chronic OSPW and ozonated OSPW exposures, the ability of goldfish to resist *Vibrio fischeri* exposures, to maintain olfactory function , and to respond immunologically following exposure were assessed. It was found that upon ozonation of OSPW, both the amount of NAs and related species, and the toxicity of the sample solution, decreased as the ozonation concentration increased. At an ozone dose of 50 mg/L, the amount of NAs and oxy-NAs were reduced to sub-ppm level. Ozonation reduced the toxicity of *Vibrio fischeri*. In terms of fish olfaction, the data suggest that OSPW interferes with the perception of odorants by likely acting as odorant(s), and that within a brief timeframe it does not exert toxicity. It was also found that macrophages from fish exposed for one week to ozonated OSPW exhibited higher reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI). However, after prolonged (12 week) exposure of fish to ozonated OSPW, the antimicrobial responses of macrophages were significantly reduced when compared to those obtained from non-exposed (control) fish. This study shows that ozonation is a promising treatment approach to accelerate the decontamination and detoxification of OSPW.

385 Increasing Wetland Diversity and Riparian and Aquatic Plant Diversity in Oil Sands Reclamation

C.A. Daly, Suncor Energy Inc. / Closure Planning & Reclamation

Oil sands mining causes disturbance to the boreal forests of northeastern Alberta, Canada. Industrial companies are required to reclaim degraded land back to ecosystems with equivalent land capability. Prior to mining, approximately 50% of the Athabasca Oil Sands area was composed of wetlands, mainly peatlands and conifer swamps. Suncor Energy Inc. (Suncor) is therefore focused on finding innovative ways to recreate wetland ecosystems through research. Recent examples of innovation in oil sands wetland reclamation are illustrated by the following projects: (1) The Suncor Pilot Fen Wetland Watershed; (2) a swamp reclamation investigation; and (3) a culturally significant marsh plant propagation program. This presentation will review how the three above mentioned wetland reclamation research projects are improving ecosystem diversity and riparian and aquatic plant community diversity in oil sands reclamation.

386 Development of triggers and critical effect sizes for adaptive regional monitoring programs

K.R. Munkittrick, Canadian Rivers Institute, Biology; T. Arciszewski, University of New Brunswick, Saint John / 5000+; M.E. McMaster, Environment Canada / National Water Research Institute

Preventing environmental damage associated with development requires that real and relevant changes are identified before they become significant or difficult to reverse. Detection of meaningful change has been challenged by inconsistencies in philosophy and design among monitoring, assessment, and baseline environmental programs that makes regional analysis of data complicated. It is possible to combine local and regional monitoring frameworks into a single linked, tiered, and triggered monitoring framework for environmental effects using linked effects-based and stressor-based tools. Our experience with the development of guidance for implementing local, regional, and national monitoring programs has led to the design and development of a philosophy for an adaptive monitoring strategy, that explicitly links site-specific environmental effects monitoring and regional cumulative effects monitoring programs with a series of decision triggers to improve the sensitivity and management responses. This presentation will explore the philosophy behind (and provide generic guidance for) critical effect sizes and trigger thresholds that can be used to develop an adaptive approach to regional monitoring that can focus resources on critical areas of concern using three case studies: data collected over 25 years, across more than 35 reference collections over two decades, and across 600 km of a river basin over a decade.

387 Ambient air monitoring for polycyclic aromatic compounds in the Athabasca Oil Sands Region for source and deposition assessment

J.K. Schuster, Atmospheric Science & Technology Directorate; T. Harner, Environment Canada / Atmospheric Science & Technology Directorate; K. Su, Environment Canada; A. Eng, Environment Canada / Atmospheric Science & Technology Directorate; J. Charland, Environment Canada; C. Austin, Environment Canada / Atmospheric Science & Technology Directorate

An air monitoring program for measuring polycyclic aromatic compounds (PACs) was established in November 2010 which now supports the Joint Canada/Alberta Implementation Plan for Oil Sands Monitoring. The program relies on local and remote sites operated by WBEA and comprises 17 passive air sampling sites, with 3 sites having co-deployed active samplers. These data contribute to the assessment of cumulative environmental effects across the oil sands region, associated with deposition of PACs, and provide a 'baseline' against which the impacts of expanded mining activities can be measured. PAC classes targeted in the study include: i.) polycyclic aromatic hydrocarbons (PAHs) ii.) alkylated PAHs, and iii.) parent and alkylated dibenzothiophenes (DBTs). These PAC classes are of concern due to their toxicity. The alkylated PAHs and DBTs are associated with petrogenic sources in the oil sands. Preliminary results are presented for air samples collected from the first 18 months of sampling (November 2010 – June 2012). The parallel deployment of passive and PS-1 type active samplers at 3 sites allows continuous validation of the integrated air concentrations derived from the passive samplers against the concentration snapshots presented by the active sampler. The data also reveal a seasonal trend in the PAH air concentrations, with higher concentrations observed during the winter months, which was markedly less pronounced for the alkylated PAHs. The data have been applied to a deposition model to assess deposition fluxes of PACs to aquatic and terrestrial surfaces across the oil sands region. PAC air concentrations show a logarithmic decline with distance from an identified major source in the area, whereby the correlation is stronger for the alkylated PAHs and DBTs. Average concentrations in air decreasing by half every 90 km, 70 km and 40 km distance from the major source region for PAHs, alkylated PAHs and DBTs respectively. This trend was also reported for snow and lichen samples in the area. PAH levels observed close to the source areas were found to be comparable to levels reported for an urban centre such as Toronto. Average air concentrations observed since November 2010 are about 30 ng/m^3 for PAHs, 70 ng/m^3 for alkylated PAHs and 15 ng/m^3 for DBTs at sites in less than 20 km distance to the major mining activities, and 20 ng/m^3, 30 ng/m^3 and 3 ng/m^3 at sites located within 70-110 km distance.

388 Current and Long-term Trends in Atmospheric Deposition of Inorganic Contaminants and Methyl Mercury to the Alberta Oil Sands Region

J.L. Kirk, Environment Canada / Research Scientist; D.C. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division; A. Gleason, Environment Canada; R.A. Frank, Environment Canada / AEPRD; X. Wang, M. Tanguay, J. Backer, G. Lawson, A. Sett, Environment Canada

Atmospheric deposition is a source of contaminants to landscapes and waterbodies of the Alberta Oil Sands region. Potential emission sources are complex and include bitumen upgrading facilities, open pit mines, exposed coke piles, deforested areas, and volatilization from tailings ponds. In addition to polyaromatic compounds, there is concern regarding inorganic contaminants, including the 13 priority pollutant elements (PPEs; Sb, As, Be, Cd, Cr, Cu, Pb, Hg, Ni, Se, Ag, Tl, and Zn), and nitrogen (N), phosphorous (P) and sulfur compounds. We have monitored atmospheric deposition of inorganic contaminants to the Alberta Oil Sands region from 2011-2013 using both snowpack measurements and dated lake sediment cores to examine spatial and temporal patterns in contaminant deposition. Springtime snowpack measurements demonstrate that aerial loadings of many of the inorganic contaminants examined increased with proximity to the major development area. Loadings of all PPEs were elevated near site AR6, which is located on the Athabasca River adjacent to major developments and upgraders (maximum loadings of As, Cr, Ni, and Zn, for example, were 360, 1170, 1120, and 3270 μg m^{-2}, respectively) compared to far field sites. Loadings of most PPEs were, however, consistently lower than at other impacted sites, such as near Rouyn-Noranda metal smelter in Quebec Canada. Co, Mn, and V, which were reported as being released to the atmosphere in high quantities from the upgraders, numerous crustal and rare earth elements (Al, Fe, Ce, La and Y), total P and N, and sulfate were also elevated in the development area. Hg and methyl Hg (MeHg), which are of particular concern because MeHg is a potent neurotoxin that bioaccumulates through foodwebs, were also elevated at numerous sites reaching over 1000 and 19 ng m^{-2}, respectively, and decreasing to 103 ± 42 and 1.2 ± 0.2 ng m^{-2} at far field sites. Assuming that < 100 and 2.0 ng m^{-2} represent THg and MeHg loadings "un-impacted by oil sands developments", we estimate that ~19, 000 km^2 is currently impacted by airborne Hg emissions originating from Oil Sands developments with areas of maximum deposition centered northeast and southeast of AR6 and covering parts of the Muskeg and Steepbank rivers. Spatial depositional patterns of different contaminants will be compared and results from analysis of highly-resolved, dated lake sediment cores spanning the last 50-100 years will be presented to place modern contaminant deposition in the context of long-term trends.

389 Fish Health in the Alberta Athabasca Oil Sands

M.E. McMaster, Environment Canada / National Water Research Institute; H. Keith, Hatfield Consultants; G. Tetreault, Environment Canada / Aquatic Ecosystem Protection Research Division; J. Bennett, Environment Canada / National Water Research Institute; J. Ings, M. Evans, D. Marcogliese, Environment Canada; D.B. Simmons, Denina Simmons Scientific Consulting / Aquatic Contaminants Research Division; J.P. Sherry, Environment Canada / Water Science & Technology Directorate; M. Hewitt, Environment Canada / National Water Research Institute; R.A. Frank, Environment Canada / AEPRD; J.L. Parrott, Environment Canada / Water Science and Technology Directorate

As part of theJoint Canada-Alberta Oil Sands Monitoring Program (JOSMP), fish health within theAthabascaRiverwatershed is being evaluated using methods developed for the Canadian Environmental Effects Monitoring program. Fish health assessments are being integrated with assessments of benthic invertebrate communities, water and sediment chemistry, toxicology and atmospheric deposition in order to evaluate ecosystem health. Data will provide a baseline against which future changes in fish populations will be judged and compared to historical studies to assess change. Information will be used to develop site-specific cumulative effects monitoring approaches and will contribute to the development of better predictive capabilities for oil sands environmental impact predictions. Additional physiological endpoints are also being evaluated in fish from these sites in attempts to understand mechanisms of action and potential source identification of changes. On theAthabascaRivermainstem, white sucker have been selected as a large bodied sentinel species and trout perch as a small bodied sentinel species, while on tributaries studied to date, the slimy sculpin is being used. Walleye are also being used for fish contaminant monitoring within the mainstem as they are consumed by resident populations. Where possible our study design collects fish at sites off the oil sands deposit (reference), at sites within the natural deposit but upstream of

development and at sites downstream of development so that fish are either unexposed, exposed to naturally occurring oil sands related compounds, or exposed to a combination of natural and anthropogenic inputs. Design of the program is to look for effects, to confirm those effects, then to use developed critical effect sizes to make decisions on steps forward depending on the source of the changes. To date, on one developed tributary slimy sculpin have demonstrated a graded response to development with reductions in overall condition and investment into gonadal growth, but larger livers and induced mixed function oxygenase activity indicative of potential exposure to PAH related compounds within both the natural deposit and within the developed regions of that tributary. Toxicology from sediments collected at these locations support these findings and comparisons will now be made to benthic invertebrate communities and water chemistry. Data from the mainstemAthabascawill also be discussed.

390 Alterations in the thyroid system of tree swallow (Tachycineta bicolor) nestlings raised on oil sands mine-impacted areas: preliminary investigations

K.J. Fernie, Environment Canada / Ecotoxicology and Wildlife Health; D. Chen, Carleton University / Chemistry, Ecotoxicology and Wildlife Health Division, Wildlife and Landscape Directorate, Science and Technology Branch, Environment Canada, National Wildlife Research Centre, Carleton University / Department of Chemistry, Southern Illinois University Carbondale / Cooperative Wildlife Research Laboratory; T. Harner, Environment Canada / Atmospheric Science & Technology Directorate; L. Cruz-Martinez, University of Calgary / Dept. of Ecosystem and Public Health, Faculty of Veterinary Medicine; L. Peters, Stantec Consultants Ltd.; J.E. Smits, University of Calgary / Dept of Ecosystem and Public Health; Faculty of Veterinary Medicine; C. Soos, Environment Canada / Ecotoxicology and Wildlife Health; V.P. Palace, Department of Fisheries & Oceans Canada, Stantec Consultants Ltd.

Alberta's Oil Sands represent an economically significant resource for Canada. In order to assess the impacts of the ongoing extraction, processing and transportation of bituminous products, a multi-agency environmental monitoring program has been put in place. Recent studies suggest that birds and amphibians resident on areas affected by oil sands mining activities may have disrupted thyroid hormone levels. However, a thorough examination of the thyroid axis requires examination of circulating thyroid hormones along with the glandular structure, plus hepatic biotransformation enzymes required to convert inactive to active forms of the thyroid hormones. The current study examined each of these three diagnostic indicators in tree swallow (*Tachycineta bicolor*) nestlings resident on oil sands mine-impacted areas and compared them to nestlings on nearby reference sites. Specifically, thyroid gland histology (i.e., colloid area, epithelial cell heights), hepatic deiodinase enzyme activity, and circulating total and free thyroxine were determined in each nestling. Tree swallow nestlings are fed predominantly with nearby emergent aquatic insects. The study sites were near industrial activities related to extraction of the oil sands, with a reference site approximately 80 km south of any industrial activity. Initial data from 2012 shows that, while circulating thyroid hormone concentrations were not significantly different among birds from these sites, differences in thyroid glandular structure and biotransformation enzymes indicated increased activity of the thyroid axis in nestlings from industrial sites. Airborne polycyclic aromatic hydrocarbons (PACs) including alkylated PAHs, were measured at each breeding colony and concomitantly in the tissue of individual nestlings and will be statistically examined relative to thyroid responses. These data will be corroborated with additional research in 2013.

391 Mercury trends in colonial waterbird eggs downstream of the oil sands region of Alberta, Canada

C.E. Hebert, Environment Canada / Science and Technology Branch, Ecotoxicology and Wildlife Health Division; D. Campbell, R. Kindopp, S. MacMillan, Parks Canada Agency; P. Martin, E. Neugebauer, Environment Canada; L. Patterson, J. Shatford, Parks Canada Agency

Colonial waterbird eggs were collected from two sites in northern Alberta and one site in southern Alberta, Canada. Northern sites were located in receiving waters of the Athabasca River which drains the Oil Sands industrial region north of Fort McMurray, Alberta. Temporal trends (1977, 2009-2012) in egg mercury (Hg) levels were assessed as were egg stable nitrogen isotope values ($d^{15}N$) as an indicator of dietary change. In northern Alberta, there was a general trend across species (California Gulls, Ring-billed Gulls, Caspian Terns, Common Terns) of increased egg Hg concentrations in 2012 compared to data from the earliest year of sampling for each species at each site. In southern Alberta, Hg concentrations in California Gull eggs declined through time, suggesting the pattern observed in northern Alberta is not consistent across the region. Bird dietary change was not responsible for any of these trends. Neither were egg Hg trends related to recent forest fires. Differences in egg Hg temporal trends between northern and southern Alberta combined with greater Hg levels in eggs from northern Alberta identified the importance of local Hg sources in regulating regional Hg trends. Hg concentrations in gull and Common Tern eggs were generally below generic thresholds associated with toxic effects in birds. However, in 2012, Hg levels in the majority of Caspian Tern eggs exceeded the lower toxicity threshold. Increasing Hg levels in eggs of multiple species nesting downstream of the oil sands region of northern Alberta warrant continued monitoring and research to further evaluate Hg trends and to conclusively identify sources.

Remedy Effectiveness Assessments and Monitoring Contaminated Sediment Remediation: Part A

392 Application of polyethyelne devices (PEDs) for monitoring PCBs at a freshwater sediment remediation site

M.J. Benotti, Analytical and Environmental Chemistry, Battelle; L.F. Lefkovitz, Battelle; M.A. Mills, USEPA, ORD / Remediation and Pollution Control Division, NRMRL

The use of passive samplers to assess the presence and distribution of hydrophobic organic contaminants (HOCs) in aquatic environments has increased markedly of late. While a number of different types of materials may be used as passive samples, low density polythelene, or polyethylene devices (PEDs) have proven to be useful and cost effective tool for such applications. This presentation will describe the use of PEDs in an ongoing, multi-year, baseline study characterizing the distribution of PCBs in a riverine system and discuss different uses of the data that has been generated. Broadly speaking, utility and relevance of using PEDs a) to characterize distributions of HOCs, b) to determine integrated site water concentrations, and c) for source attribution studies will be discussed. PEDs were deployed in both the water column and in piezometers to characterize surface and pore water PCB concentrations. Waterborne concentrations can be calculated based on the loss of PRCs (performance reference compounds) during deployment. Alternative methods of determining water concentrations from the PEDs will be explored. Results from PEDs deployed in the water column will be compared to actual water concentrations measured in grab samples, and PEDs in the piezometers will be compared to groundwater concentrations measured in adjacent upland wells.

393 Monitoring of PAHs in Groundwater and Surface Water with Low Density Polyethylene Films – Experiences, Observations and Future Directions

H.L. Lord, M. Cojocar, L. Khachatryan, Maxxam Analytics International Corporation / Scientific Services; B. Chubb, Maxxam Analytics International Corporation; T. Obal, Maxxam Analytics International Corporation / Scientific Services

The use of passive sampling to monitor environmental pollutants has gained increasing attention in recent years. Numerous device configurations have been proposed and tested. Low Density Polyethylene film (LDPE) was shown to be valuable as a component of the high capacity semi-permeable membrane devices (SPMD), and subsequently as a lower capacity passive sampler on its own. The uptake processes identified for SPMD proved to be equivalent for LDPE, aiding the fairly rapid development of this alternative technology. In this presentation we outline our recent experiences in LDPE passive sampler design, deployment and analysis and describe our view of the next steps in the development of this technology. LDPE films were deployed for one month in groundwater monitoring wells or surface waters at sites with known or suspected contamination with polycyclic aromatic hydrocarbons (PAH). Laboratory experiments were conducted to evaluate LDPE/water partition coefficients (K_{PE}). After deployment the films were extracted and either literature or laboratory-determined K_{PE} were used to estimate water concentrations. Spot water samples were also taken in some cases. It was found that calibration by use of the partition coefficient only was appropriate for lower log K_{ow} PAH. At moderate log K_{OW} small deviations from expected performance were seen likely due to either higher inaccuracies in the K_{PE} determinations in this range of log K_{OW} or equilibration not having been reached. At higher log K_{ow} the data became

unreliable and an assessment of degree of fractional uptake was required to correct the calibration of water concentration. Fractional uptake estimates may be measured during deployment by the use of performance reference compounds included in the film, or estimated prior to deployment by modeling. An understanding of the sources and magnitudes of inaccuracies in passive sampling data is valuable both for providing estimates of measurement uncertainty in data obtained, and in the rational design of passive sampling ahead of deployment. This presentation will explore these issues in relation to our recent findings and make practical recommendations for implementation.

394 Selecting performance reference compounds (PRCs) for polyethylene passive samplers deployed at contaminated sediment sites

R.M. Burgess, USEPA / Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory / ORD / NHEERL-Atlantic Ecology Division; M.M. Perron, EPA; L. Fernandez, USEPA; M.G. Cantwell, Atlantic Ecology Division

Use of equilibrium passive samplers for performing aquatic environmental monitoring at contaminated sediment sites, including Superfund sites, is becoming more common. However, a current challenge in passive sampling is determining when equilibrium is achieved between the sampler, target contaminants, and environmental phases. A common approach is the use of surrogate contaminants, called performance reference compounds (PRCs), to indicate the degree of equilibrium achieved for target contaminants during the deployment. However, there remain several research and logistical issues related to the use of PRCs. One of these logistical issues is the cost associated with purchasing PRCs. In an effort to address PRC expense, this investigation (1) compared the performance of inexpensive PRCs (e.g., deuterated PAHs) and expensive PRCs (i.e., 13C-labelled PCBs) to estimate dissolved PCB concentrations in deployments at freshwater and marine sites, and (2) evaluated the use of less PRC relative to conventional quantities for estimating dissolved PAH and PCB concentrations. At saltwater sites, differences between total PCB concentrations calculated using 13C-labeled PCBs and deuterated PAHs were less than 0.1 ng/L with differences ranging from -0.08 to 0.08 ng/L. At freshwater sites, concentrations calculated using deuterated PAHs were less than those calculated using 13C-labeled PCBs with differences ranging from -0.92 to 0 ng/L and most approximately -0.4 ng/L or less. Similar findings were observed on an individual congener basis. Calculated total PAH concentrations were very similar (e.g., 17.1±0.85, 17.9±0.72, and 17.1±0.79 ng/L) for the three quantities of PRC evaluated (i.e., conventional, large, and small, respectively). Similarly, calculated total PCB concentrations were 99.5±5.8, 93.7±6.7, and 88.2±6.2 pg/L for the three quantities of PRC assessed. No statistical differences were observed between PRC quantities for total PAHs or total PCBs. Individual PCB congeners and PAH molecules demonstrated comparable behavior. Results of this investigation provide evidence that using inexpensive and smaller quantities of PRCs can be successfully performed to yield substantial cost savings without sacrificing scientific accuracy.

395 Pilot-Scale Demonstration of Reduced PCB Bioavailability with Activated Carbon Amendments in a Phragmites Marsh

N.A. Andrade, Civil and Environmental Engineering; J. Sanders, University of Maryland Baltimore County; C.A. Menzie, B. Amos, Exponent; S.S. Brown, The Dow Chemical Company / Department of Toxicology; C.C. Gilmour, Smithsonian Env. Research Center; C. Greene, Parsons Corporation; U. Ghosh, University of Maryland Baltimore County / Department of Chemical, Biochemical, and Environmental Engineering

Amendment of contaminated sediments with activated carbon (AC) is an emerging technology utilized to reduce bioavailability of hydrophobic organic compounds. Recent work has proven it to be effective in laboratory scale as well as large scale; however, additional pilot scale experiments are essential to address scaling-up and site-specific issues. A New Jersey *Phragmites* marsh impacted with PCBs and other contaminants is being considered for remediation. A pilot study was initiated during summer 2012 to evaluate the efficacy of three types of AC amendments in reducing contaminant bioavailability. Tested treatments were granular AC, granular AC with a thin sand top layer, and a pelletized AC (SediMite) which were added to the surface of three 100 m^2 plots; a control plot was also established. A holistic approach was taken to evaluate the effectiveness of amendments, which included analysis of sediment, epibenthic native community, field exposed non-native amphipods, and porewater concentrations through the use of polyoxymethylene passive samplers. The focus of this presentation is on the assessment of amendment persistence and performance in reduction of PCB bioavailability. Black carbon analysis performed in sediment cores from treated and untreated plots demonstrated that treatments remained in place one month after application and also after a rare storm event, showing the treatments can be successfully used in the marsh environment. After treatments were applied, accumulation of PCBs in native organisms was reduced by 33% in the AC treatment, 28% in the AC plus sand treatment, and by 55% in the SediMite treatment. Bioaccumulation of PCBs in non-native caged amphipods versus control was reduced 57%, 47%, and 98% in the AC, AC plus sand, and SediMite treatments, respectively. Reductions in PCB accumulation in organisms paralleled reductions in porewater concentrations measured by the use of passive samplers. Additional measurements are underway for evaluation of the treatments one year after application.

396 Assessing Recovery from DDT-Contaminated Sediment in Lago Maggiore, Italy

D. Lin, Y. Cho, R.G. Luthy, Stanford University / Department of Civil and Environmental Engineering; E. Eek, Norwegian Geotechnical Institute; A. Oen, Norwegian Geotechnical Institute

This paper presents a case study in application of novel and traditional measurement techniques to assess the status of natural attenuation for contaminated sediment in Lake Maggiore, Italy. The status of natural attenuation was assessed using different types of measurements that provide multiple lines of evidence on the recovery process. The study site was previously contaminated with DDT from a manufacturing plant located upstream on one of the lake's tributaries. Current understanding of the lake is that the site is net depositional and incoming sediment is clean, and natural attenuation will reduce the risks from DDT in the lake. We employed innovative design methods using passive samplers to measure the sediment-to-water flux of DDT from the sediment bed, as well as to measure the DDT concentration profile in the overlying water and within the sediment porewater. We combined these findings with more traditional field measurement methods, including sediment traps and sediment cores, to evaluate whether the data were internally consistent. Sediment traps were used to measure the sedimentation rate, and the samples were analyzed to understand whether the incoming sediment contained DDT. Sediment cores were analyzed at up to 1 cm resolution to assess how effectively contaminated sediment is buried below the surface. All of these methods of measuring the source and transport of DDT within this freshwater lake are being used to guide future work on monitoring and managing DDT in the lake. We expect this toolset to be applicable for other contaminated field sites, and can be used to measure sediment remediation efficiency as well.

397 Contaminant mass transfer model to assess the effectiveness of in-situ activated carbon treatment in sediments

Y. Choi, Y. Cho, R.G. Luthy, Stanford University / Department of Civil and Environmental Engineering

In-situ activated carbon (AC) treatment is a promising technique to treat hydrophobic organic contaminants in sediments. However, uncertainties on the effectiveness of the treatment exist due to limited field experience. The long-term performance of the treatment is in question since the effectiveness in pilot-scale field trials has not been monitored for more than five years. This calls for a reliable method to assess the long-term effectiveness of in-situ AC treatment under various field conditions. In this study, a previously developed contaminant mass transfer model is modified, validated with experimental results, and applied to simulate the effectiveness of AC treatment under various field application scenarios for contact times up to 25 years. The model simulates mass transfer processes of hydrophobic organic contaminants among and within sediment, AC, pore-water, and passive sampler using parameters determined by a series of laboratory measurements. Sediment column studies mimicking possible field application procedures are conducted to validate the model. The results of the sediment column studies show 96-99% reduction in uptake by polyethylene passive samplers after 24 months of AC-sediment contact. The contaminant mass transfer model accurately reproduces the experimental results obtained in the column studies for four polycyclic aromatic hydrocarbons. The model successfully addresses the effect of AC particle size, AC mixing regime and distribution, contact time, and pore-water movement on the effectiveness of the treatment observed in the column studies. The modeling results suggest a strong dependency of the treatment effectiveness on AC particle size and

distribution in the short term (i.e., months), but the long-term effectiveness (i.e., years) is not substantially affected by those factors. For all simulated AC treatment scenarios, the model predicts more than 99% reduction in pore-water concentration for benz[a]anthracene after 25 years.

398 Two sides of in-situ management of contaminated sediments by sorbent amendments

J. Akkanen, University of Eastern Finland / Department of Biology; J.V. Kukkonen, University of Jyvaskyla / Department of Biology; I. Nybom, University of Eastern Finland / Department of Biology

Controlling the bioavailability of contaminants in contaminated sediments has been quickly developed to be a considerable choice for sediment remediation. The remedial actions are done by adding sorbents to the sediments to reduce biouptake, transport and thus risks of the contaminants by strong sorption. Especially in the case of neutral lipophilic organic chemicals, activated carbon has been shown to be effective in reducing the exposure of benthic organisms. This was first demonstrated in laboratory and later in field experiments as well. The efficiency to reduce contaminant bioavailability appears to be chemical congener, sorbent dose and type specific. Sediment characteristics play a role too. In addition to these beneficial effects, there are also secondary implications, which can be manifested in adverse effects in the sediment-dwelling organisms. The magnitude of these effects appears to be sediment, organism and sorbent specific. Sorbent properties such as sorption capacity and particle size are important. Less selective sediment feeders are more susceptible to these adverse effects and the effects are stronger in sediment being less suitable as habitat for the organisms. However, in sediments that are acutely toxic the amendments can improve well-being of the organisms. The mechanisms of these adverse effects are still partly unclear, but there are indications that reduction of nutrient availability can be one. In addition, other mechanisms and long term implications can be also discussed. Therefore, the usage of sorbents for remediation purposes requires case specific assessments for to evaluate both positive and negative effects.

399 Remedy Effectiveness Monitoring Using Fish: Corresponding Site Boundaries and Fish Exposure Areas

M.J. Erickson, Arcadis US, Inc.; K. Gustavson, US Army Corps of Engineers

Reducing fish tissue contaminants is often a remedial action objective (RAO) at contaminated sediment sites, particularly those impacted with PCBs or other persistent hydrophobic contaminants. Sediment remediation is often selected to eliminate or lessen exposures to those contaminants. However, there are many challenges associated with determining and targeting the media and locations that actually drive contaminant burdens in fish. One aspect of this challenge is understanding the spatial overlap between the site and the exposure area of the fish (the site: exposure area ratio). For sites that represent a small proportion of the exposure area, this ratio may be much less than 1. At such sites, sediment remediation may have undetectable influences on fish tissue, especially for sediments where non-site sources contribute to contaminant burdens. Many relatively small sites in large urban or industrialized waterways fall into this category. At such sites, is it appropriate to chose fish tissue reduction to specific risk-based targets as a remedial goal? Moreover, how can the effectiveness of a remedy in achieving this goal be evaluated when fish migration occurs over large areas? Will future reductions be happenstance as opposed to a remedial benefit? What if fish tissue does not decline to target levels – should the remedy be deemed ineffective? In these scenarios, remedies may not be able to be proven effective or ineffective in relation to the ultimate goal. How should this problem be addressed in remedy effectiveness monitoring? What about in remedy selection, RAO development, and goal setting? The issue of effectiveness monitoring is integrally connected with RAO and goal setting for such sites. When achievable goals are not set, this creates a disincentive for effective monitoring of effectiveness – both for the regulating and regulated communities. This talk addresses these questions in examining the challenges and tradeoffs inherent in selection of remedial goals and monitoring approaches and methods, in particular for these types of sediment sites. Examples will be presented to illustrate these important issues and challenges. We make the case for careful selection of remedial goals and corresponding monitoring approaches at such sites, and discuss alternatives to defining fish tissue targets for these types of sites.

Policy and Litigation in an Uncertain World

400 How policies can affect the publication process in LCA?

L. Patouillard, IFP Energies nouvelles / Economics and environmental evaluation department; B. Cheze, IFP Energies nouvelles

The United States (US) and the European Union (EU) have developed regulations promoting biofuel production in order to lower greenhouse gas (GHG) emissions in the transport sector. Both regulations set minimum thresholds for life cycle GHG emission savings compared to a fossil fuel as the only quantitative environmental criteria that biofuels should meet. However, their designs are quite different. Indeed, the Renewable Energy Directive (RED) in the EU has been developped as a complementary policy of a Low Carbon Fuel Standard (LCFS) whereas there is no national LCFS framework in the US. This paper investigates if Life Cycle Assessment (LCA) literature of advanced biofuels (i.e., biomass-based fuels from lignocellulosic materials and microalgae) that meets political targets are more likely to be published. Furthermore, the influence of the public policy design on the publication process is also examinated. Thanks to specific set of analytical techniques (Funnel graphs) and specific econometrics method (meta-regression analysis), potential publication biases in LCA GHG emission results of advanced biofuels are investigated in order to be more conclusive on its environmental performances and to support policy makers. This quantitative literature review is based on 47 LCA studies providing 593 observations. Results reveal the existence of an asymmetrical publication bias of US studies to rather publish results under their political minimum thresholds for life cycle GHG emission savings. Such phenomenon does not exist in the EU. It highlights the influence of public policy designs on the publication process. Then we perform harmonisation correcting from identified biaises. Mean value and Confidence Interval of LCA GHG emissions for Biomass to Liquid (BtL), Ethanol and G3 biofuel are estimated to 12.8 [10.8;14.9], 48.6 [48.1;49.0] and 32.3 [11.4;53.2] gCO_2eq/MJ of biofuel respectively. Even if this range of values is lower than fossil reference (about 83.8 in gCO_2eq/MJ), only BtL still comply with the GHG emission reduction thresholds defined in the US and EU regulations.

401 An Approach for Developing Updated Selenium Criteria for the Protection of Freshwater Aquatic Life

S.D. Baker, GEI Consultants, Inc. / Ecotoxicologist/Wildlife Biologist; S.P. Canton, GEI Consultants, Inc. / Ecological Division

While the USEPA is in the process of revising the national ambient water quality criteria (AWQC) for selenium (Se), it is unknown when a new draft will be available. The release has been delayed multiple times over the last several years, and an estimated release date has not been provided to the public. However, derivation of updated Se criteria is currently scientifically defensible due to new acute water-column toxicity and tissue-based chronic toxicity data made available since the current criteria (USEPA 1987) and the last draft criteria (USEPA 2004) were released. Therefore, interested states may develop their own updated criteria instead of relying on the outdated and inappropriate Se criteria from 1987. Such an effort to develop updated state-wide acute and chronic Se criteria was recently conducted in Kentucky. The resulting proposed water-column based acute criterion takes into account the fraction of selenite and selenate and considers sulfate's toxicity-reducing effect on selenate. Tissue-based chronic criteria were developed, as it is expected that USEPA's updated chronic criterion will include a component based on Se concentrations in fish tissues. To provide stakeholders with options for assessment evaluations, we developed chronic criteria based on either whole-body or egg/ovary concentrations. However, given the potential expense and difficulty that required tissue collection will pose for the regulated community, we proposed a multi-step criterion that begins with screening of water-column data to weed out locations with low Se and limited risk to aquatic life. The current national chronic criterion of 5.0 µg/L will be used as a threshold, and if exceeded, will trigger the requirement to collect fish tissues (whole-body or egg/ovary) to assess attainment. These proposed criteria present a significant improvement over the outdated 1987 USEPA Se criteria currently in use by most, if not all, states. In addition, it is expected that the Se criteria being developed by USEPA will follow a similar approach.

402 Importation of Industrial Chemicals in Emerging Markets: A Unified Strategy for Data Compilation and Generation

W.T. Mehler, Gradient, Gradient Corporation; J. Kneeland, A.S. Lewis, M. Sharma, Gradient Corporation

For companies involved in the global importation and exportation of chemicals, one of the key challenges is identifying and staying current with the developing landscape of new chemical import requirements. Companies attempting to establish themselves in emerging markets, if proactive, can expedite the registration process by understanding how data can be identified and generated in a manner that will be broadly applicable across multiple jurisdictions. Here, we compare data requirements, timelines, and application costs associated with new chemical importation compliance in three emerging markets and present an unified strategy for meeting country-specific requirements that maximizes data generation and analyses. Our evaluation was focused on industrial, non-cosmetic chemical importation, in the following jurisdictions: Australia, New Zealand, China. Requirements will be compared against those of the European Union (EU) through REACH. While the requirements among the jurisdictions reviewed share some features (*e.g.*, need to notify before importation), the extent of data needed to register a compound can differ significantly, particularly for high import volume compounds. While some jurisdictions simply require that notifications include existing toxicity data (even if very limited), others require extensive animal and ecotoxicity testing before importation (and, in some cases, even after importation if the conditions of volume of use change significantly [e.g., Secondary Notifications in Australia]). The extent to which toxicity data generated for analog compounds can be used to waive testing also varies among jurisdictions. Moreover, in some jurisdictions there are additional constraints for conducting toxicity testing. In China, for example, certain toxicity testing must be conducted in-country. Given the current differences in test requirements across jurisdictions, thoughtful and streamlined data compilation and generation is crucial to maximize time and resources and avoid the need for duplicative testing or registration delays.

403 Trace Organic Compounds and the Regulatory Intersection of Policy and Science in the Water and Wastewater Industry

M.E. Sadler, B.D. Stanford, Hazen and Sawyer, P.C.

The occurrence, potential public health effects, and environmental impacts of emerging contaminants have been portrayed as a growing threat to water and wastewater providers. These issues surfaced in this country nearly 60 years ago, but have gained notoriety during the past two decades as analytical techniques have broken the parts per quadrillion (pg/L) barrier. Emerging contaminants include a wide variety of chemicals that have been largely outside the scope of environmental regulation. In response to the issue, the Environmental Protection Agency (EPA) has been methodically working towards gathering information and data in order to make decisions on future regulatory action. Therefore, it is necessary for the scientific and management community in the water and wastewater field to be armed with an understanding of current regulation and policy as well as the potential for future regulation among the applicable regulatory programs. Currently, no single regulatory program in the US considers the totality of the potential risks of endocrine disruptors and other emerging contaminants. Are there risks to human health and aquatic life that should be addressed through the Safe Drinking Water Act and the Clean Water Act? Are indicator compounds the appropriate mechanism to address future compliance issues for various groups of compounds? EPA has made significant strides in refining validated analytical methods, sensitive ecological effects test methods, occurrence data, treatment data, and environmental fate data. Additionally, the growing public awareness of the emerging contaminants issue and escalating research may increase the pressure for regulation. This paper will focus on the regulatory mechanisms for drinking water regulation via the Safe Drinking Water Act and water quality standards and National Pollutant Discharge Elimination System (NPDES) permit limits development via the Clean Water Act. This paper will also highlight current research in the water and wastewater industry with respect to advanced treatment technologies. Future regulation of emerging contaminants will be driven by policy choices and by the necessity and/or efficacy of advanced treatment and the associated cost of implementation.

404 No More Excuses: It is Time to Incorporate Uncertainty Analyses in Pesticide Risk Assessments

D.R. Moore, Intrinsik Environmental Sciences (US), Inc; M.F. Leggett, CropLife America

A committee of the National Academy of Sciences (NAS) recently released a report entitled, "Assessing Risks to Endangered and Threatened Species from Pesticides". In that report, the NAS committee made several recommendations to the EPA and US Government Services on how to deal with uncertainty in assessments of pesticide risks to listed species. The recommendations of the NAS committee echoed observations of past NAS committees that without uncertainty analysis it can be quite difficult to determine the conservativeness of risk estimates. The panel stated that a major difficulty with EPA's approach was that it did not supplant or supplement artificially precise single estimates of risk ("point estimates") with ranges of values or quantitative descriptions of uncertainty. Often EPA failed to even provide qualitative statements of uncertainty. The same can be said of the Biological Opinions produced by the Services. It was the NAS's opinion that lack of a rigorous uncertainty analysis obscures the uncertainties inherent in risk estimation although the uncertainties themselves do not go away. Although lack of an uncertainty analysis has been an issue for over two decades, little has changed in terms of how uncertainty is routinely addressed in pesticide risk assessments due to the perception that uncertainty analyses are difficult and resource intensive. The use of conservative models, inputs and assumptions is a conventional approach to deal with sources of uncertainty in ecological risk assessments. The risk assessments conducted by EPA and the Services have been strongly biased to the side of conservatism (by design). In this paper the conservative assumptions used by EPA and the Services are examined with a view to assessing the utility of incorporating the NAS recommendations for refining future pesticide assessments to include rigorous uncertainty analyses.

405 Where are active pharmaceutical ingredients produced and does the price of medicines correlate to diminished pollution control?

L. Gunnarsson, J.D. Larsson, University of Gothenburg / Department of Infectious Diseases, Institute of Biomedicine

Pharmaceutical companies increasingly outsource their manufacturing of active pharmaceutical ingredients (API) to low income countries. We have previously shown that some bulk drug manufacturers in Patancheru, India severely pollute the environment. Recent examples from other regions indicate that this is not an isolated problem. However, the current legal framework does not force companies to publically disclose information on by whom, where and under what circumstances the API of a given product is produced. A use of less environmentally responsible sub-contractors is therefore not easily identified by third parties. At the end of the day, this lack of transparency effectively prevents prescribers and the general public to make environmentally informed decision, and thus reduces financial incentives for API manufacturers to invest in green production technologies. The Swedish Medical Products Agency has information on where and by whom the API is produced for products approved for the Swedish market, data which can be accessible for research purposes, given that specific companies and products are not revealed. We have received data on the production site as well as sales statistic for about 60% of all medicinal products (human and veterinary) sold in Sweden in 2010. The aims were to describe where the APIs in pharmaceutical products, sold in Sweden, are produced and how the price of interchangeable products correlate to the country of origin of the API. We will also investigate if price correlates to corruption indexes (www.transparency.org) and environmental performance indexes (http://epi.yale.edu/) in the manufacturing country. Such surrogate measures are used as data on pollution is lacking for most sites. Furthermore, we will analyze if there are general differences between the countries of origin of the APIs for generics and original products. To date, all data has successfully been compiled into a workable database and the analysis is ongoing. Forty-eight per cent of the investigated products have stated a possible production country with poor corruption index (< 4) and 34% have stated a production country with poor environmental performance index (< 50). We believe our analyses will highlight some of the international aspects and regulatory challenges linked to pharmaceutical production. The study also exemplifies how recent scientific findings call for an evolution in regulations that meets the expectations of the general public.

406 Emerging Contaminant Policy: consequences of limited science

R. Klaper, University of Wisconsin-Milwaukee / School of Freshwater Sciences

Emerging contaminants such as pharmaceuticals, personal care products, flame retardants, and manufactured nanomaterials have all been measured in a variety of environments as well as in many animals, including humans, causing great concern over their potential impacts. However, they are often found in relatively small concentrations which leads to questions as to how much of an impact they may have on humans and the environment and therefore if and how regulation of these contaminants should be developed. Science has shown that some of the chemicals in this group including hormones, neuropharmaceuticals and potentially select nanomaterials may have an impact even at the low concentrations due to their high reactivity or their specificity for key metabolic pathways. By in large key information regarding the impacts of emerging contaminants is missing which creates issues when determining proper regulation. There is often information on acute toxicity of single chemicals, but studies of mixtures, which is common exposure scenario in the environment, are difficult and lacking. There is also a lack of information on the effects on reproduction, immune function, growth and other sublethal endpoints. With the multitude of chemicals in the marketplace it is clear that a chemical-by-chemical approach to science and regulation may not be feasible. This presentation will discuss these issues as well as their implications for currently regulatory frameworks as well as cleanup or removal programs such as pharmaceutical take-back programs and wastewater treatment technologies.

Pyrethroid Pesticides in Aquatic Environments: Exposure, Effects, and Mitigations: Part A

407 Spatial extent of depositional and non-depositional areas in Pleasant Grove Creek California with concurrent pyrethroid and benthic macroinvertebrate assessments

L.W. Hall, University of Maryland / Wye Research & Education Center; R. Anderson, W. Killen, University of Maryland

The objectives of the 2012 Pleasant Grove Creek study were to: (1) determine the % of depositional and non-depositional area for the entire stream using the same 99 randomly selected sites that had been previously used two years before in 2010 and compare the results from both years; (2) measure 8 pyrethroids from 12 randomly selected depositional and 12 non-depositional areas; (3) concurrently collect, identify and compare benthic macroinvertebrates from the depositional and non-depositional areas where pyrethroids were measured and (4) measure physical habitat metrics at each site where benthic macroinvertebrates were collected. Five percent of the 99 sites were classified as predominately depositional areas in 2012 and the percent depositional area declined 43% from 2010 to 2012. Mean values for 7 of the 8 pyrethroids were approximately 2x to 11x greater in depositional areas compared with non-depositional areas. Selected benthic metrics were not statistically different between samples collected in depositional and non-depositional areas thus suggesting that pyrethroids are not a major stressor impacting resident communities. Physical habitat scores were rated as poor and similar between sites in depositional and non-depositional areas. Results from this study illustrate the importance of determining the spatial extent of depositional areas for water bodies where hydrophobic chemicals, such as pyrethroids, are suspected to be a stressor.

408 Risk Assessment Approaches for Evaluating Benthic Aquatic Invertebrate Exposure to Pyrethroids

A. Blankinship, K. Sappington, K. White, E. Gelmann, USEPA

Since 2007, the US Environmental Protection Agency (USEPA) has conditionally required whole-sediment toxicity testing to support the registration of pesticides in the United States. The conditions for requiring whole-sediment toxicity testing depend on a pesticide's use pattern, environmental fate and transport properties, and toxicity in relation to potential exposure. Based on these considerations, whole-sediment benthic invertebrate toxicity testing is generally required for synthetic pyrethroids. The Agency's approach to address potential risk to sediment-dwelling organisms exposed to pesticides has evolved over the past several years. Aspects of the approach include identifying an optimized battery of sediment toxicity tests and the conditions upon which to request such testing. In addition, the Agency is developing a process for integrating sediment-toxicity values with potential exposure to evaluate possible risk concerns. This presentation will provide the current thinking within EPA's Office of Pesticide Programs regarding sediment toxicity testing for pesticides such as synthetic pyrethroids and the process for assessing risk to sediment-dwelling organisms.

409 Addressing uncertainty in aquatic exposure assessments of agricultural pyrethroid use on multiple crops

A. Ritter; D. Desmarteau, C.M. Holmes, M. Cheplick, Waterborne Environmental, Inc.; P. Hendley, Phasera Ltd.; M.G. Dobbs, Bayer CropScience / Ecotoxicology Group; S.H. Jackson, BASF Corporation / Principal Scientist; J.M. Giddings, Compliance Services International

US Environmental Protection Agency (USEPA) Tier II modeling for aquatic exposure assessments is designed to account for uncertainties by using conservative assumptions about chemical behavior and the receiving environment. In order to reduce the uncertainties in aquatic exposure assessments, a tiered approach is recommended that proceeds from simpler aquatic exposure modeling to more refined modeling as well as probabilistic analysis. This presentation will address exposure variability and uncertainty in the context of use of pyrethroids on crops across the United States. Initially, pyrethroid concentrations from Tier II (USEPA's PRZM-EXAMS model) will be shown as a baseline. Higher tier modeling will be presented for a range of crops to show the potential for exposure at different scales (field, watershed and national [presented in more detail in an accompanying presentation titled, 'National surface water vulnerability assessment of agricultural pyrethroid use on multiple crops for higher tier exposure modeling']). Probabilistic tools and methods that can be used to quantify the significance of various sources of uncertainty will be shown, including percent drift from multiple applications, percent crop area, percent crop treated, variations in soil/weather, variation in field to water body proximity and area ratios, timing of application across watersheds, and other variables. This analysis provides information about the likely location and magnitude of pyrethroid residues in the sediment and water column of US water bodies. It will also provide additional value for risk assessment by increasing understanding of the potential for runoff and drift transport of sediment and agricultural chemicals for many different types of crops and will help put standard modeling scenarios into a national context.

410 Beyond the screening level: higher-tier ecological risk assessments of pyrethroid insecticides

J.M. Giddings, Compliance Services International; P. Hendley, Phasera Ltd.; M.G. Dobbs, Bayer CropScience / Ecotoxicology Group; C.M. Holmes, A. Ritter, Waterborne Environmental, Inc.; M.F. Winchell, Stone Environmental, Inc. / Senior GIS Specialist; G. Mitchell, FMC Agricultural Solutions; K.S. Henry, Syngenta Crop Protection, LLC / Ecological Sciences

Conservative screening-level approaches to assessing the risk of pyrethroid insecticides to aquatic biota suggest the possibility of concern for some taxa under some use scenarios. To address these concerns, the Pyrethroid Working Group is conducting higher-tier risk assessments that incorporate refined analysis of aquatic exposure and ecological response. Refined exposure analysis is proceeding along six paths: (1) use of more relevant models to incorporate the strong adsorption behavior of the pyrethroids and the resulting importance of sediment transport and deposition; (2) probabilistic modeling to account for uncertainty and variability in critical exposure parameters; (3) a nationwide exposure modeling analysis of the potential for pyrethroid runoff and drift into individual stream reaches and catchments; (4) simulation of processes affecting exposure in flowing water, especially sediment movement and chemical pulses; (5) high-resolution landscape modeling of flowing and static water bodies in selected vulnerable watersheds; and (6) modeling of pyrethroid runoff from urban residential neighborhoods. Higher-tier assessment of aquatic effects includes (1) analysis of the extensive database of single-species ecotoxicology data (including Species Sensitivity Distributions); (2) results from microcosm and mesocosm studies; (3) modeling of invertebrate population responses to dosing and potential indirect effects on fish; and (4) bioassessments of urban stream communities. This presentation will provide an overview of the assessments and discuss the likelihood of pyrethroid exposure and ecological effects in agricultural and urban environments.

411 Methodology for Derivation of Pesticide Sediment Quality Criteria for the Protection of Aquatic Life

K.J. Trunnelle, UC Davis / Department of Environmental Toxicology; T.L. Fojut, Central Valley Regional Water Quality Control Board / Environmental Toxicology; R.S. Tjeerdema, University of California – Davis / Environmental Toxicology

Sediment toxicity caused by pyrethroid pesticides has been documented throughout California, as well as other areas across the United States. In order to evaluate sediment pyrethroid concentrations in ambient sampling and set objectives that will be protective of aquatic life, numeric sediment quality criteria for pyrethroids are desired by regulators. Sediments represent an integral component of aquatic ecosystems that provide habitat and food sources for aquatic life. Although many international and local governments have regulations in place to protect aquatic life, the majority of these are focused on or are based upon criteria for conserving water quality, and these have been in place for many years. After release to the aqueous environment, however, pyrethroids and other hydrophobic organic chemicals tend to accumulate in sediments, where they may cause toxicity to aquatic life, even when water quality criteria are met. Sediments comprise a complex medium and pose unique challenges for those who would like to develop single numeric concentrations below which aquatic life is protected. The goal of this project is to develop a methodology for derivation of numeric pesticide sediment quality criteria for the protection of aquatic life. This project was focused on the Sacramento and San Joaquin River basins of California, but the methodology is applicable in all freshwater environments. The new methodology, termed the University of California Davis Sediment Methodology (UCDSM), utilizes data from spiked-sediment toxicity tests to derive criteria, similarly to methods for deriving water quality criteria. Because there are much fewer toxicity test data available for sediments than for water, the UCDSM draws on knowledge and data from aqueous toxicity tests and criteria derivation methodologies. Field-collected sediments are more heterogeneous and complex than ambient waters; therefore, accounting for bioavailability is necessary for both sediment criteria derivation and compliance sampling. The UCDSM was used to derive bioavailable sediment quality criteria for bifenthrin, esfenvalerate and permethrin, which are pyrethroid pesticides of particular concern in the Sacramento and San Joaquin River basins. The derived BSQC were compared to pyrethroid sediment concentrations measured in California to determine where mitigation measures may be needed.

412 National surface water vulnerability assessment of agricultural pyrethroid use on multiple crops for higher tier exposure modeling

C.M. Holmes, Waterborne Environmental, Inc.; J. Amos, Waterborne Environmental, Inc.; M. Cheplick, A. Ritter, Waterborne Environmental, Inc.; P. Hendley, Phasera Ltd.; M.G. Dobbs, Bayer CropScience / Ecotoxicology Group; S.H. Jackson, BASF Corporation; J.M. Giddings, Compliance Services International

Pyrethroids have been used on numerous agricultural crops across the United States to control insect pests and improve food yield and quality for over two decades. This presentation outlines a national scale assessment to characterize the potential for pyrethroids to enter flowing surface waters in order to identify, characterize and prioritize watersheds for regulatory exposure modeling as part of the USEPA Registration Review process. Over two million catchments within the National Hydrography Dataset (NHDPlus) were characterized using geospatial data to develop metrics related to potential surface water exposure from spray drift and erosion transport of pyrethroids. The NHDPlus catchments were aggregated into larger watersheds to meet objectives specific to pyrethroid risk assessments (e.g., watershed area, stream flow) for national ranking. Other datasets used included USDA NASS Cropland Data Layer, NRCS Soil Survey Geographic database, USGS National Elevation Dataset, and national pyrethroid use estimates. Metrics generated included modeled estimates of pyrethroid mass deposition from applications made near surface water (AgDRIFT®) and pyrethroid mass potentially entering surface water via erosion from treated fields within the catchment (PRZM). Modeling results were modified based on the amount of crop with each catchment and their proximities to the water bodies as well as estimates of the fraction of crop treated with pyrethroids. National watershed ranking was performed using average annual pyrethroid mass transported to surface water in addition to potential seasonal loadings. Estimates were made considering individual crops as well a combination of all crops on which pyrethroids may be used. Ultimately, national rankings were used to identify watersheds representative of high crop-specific exposure scenarios across the US. This national exposure estimate is part of a comprehensive suite of aquatic exposure assessments for pyrethroids (Ritter et al., same session) and will be a key component of a full ecological risk assessment (Giddings et al., same session). In addition, this multi-crop national assessment will generate comprehensive national estimates of the distribution and location of various crops within agricultural watersheds and will therefore allow the standard scenarios used for preliminary pesticide exposure assessments to be put into context.

413 Optimization of a surface wiping method for estimating runoff-transferable pesticide residues from impervious surfaces

R. Reif, University of California Riverside / Department of Environmental Sciences; J. Gan, University of California Riverside / Environmental Sciences / Department of Environmental Science

Residues from pesticides (pyrethroids, fipronil, organophosphates and metabolites) are frequently found in residential runoff as a result of their current and past use for pest control. Recent studies have showed that >80% of pesticides in runoff water are associated with suspended particles >0.7 µm. In outdoor environments, hard surfaces such as concrete from driveway, sidewalk or street are normally covered with dust particles which may act as carriers of pesticides. Therefore, the availability of simple and noninvasive methods to estimate the contribution of loose particles to the pesticide load in urban runoff is crucial. Although vacuuming is also an efficient method, surface wiping could be more portable, rapid, foulproof and inexpensive. In this study, we tested wipes made of different materials, as well as the solvent used to pre-wet the wipe. To carry out the study under controlled conditions, 10 concrete slabs (60 × 40 cm) were prepared to simulate urban impervious surfaces, and dust previously contaminated with pesticides at environmentally relevant concentrations was then applied onto the surfaces. Each slab provided surface area enough for 6 different trials. After wiping, pesticides were extracted and quantified. One of the parallel slabs was vacuumed and the collected dust particles were similarly analyzed for pesticide levels. The performance of the surface wiping method was statistically compared against the results of the surface vacuum method. The end product of this work is a standardized and optimized surface wiping method that can be easily adopted for estimating runoff-transferable pesticide residues on hardscapes in the urban environments.

414 Pyrethroid and organochlorine pesticides in sediments and biota of the Lagunas de Chacahua National Park, Mexico

C. Chapa Balcorta, Universidad del Mar / Oceanología; J. Gan, University of California Riverside / Environmental Science; J. Fang, University of California, Riverside / Environmental Science; R. Guerra, Universidad del Mar / Resources Institute

The "Lagunas de Chacahua" National Park is an area of great importance for supporting wildlife as well as human communities. Recognized as a RAMSAR site, this place is the oldest protected area in Mexico. However at its surroundings, there has been heavy pesticide use for both agriculture and malaria control. Chlorinated pesticides and pyrethroids are among the used compound groups in the area and could pose a risk for the native and migratory biota as these pesticides may bioaccumulate. To assess the pesticide levels for the first time in this area, samples of sediment and tropical mussel (*Mytella strigata*) tissues were collected at multiple sites inside the lagoons (Chacahua, Pastoría and Palmarito), over a one-year time span. Of the 23 compounds analyzed, only 14 were detected in sediment samples, of which Bifenthrin was the most abundant (0.13- 722 ng g^{-1}) while pp-DDE was the pesticide detected at most sites. Concentrations were higher during summer compared with the winter season. 19 pesticides were detected in the mussel samples with highest occurrence and concentration during winter. Biphenthrin presented the highest concentrations (0.1-264 ng g^{-1}), while Alachlor was the most abundant organochlorine pesticide, ranging from 0.1- 245 ng g^{-1}. Additionally, Deltamethrin was the compound with the highest bioaccumulation factor (116). As *Mytella strigata* is a shellfish consumed both, by wildlife and human communities, it is a source of pesticide exposure, and might result in further environmental and health impacts.

What Do We Know About the Ecological Risk of Personal Care Product Ingredients?

415 Evaluation of down-the-drain modeling approaches for the assessment of triclosan inputs to freshwater aquatic environments

A.L. Perez, Cardno ChemRisk; A.J. Slocombe, M.G. Nelson, L.A. McCarthy, K.M. Unice, Cardno ChemRisk, LLC

Triclosan (TCS) is a high-volume use antimicrobial chemical used in numerous consumer products. For the past decade, TCS has been detected at variable concentrations in waste streams and freshwater aquatic systems in the US which creates challenges for ecological risk assessment. In the current analysis, we investigate the effectiveness of down-the-drain predictive models E-FAST and iSTREEM, which are designed to estimate chemical concentrations found in environmental waters and doses to aquatic life for use in a screening level assessment. Modeled values for TCS were compared with over 2200 reported field concentrations from fresh water systems collected from 2000 through 2012 in the US reported as effluent-impacted and as environmental with no information regarding inputs from waste streams. Measured TCS concentrations in effluent-impacted waters ranged from less than the limit of detection (LOD) to 2.3 µg/L (median 0.036 µg/L), while environmental concentrations ranged from below the LOD to 1 µg/L (median 0.00036 µg/L). The central tendency predictions of the iSTREEM model agreed well with the environmental distribution from our data and the effluent-impacted corresponded more closely to the low-flow rate distributions. For the input parameter distributions used in iSTREEM, the median concentrations for best and worst case scenarios ranged from below current technically feasible LODs to 0.009 µg/L for low-flow rates. Aquatic exposure estimates from E-FAST ranged from 0.0004 to 2 µg/L for the 50th percentile and from 0.01 to 46 µg/L for the 10th percentile and generally overestimated measured TCS concentrations. This analysis showed that flow rate was an important determinant of TCS concentration in environmental waters, and a potential contributor to observed exceedances of the no observable effect concentration (NOEC) of 0.5 µg/L for algal growth.

416 SSD Analysis of High Quality Triclosan Chronic Ecotoxicity Data – Roles of Data Quality and Quantity to Derive Appropriate PNECs

S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization; G. Carr, The Procter & Gamble Company / Quantitative Sciences; M.C. Capdevielle, Colgate-Palmolive Company / Environmental Occupational Health & Safety Dept.; P. De Leo, American Cleaning Institute; S. Pawlowski, BASF, BASF SE; C. D'Ruiz, Henkel Beauty Care / R&D/North America Regulatroy Affairs; B. Montemayor, Canadian Cosmetic, Toiletry and Fragrance Association

Triclosan (TCS) is an anti-microbial chemical used in a wide array of consumer, health and beauty product applications. It is widely dispersed in aquatic environments, is routinely monitored as a archetypical personal care product ingredient and is often characterized as a "Chemical of Emerging Concern". Environmental hazard and risk assessments of TCS have repeatedly been the subject of investigations since its introduction into the market in the 1970s and by the early 2000s sufficient chronic ecotoxicity data had been generated to develop Species Sensitivity Distributions (SSDs) making it one of the more well studied High Production Volume chemicals. Published SSDs and Predicted No Effect Concentrations for ecosystems have routinely used highly inclusive toxicity data collections resulting in PNECs that vary by approximately an order of magnitude (approximately 100-800 ng/L using probabilistic approaches). In this talk, we review the available chronic toxicity data that has been used in various contexts and apply stringent quality and validity criteria for inclusion in the SSD. Explanations of outcomes over time will be put into context with a current, robust SSD HC5 (Hazardous Concentration to 5% of species) of 534 ng/L using current SSD-generating tools. Monte-carlo simulations, leave-one-out, and add-one-in statistical analyses were used to inspect the potential of the present HC5 to be altered by generating additional chronic toxicity data and we will generalize the observed phenomena by comparison to other, well studied chemicals with robust Species Sensitivity Distributions (e.g., linear alkylbenzene sulfonate, zinc pyrithione, and C16/17 High Solubility Alkyl Sulfate). We conclude the latest triclosan HC5 is the most robust available, will only marginally benefit from additional ecotoxicity data for the aquatic compartment and will be useful in environmental risk assessments of TCS globally.

417 The effect of pH on the toxicity and uptake of triclosan: can we predict intracellular distribution?

C. Rendal, Unilever / Safety and Environmental Assurance Centre; J. Roberts, Unilever; O. Price, Unilever / Colworth Science Park; N. Bettles, Unilever / Safety and Environmental Assurance Centre; R. van Egmond, Unilever / SEAC

The environmental effects of the common antibacterial agent triclosan have been a subject of focus over the past decade due to its widespread use in personal care products. The compound is highly toxic to aquatic organisms and accumulates in biota. Furthermore, triclosan is a weak acid and is known to exhibit pH sensitive toxicity due to differences in the uptake rate and toxicity of neutral and ionized species. Reviews of ecotoxicological data have established that freshwater micro-algae are amongst the most sensitive non-target species to triclosan; however, despite the large number of investigations, the specific mode of action in non-target species such as algae is still not fully understood. Moreover, species sensitivity distributions reveal that the sensitivity of algal species may vary by a factor of 200 from one species to another based on EC10 values from growth inhibition tests. We present the results of a series of pH specific toxicity tests with one of the most sensitive species – the freshwater microalgae *Scenedesmus subspicatus*. The results of the study confirm that the toxicity of triclosan is sensitive to pH with a measured EC10 value at pH 7.0 over four times lower than at pH 8.5, indicating that the neutral species is significantly more toxic than the ionized form. By applying a QSAR for baseline toxicity and considering the toxic ratio, the results also confirm that triclosan acts through a specific mode of action on *Scenedesmus subspicatus*. Understanding where and how triclosan accumulates can be a key factor in determining why the sensitivity of algae species is so diverse, and may help to identify where the molecular initiating event leading to an adverse outcome could occur. We apply a cell model based on the Fick-Nernst-Planck equations to investigate the bioconcentration and intracellular distribution of triclosan within the various internal organelles of the alga cell including mitochondria, chloroplasts and vacuoles. However, there are a number of challenges related the use of such a model. These challenges are mostly related to characterizing the various organelles within the cell in terms of size, lipid content, pH and membrane charge. Another complicating issue is the growth cycle of cells and the biochemical changes occurring in algae in the presence or absence of light, which may affect the uptake and internal distribution of chemicals.

418 What is the concentration of microplastics in our rivers?

A. Boxall, University of York / Environment Department; C. Johnson, R. Cross, S. Lambert, University of York; R. Williams, V. Keller, CEH

Microplastics are used widely in consumer products and following use can be released to the wastewater system. There is increasing concern over the potential adverse impacts of these materials on freshwater and marine systems. Our understanding of the levels of exposure of these materials in aquatic systems is however limited meaning that it is difficult to assess whether a risk really exists or not. The aim of this study therefore was to establish the level of exposure of microplastics in UK rivers. Initially a consumer survey was performed to identify the level of use of microplastic containing products by the UK population. Experimental studies were then performed to characterise the size of microplastics in different product types and to assess the potential for removal of these in wastewater treatment processes as well as the rate of deposition in receiving waters. The results were then used alongside the spatial exposure model LF2000-WQX model to estimate levels of microplastics across a typical UK catchment. Around 40% of the population were found to use facial scrubs resulting in a per capita emission rate of 0.02 g microplastic per day. Depending on particle size, between 31-72% of the particles were removed in a typical treatment process and mean settlement velocities in river waters ranged from 1.0 – 1.5 mm/s. When these data were used to estimate exposure, mean predicted concentrations for the catchment ranged from 2.2 – 5.3 µg/l. To the best of our knowledge, these are the first estimates of microplastic concentrations in river catchments. The results should be invaluable in establishing whether microplastics pose a risk and in the design of studies into the ecotoxicity of microplastics.

419 Global environmental exposure model applicable to any countries for predicting river concentrations of down-the-drain chemicals

M. Yamane, Y. Honda, Kao Corporation / Safety Science; T. Kawaguchi, Nihon Suido Consultants Co., Ltd.; N. Nishiyama, Kao Corporation / Safety Science

Developing countries show significant economic growth and population increase, therefore, released volume of down-the-drain chemicals is assumed to be increased continuously, and environmental risk might arise in these countries. However, it is very difficult to evaluate exposure analysis of down-the drain chemicals in developing countries because environmental exposure model does not exist in these countries. This new model is distributed hydrological model applicable to any countries and regions. Model is constructed mainly by two parts, one is the Hydro-BEAM (Hydrological River Basin Environment Assessment Model) to estimate river discharge and the other is multimedia environmental fate model to estimate chemical concentrations. The model estimates river discharge by using GIS (geographical information system) data, which can be freely downloaded from websites. GIS data includes altitude, land use, water flow direction, population density and meteorological elements such as precipitation, air temperature. The model also predicts daily chemical concentrations with spatial resolution of approximately 1 km grid. Estimated river discharge is well agreement with the observed data. River concentrations of major surfactant, LAS (Linear Alkylbenzene Sulphonate), also show good agreement with the observed data in Japan. We evaluated the exposure analysis in Java Island, Indonesia as the first targeted area using this model. The number of grid covering Java Island is 132791 and divided into 2190 river zones. Mean and 95 percentile of LAS concentrations in Java Island are higher than Japan, because the coverage of Waste Water Treatment Plant (WWTP) is very limited in this country. This model can be very useful tool for evaluating the detailed environmental exposure analysis of down-the–drain chemicals in any countries and regions, especially, for countries where a high tier model does not exist.

420 Ecological risk of the fragrance ingredient HHCB (Galaxolide) to aquatic organisms

S.A. Laessig, K. Anitole, E. Wong, F. Arnold, L. Blake-Hedges, B. Boethling, E. Clark, M. El-Zoobi, C. Lin, A. Pfahles-Hutchens, E. Sommer, USEPA; J. Koh, Ministry of Environment(MOE), Republic of Korea

HHCB is a widely used polycyclic musk fragrance ingredient present in consumer products including perfumes, cosmetics, shampoos, lotions, detergents, fabric softeners, and household cleaners. EPA recently evaluated HHCB as part of the TSCA Work Plan Chemicals risk assessments and released a draft for public comment which is currently in peer review. HHCB is imported to the US by four importers in a volume of 1 to 10 million pounds per year. The use of commercial and consumer products that contain HHCB results in a continual disposal of HHCB to municipal wastewater before release to the environment. HHCB is not readily biodegradable and binds strongly to sediment, soil, and sludge. HHCB is considered moderately persistent and moderately bioaccumulative even though it is biotransformed to less toxic, less bioaccumulative, polar metabolites. HHCB has high acute aquatic toxicity for fish, invertebrates, and algae. Chronic toxicity of HHCB in aquatic organisms is moderate to high, with effects on adult and embryo survival, behavior, respiration, and larval development in fish and invertebrates. Acute and chronic toxicity has also been tested in a variety of sediment invertebrates. Mechanistic studies in vitro and in vivo show that HHCB may cause effects by interfering with steroid hormone receptors, metabolism, and membrane transporters in fish and invertebrates. Measured levels of HHCB in wastewater, surface water, and sediment from the USGS NWIS database and the literature were used to characterize exposures in US waterways. Preliminary ecological risks were assessed by comparing levels of HHCB measured in the environment to the concentrations of concern determined from acute and chronic aquatic and sediment toxicity studies. Preliminary conclusions for aquatic and sediment toxicity from EPA's draft risk assessment of HHCB will be presented. This abstract does not represent official EPA policy.

421 The North American Environmental Risk Assessment of Major Surfactant Classes: Alkylethoxylates and Alcohols (Part 1)

S.D. Dyer, The Procter & Gamble Company / Central Product Safety; S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization; D. Versteeg, The Procter & Gamble Company / Environmental Science Department; C.E. Cowan-Ellsberry, CE2 Consulting, LLC / Central Product Safety; D.C. McAvoy, University of Cincinnati / Environmental Engineering; P. Dorn, Shell Health; H. Sanderson, Aarhus University / Environmental Science, National Environmental Research / Department of Policy Analysis; D. Ferrer, K. Stanton, American Cleaning Institute

It has been more than 20 years since environmental research for major surfactants was summarized and published. Sponsored by the American Cleaning Institute, a revised assessment of the major anionic and non-ionic surfactants, and their source alcohols, has recently been conducted. The major chemical and surfactant classes include: long-chain alcohols, alkylethoxylates, alkyl sulfates, alkylethoxysulfates, and linear alkylbenzenesulfontes. ACI and its member companies have spent no less than 30 million USD on the assessment and reporting of the environmental safety data of these substances over the past 5 decades via ACI and its predecessor association's activities. The projects span the development of analytical, modeling and sampling methods, as well as fate, effects, and monitoring studies. Well over 250 peer-reviewed and publicly available papers and reports have been published due to the efforts of ACI its predecessor associations and its member companies. This presentation will: concisely report the most relevant environmental data generated regarding long-chain alcohols and alkylethoxylates over the recent decades; demonstrate the advancement and increased understanding of the risk assessment of these surfactants and alcohols, as well as how to conduct risk assessments for categories of compounds; provide an overview of the key scientific findings; and reaffirm the industry's commitment to transparency and scientific advancement.

422 The North American Environmental Risk Assessment of Major Surfactant Classes and their Alcohols: The Anionics (Part 2)

S.D. Dyer, The Procter & Gamble Company / Central Product Safety; S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization; D. Versteeg, The Procter & Gamble Company / Environmental Science Department; C.E. Cowan-Ellsberry, CE2 Consulting, LLC / Central Product Safety; D.C. McAvoy, University of Cincinnati / Environmental Engineering; P. Dorn, Shell Health; H. Sanderson, Aarhus University / Environmental Science, National Environmental Research / Department of Policy Analysis; D. Ferrer, K. Stanton, American Cleaning Institute

Surfactants are amongst the largest volume materials that are used by consumers and disposed of down the drain. It has been more than 20 years since environmental research for major surfactants was summarized and published. Sponsored by the American Cleaning Institute, a revised assessment of the major anionic and non-ionic surfactants, and their source alcohols, has recently been conducted. The major chemical and surfactant classes include: long-chain alcohols, alkylethoxylates, alkyl sulfates, alkylethoxysulfates, and linear alkylbenzenesulfontes. ACI and its member companies have spent no less than 30 million USD on the assessment and reporting of the environmental safety data of these substances over the past 5 decades via ACI and its predecessor association's activities. The projects span the development of analytical, modeling and sampling methods, as well as fate, effects, and monitoring studies. Well over 250 peer-reviewed and publicly available papers and reports have been published due to the efforts of ACI its predecessor associations and its member companies. This presentation will: concisely report the most relevant environmental data generated regarding the anionic surfactants (alkyl sulfates, alkylethoxysulfates and linear alkylbenzensulfonates) over the recent decades; demonstrate the advancement and increased understanding of the risk assessment of anionic surfactants, as well as how to conduct risk assessments for categories of compounds; provide an overview of the key scientific findings; and reaffirm the industry's commitment to transparency and scientific advancement.

Fate, Transport, and Toxicity of Wastewater-borne Contaminants

423 Overview of the Fate, Transport, and Toxicity of Wastewater-borne Contaminants

G.S. Toor, University of Florida, Gulf Coast Research & Education Center / Soil & Water Science Department

The remarkable increase in human life-span over the last century would not have been possible without the discovery and use of chemicals. These discoveries have had tremendous benefits to our society. In the last few decades, there has been increasing concern of existence of many chemicals and their associated environmental impacts. As a result, a plethora of studies detected these chemicals (now known as emerging contaminants or contaminants of emerging concern) in a variety of environmental media such as water,

sediments, plants, non-target aquatic and terrestrial organisms. We now recognize that these chemicals are present in most environmental matrices and our improved methods of analyses allow us to detect the small concetrations (ng to ug/L) of these chemicals. We know that some chemicals are naturally degraded while others resist degradation and can exist for a longer period of time in environmental matrices. A variety of surface and sub-surface pathways of contaminant transport exist. It is recognized that domestic wastewater is a rich source of these chemicals in our waterways. Wastewater in most of the world is largely managed in two ways: (1) decentralized treatment (mostly in rural areas) where wastewater is collected from an individual house or business, treated, and dispersed onsite in groundwater via soil profile by onsite wastewater treatment systems (commonly called septic system) and (2) centralized treatment where wastewater from large population (city, county) is collected, treated, and discharged in surface waters via streams/rivers by wastewater treatment plants. Thus, situations where these emerging contaminants can be more concentrated are aquatic systems as most of the treated wastewater is discharged in nation's surface water bodies and terrestrial systems (soil) that receive regular inputs of bio-solids and recycled wastewater. Research shows that several of these contaminants can be toxic to aquatic organisms. Some research shows that some plant species can bioaccumulate these contaminants. This overview presentation will set the stage for this symposium and articulate our major known and unknowns as related to fate, transport, and toxicity of wastewater-borne contaminants.

424 Reuse of Decommissioned Wastewater Facilities Supplied with Reclaimed Water for Aquaculture

S. Mims, R. Cuevas, Kentucky State University

The use of reclaimed water for aquaculture is an innovative approach to secure food production through the integration of decommissioned wastewater treatment plants with sustainable agricultural technologies. With better methods for processing wastewater, many municipalities are now building new, larger facilities, and decommissioning the old ones, many of which have tanks and ponds that could be converted for fish culture. Many are being needlessly demolished when recycling them as fish production facilities could save the community demolition costs, create new jobs and generate revenue. The new facilities are being built adjacent to the old, and would allow reclaimed water to be used for aquaculture. One concern of using reclaimed water for food production is the chemicals that could be present in the water especially persistent organic pollutants such as endocrine-disrupting compounds. There are different methods for the treatment of reclaimed water. During the final treatment stage, water could be disinfected by ozone and/or ultraviolet light (UV). This presentation will discuss the goal of this integrated project to use reclaimed water for aquaculture enterprises for rural municipalities and their limited resource farmers. Specific objectives are to evaluate and compare fish production, water quality and bioeconomics using ozone-treated, UV-treated, and UV combined with ozone-treated reclaimed water technology and to biomonitor fish for contaminants using reclaimed water.

425 Degradation of halogenated pollutants by anaerobic bacteria in sewers

S.L. Capozzi, Rutgers, the State University of New Jersey; L.A. Rodenburg, Rutgers University / Department of Environmental Sciences; J. Guo, Rutgers, the State University of New Jersey / Department of Environmental Science; D. Fennell, Rutgers University / Department of Environmental Sciences; A.J. Murphy, Rutgers, the State University of New Jersey

Recently we used monitoring data on concentrations of polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in wastewater treatment plant influents and effluents to demonstrate that these classes of contaminants are extensively dechlorinated somewhere within the wastewater collection system, presumably by anaerobic bacteria in sewer sediments. Here, we present data from model sewer systems consisting of sewer sediment spiked with PCBs, PCDD/Fs, as well as several flame retardants. Sewer microcosms were maintained under methanogenic conditions. Measurable dechlorination of PCBs was observed after 35 days, with no apparent lag time. The results suggest that sewers serve as an anaerobic pretreatment zone where recalcitrant halogenated pollutants are dehalogenated by bacteria. In the case of PCBs, this dehalogenation reduces the dioxin-like toxicity of the PCB mixture by up to 96% and produces low molecular weight products that can be degraded aerobically in the wastewater treatment plant or can volatilize and then be destroyed by reactions with hydroxyl radicals in the atmosphere.

426 Fate, Transport, and Toxicity of Wastewater-borne Contaminants by Example of Widely Used Persistent Antimicrobials

R.U. Halden, Arizona State University / Center for Environmental Security, The Biodesign Institute at Arizona State University

Contaminants of Emerging Concern (CECs) such as pharmaceuticals and personal care products (PPCPs) are in widespread use in the US and abroad. A particularly large and steadily growing market segment within this group of compounds are antimicrobials added to consumer products of daily use. This work explored the fate, transport and toxicity of two antimicrobial agents, specifically triclosan and triclocarban, as well as related mass-produced industrial chemicals that are subject to disposal down the drain. The study provides an overview of the current state of knowledge concerning the environmental fate and behavior of persistent antimicrobial agents. A meta-analysis approach of peer-reviewed research studies was employed to identify principal environmental pathways and to rank them in order of relevance to human exposure. In addition, an environmental safety assessment was conducted to pinpoint particular ecological and human health risks and to identify intervention opportunities to meet public health objectives in an environmentally responsible and sustainable fashion.

427 Uptake of pharmaceutical and personal care products by algae, cyanobacteria, and duckweed

J. Richards, University of California Riverside; J. Gan, University of California Riverside / Environmental Sciences / Department of Environmental Science

Based on surveys conducted between the years of 2005-2008, almost half of the USA population had used at least one prescription drug in the past month and this percentage is expected to increase in the future. Increased pharmaceutical and personal care product (PPCP) usage will cause the amount of PPCPs in the environment to increase due to inefficient removal from conventional wastewater treatment processes. Natural and constructed systems such as wetlands or ponds have been shown to effectively remove PPCPs from wastewater, and plant uptake has been theorized as a major component of this removal. However, these studies often do not quantify PPCP uptake and have largely ignored algae and duckweed despite their ubiquitous nature and importance as food sources in aquatic ecosystems. The objectives of this study were to: 1) Quantify the bioaccumulation of PPCPs with a wide range of properties by algae, cyanobacteria, and duckweed and 2) determine the importance of factors such as lipid content, cell biovolume, and cell surface area on the bioaccumulation of different PPCPs. In a growth chamber, cultures of two green microalgae, *Chlamydomonas sp*. and *Chlorella vulgaris,* the cyanobacteria *Microcystis aeruginosa* and the aquatic macrophyte *Lemna minor* were spiked with ^{14}C labeled carbamazepine, bisphenol A, sulfamethoxazole, or naproxen. Sampling occurred at 1, 6, 12, 24, and 96 hours for analysis of ^{14}C activity in the nutrient solution and lipid content, ^{14}C activity, cell surface area, and cell biovolume of the tissue samples. Bioaccumulation factors were calculated and relationships between lipid content, cell surface area, cell biovolume, and PPCP uptake were evaluated.

428 Fate and Toxicity of Perfluorochemicals to Aquatic and Terrestrial Organisms in a Wastewater-dominant System

I.A. Rodriguez-Jorquera, University of Florida / Soil and Water Science; G.S. Toor, University of Florida, Gulf Coast Research & Education Center / Soil & Water Science Department; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology

In wastewater dominant systems, endocrine disrupting compounds (EDCs) are known to cause toxic effects at very low concentrations to aquatic and terrestrial organisms. One commonly found emerging contaminant in waterways is the group of perflurochemicals (PFCs). We investigated the occurrence of PFCs in an urban stream that enters a wildlife refuge and the effects exerted by environmental relevant concentrations of PFCs on gene expression in wildlife (fish and birds). Alteration in gene expression, e. g. gene expression profiling, measured using microarrays, allows early detection of toxic effects and elucidation of particular mechanisms of action caused by chemicals; establishing links between toxicants and effects. We detected PFCs in all the water samples, collected downstream from a wastewater treatment plant, along the stream. The dominant PFCs were PFOS (Perfluorooctanesulfonic acid), PFOA (Perfluorooctanoic acid), and PFHxA

(Perfluorohexanoic acid). The effects of PFCs on gene expression were determined in fathead minnows and red-winged blackbirds using microarrays. We observed that these waters contain PFCs in the 300 ng/L concentration range which exerted effects on fish gene expression related with cholesterol metabolism and DNA repair. To confirm these findings, we exposed fathead minnows during 48 h through the water to three diferent PFOS concentrations ranging from 0 to 10000 ng/l and a mixture of PFCs. In addition, to understand the effects of PFCs on terrestial biota, we exposed red-winged blackbirds during 96 h through feed to three PFOS concentrations ranging from 0 to 700 ug/kg and a PFCs mixture; in an attempt to mimic common concentrations of PFCs found in these waters. No mortality was detected in either fish or bird exposures. A drop in food consumption was observed in all birds except for the control group (paired t-test; $p < 0.01$), with more decrease in birds fed the mixture of PFCs and in the high PFOS dose group (p=0.0006 and 0.000002, respectively). Further, all treated birds showed weight loss when compared with pre- exposure weights; this was greatest in the high dose treatment (paired t-test; $p < 0.0002$). Concetrations of PFCs normally found in these waterways are high enough to alter gene expression profiles in fish and birds. These profiles fit into adverse outcome pathways suggesting effects on survival and reproduction of these organisms.

429 Persistence and Mobility of PPCP/EDCs in Soil Exposed to Reclaimed Water and Biosolids

J. Gan, University of California Riverside / Environmental Sciences, University of California, Riverside / Department of Environmental Science; L. Dodgen, University of California Riverside / Environmental Science

The United States reuses 9.5×10^{11} gallons (3.6×10^{12} liters) of treated wastewater and 4.2×10^{6} tons (3.8×10^{9} kilograms) of biosolids each year for agricultural, landscape, industrial, and other applications. However, these materials often contain unregulated organic contaminants, such as pharmaceutical and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) at ng/L – mg/L concentrations. When agricultural fields are irrigated with reclaimed water or amended with biosolids, PPCP/EDCs have shown the potential to translocate into the soil matrix, where compounds will vary in persistence and mobility due to their individual physical and chemical properties and according to environmental conditions. PPCP/EDC persistence and mobility in soil will be examined using a general overview of pertinent literature and a case study of a suite of PPCP/EDCs spiked into four soils.

430 Wastewater-borne Nitrogen Transport from Septic Drainfields to Groundwater

M. De, University of Florida / Soil and Water Quality Laboratory, Gulf Coast Research and Education Center; G.S. Toor, University of Florida, Gulf Coast Research & Education Center / Gulf Coast Research & Education Center, University of Florida / Soil & Water Science Department

The knowledge about the fate and transport of nitrogen (N) below drainfields of onsite wastewater treatment systems (OWTS) is needed to protect ground- and surface- water contamination, especially in areas with porous sandy soils and shallow groundwater such as Florida. Our overall goal was to study the dynamics of wastewater-borne N transport in the vadose zone and groundwater of three OWTS. These OWTS (6-m length, 0.6 m width) included two most common systems (drip dispersal, gravel trench) and an advanced system with in-built aerobic (lingo-cellulosic) and anaerobic (sulfur) media for enhanced N attenuation. Each system received 120 L of septic tank effluent (STE) per day (equivalent to 3 L/ft^2/day). From May 2012 to May 2013, soil-water samples were collected from the vadose zone using suction cup lysimeters installed at 0.30, 0.60, and 1.05 m depth and groundwater samples were collected from piezometers installed at 3-3.30 m depth below the drainfield. The pH and EC (n = 52) was greater in STE (6.60-7.72; 0.95-1.33 dS/m) than soil-water (6.25-6.88; 0.49-0.78 dS/m) and groundwater (4.19-4.78; 0.29-0.41 dS/m). Median concentrations of chloride in soil-water samples were 68-75 mg/L in gravel trench and drip dispersal, which were slightly lower than STE (83 mg/L). Most of the N in STE was NH_4-N (42-66 mg/L), followed by organic N (5-8 mg/L), and NO_3-N (0.03-0.14 mg/L). However, NH_4-N in soil-water and groundwater was < 1.0 mg/L; suggesting that >99% of NH_4-N was converted to NO_3-N in the vadose zone. NO_3-N increased in both drainfields suggesting rapid nitrification. In advanced system, NO_3-N input to vadose zone was < 0.15 mg/L as compared to >20 mg/L in other two systems. This suggests >98% of N removal in advanced system as compared to other systems (~50%). Median NO_3-N (after plume development) in groundwater beneath gravel trench (10-12mg/L) and drip dispersal (13-20 mg/L) exceeded EPA drinking water limit of 10 mg/L, suggesting shallow groundwater contamination of Floridian aquifer. Our ongoing research includes determining mass balance of water and N, kinetics of N mineralization, characterization of organic N, elucidation of microbial pathways of N transformation, and modeling of N transport to groundwater in OWTS.

Ecological Consequences of Exposure to Pharmaceuticals: From the Laboratory to the Field

431 Tailored risk assessment of pharmaceuticals – the regulatory perpective

G. Maack, Federal Environment Agency / Ecotoxicological Assessment; J. Bachmann, Federal Environment Agency (UBA); A. Hein, Federal Environment Agency (UBA) / Pharmaceuticals, washing and cleansing agents, nanomaterials; J. Schoenfeld, Federal Environment Agency (UBA)

The authorization of human and veterinary pharmaceutical products requires the assessment of possible environmental risks before market authorisation. The procedure to assess the environmental risks is defined by several guidelines and background information, e.g., "question & answer" documents by the European Medicines Agency (EMA). Often there is the impression that for regulation well established but antiquated methods and approaches are used. Established and validated methods are the basic prerequisite for planning and legal reliability, meaning that not every scientific approach can be included in regulatory risk assessment immediately. Providing that sound knowledge about specific Mechanisms of Action (MoA) of the active ingredients of pharmaceuticals is available, the guidelines allow already leaving the standard procedure and using a tailored approach instead. Candidate groups suitable for tailored approaches are, e.g., steroidal hormones, progestins, anti cancer drugs with anti-androgenic mechanism of action and parasiticides for pasture animal treatments respectively. These are examples of substance groups with specific mechanisms of action, which need to be reflected in an environmental risk assessment. So are, e.g., for steroidal hormones the standard tests, a Fish Early Life Stage Test (OECD 210) for human pharmaceuticals or a Fish Acute Test (OECD 203) for veterinary medicinal products, not sufficient. Full or modified fish life cycle tests can be used as adequate methods to evaluate the relevant endpoints and adequate effects caused by the test substances in the aquatic environment. Similar with parasiticides; here tests with dung fauna species are necessary to adequate assess the environmental impact. A tailored approach does not only mean the necessity of additional tests, but depending on the knowledge about the Mode of Action a waiving of individual tests should be possible. However these tailored approaches are "case-by-case" decisions and not a standard procedure. Applicants are asked to contact the respective authority before the formal procedure of application is started to prevent unnecessary time conflicts and potential refusing of a market authorization. The German Federal Environment Agency favours a strategy for specific active ingredients, where a tailored environmental risk assessment reflects a realistic assessment of potential environmental impact. We present examples of tailored approaches inside and outside the standard procedure.

432 Ecological risk assessment of pharmaceuticals in Korea – Results of five year research between 2008 and 2012

K. kim, Seoul National University of Science and Technology / School of Public Health; S. Lee, S. Kim, Seoul National University / School of Public Health; K. Ji, Yongin University / Department of Occupational and Environmental Health; Y. Kho, Eulji University / School of Human & Environmental Sciences; J. Park, Soonchunhyang University / College of Natural Sciences; K. Choi, P. Kim, National Institute of Environmental Research; K. Choi, Seoul National University / School of Public Health

Pharmaceuticals in water environment have been of significant environmental health concern in Korea. We have conducted a five year research on ecological risk assessment of major pharmaceutical residues in Korean waterway. In order to identify priority pharmaceuticals of which ecological risks should be assessed, risk based prioritization was performed employing available toxicity and occurrence information. Twenty two pharmaceuticals were chosen as target compounds. Both acute and chronic toxicity tests employing algae, waterflea, and fish were conducted to derive predicted no effect concentrations. Ecological risk assessment showed that most of the target pharmaceuticals would cause miniscule risks. Hazard quotients based on

measured environmental concentrations were the highest for fenbendazole, followed by neomycin, mefenamic acid, ibuprofen, and chlortetracycline, but none of HQs was greater than one. Several research priority areas were identified for the second round of five year research. These included (1) development of prioritization scheme, (2) derivation of environmental criteria, (3) multi-generation toxicity assay for compounds of potential risks, (4) mixture toxicity, (5) risks from concentrated animal feeding operations, and (6) antimicrobial resistance buildup.

433 A tale of two studies: Examining the link between laboratory data and field experiments on the impacts of a major pharmaceutical in effluent

R. Klaper, N. Niemuth, N. Neureuther, University of Wisconsin-Milwaukee / School of Freshwater Sciences

Several laboratory studies have examined the impacts of single pharmaceuticals or other emerging contaminants in the laboratory at the individual or population scale. There is a question as to how relevant this information is to a field setting and whether the same impact may be seen in field organisms. This presentation will discuss our work on the individual and mixture impacts of several of the major emerging contaminants of sewage effluent in Milwaukee on the model fish the fathead minnow (Pimephales promelas). We then compare our results to those seen in wild populations of perch (Perca flavescens) in Lake Michigan. We examine chemical accumulation with respect to multiple endpoints including hormone levels, histology and reproduction and describe the links with individual and global gene expression in laboratory and field experiments and demonstrate the linkages or absence of links among these endpoints in some cases with field samples. Logistical details regarding sample size, timing of collection, population variation and their impact on interpretation will be discussed.

434 The Effects of Select Pharmaceuticals on Fish Reproduction

L. Beyger, University of Ontario Institute of Technology; J. Guchardi, University of Ontario Institute of Technology / Faculty of Science, Aquatic Toxicology; D.A. Holdway, University of Ontario Institute of Technology / Faculty of Science, Aquatic Toxicology

Non-steroidal anti-inflammatory drugs are the most frequently detected pharmaceuticals in the environment due to their high level of use and ease of access (Santos *et al.,* 2010). As such, the study of environmentally relevant concentrations of such pharmaceuticals on fish reproduction is important. A study was conducted using Florida flagfish (*Jordanella floridae*) breeding harems to monitor the reproductive effects of exposure to ibuprofen (0.1 µg/L), naproxen (0.1 µg/L), and 17α-ethynylestradiol (10 ng/L) alone, and in a mixture. Flagfish were set-up into breeding harems with 2 males and 4 females per treatment and each treatment was run in triplicate alongside controls. The experiment was conducted in 70L aquaria with pre-exposure (21-d), exposure (21-d), and post-exposure (7-d) phases during which the reproductive endpoints were monitored. Of particular interest were the reproductive endpoints of fertilization, hatchability and egg production. There was a significant decrease ($p \leq 0.05$) in fertilization for flagfish exposed to 0.1 µg/L naproxen, and 10 ng/L 17α-ethynylestradiol. Hatchability and egg production showed no significant effects from exposure to the pharmaceuticals. While reproductive effects are well known for 17α-ethynylestradiol, to the best of our knowledge the effects of naproxen on fertilization at the environmentally relevant concentration of 0.1 µg/L has not previously been noted. This finding indicates that more research is necessary into the potential reproductive effects of naproxen on fish and other aquatic organisms.

435 Population failure in fish from estrogen contaminated environments is regulated by direct and parental effects on survival and fecundity

A. Schwindt, Colorado State University / Fish, Wildlife, and Conservation Biology; D. Winkelman, Colorado State University

Increased demands on freshwater ecosystems throughout the arid western USA are coincident with an increasing human population. For example, in northeastern Colorado flow in the South Platte River is up to 90% wastewater effluent most of the year. The effluent contains a complex mixture of chemicals that can disrupt hormone systems in fishes. One component of the effluent, 17α-ethinylestradiol (EE2) the synthetic estrogen in birth control, is known to disrupt fish reproduction. While the effects of EE2 on individuals are well known, the consequences for populations are just now being assessed. For these studies we evaluated the effects of EE2 exposure on fathead minnow (*Pimephales promelas*) populations over the course of three generations. We constructed 28, 1100L aquatic mesocosms to house the fish populations at the Colorado State University Foothills Fisheries Laboratory. In the F0 generation, each mesocosm received five male and five female fish exposed to measured EE2 concentrations ranging from 0, 3.2, 5.8, and 10.9ng/L for 102 days (n=7). Survivorship and the numbers of eggs, embryos and offspring were quantified. Following the mesocosm study the F1 fish were divided into two groups. One group continued to receive the concentration of EE2 in the mesocosm and in the other the exposure was stopped. F1 reproductive output was assessed after 203 days and survival was estimated in the F2 fish. The effects of EE2 exposure differed depending on the life stage and exposure history. In the F0 generation EE2 reduced adult male survival but had little effect on reproduction. In the F1 generation, fish allowed to recover from EE2 exposure displayed reduced reproductive capacity. In F1 fish subjected to lifetime exposure no successful reproduction was evident. More striking were the effects on the F2 generation. Survival of the F2 fish whose parents were exposed early in life was significantly less than the F2 fish whose parents were exposed only as adults. The effects of EE2 on the F2 generation resulted from exposure through the parental germ line. EE2 exposure may cause population failure by several mechanisms and even though fish may no longer be directly exposed to EE2, effects are still evident.

436 Anatomical, Behavioral, and Molecular Responses of Minnows Exposed to Pharmaceuticals Using Field and Laboratory Approaches

H.I. Schoenfuss, D.C. Rearick, St. Cloud State University; E.T. Furlong, US Geological Survey / National Water Quality Laboratory; M.M. Schultz, College of Wooster / Department of Chemistry; D. Martinovic, University of Saint Thomas, University of St. Thomas / Dept. of Biology, USEPA, Mid-Continent Ecology Division / University of St. Thomas / Biology; P. Phillips, US Geological Survey; S.E. Bartell, Normandale College Community College; D.W. Kolpin, USGS

Pharmaceuticals and their metabolites are present in low concentrations in most municipal treated wastewater effluents. However, pharmaceutical production can locally increase the occurrence and concentrations of some pharmaceuticals in the treated wastewater stream and ultimately receiving aquatic environment with unknown consequences. Using existing data from such an effluent, we assessed the biological potency and effects of several classes of pharmaceuticals and their mixture and conducted field and laboratory experiments. In the first experiment, larval and mature fathead minnows were exposed to environmental concentrations of several classes of pharmaceuticals including sleep aids, muscle relaxants, antidepressants, opioids, opiate agonists and their complex mixture. In the second experiment, fish were exposed to environmental effluent on site (adults) and laboratory static renewal exposures in the laboratory using serial diluted grab samples (larvae). In both studies, we examined anatomical (organosomatic indices, histopathology, reproduction), behavioral (adult nest defense aggression, larval escape performance), molecular (vitellogenin induction), and immunological endpoints (spleen and kidney flow cytometry) following 21-day exposure. Larval fish appeared unimpacted by all exposures. In contrast, laboratory exposure revealed significant differences in hepatosomatic indices (HSI) for both sexes, with a more prominent effect on female fathead minnows. Control females exhibited lower HSI compared to the opiate agonist, antidepressant, muscle relaxant, and pharmaceutical mixture. Additionally, female fish exposed to the complex pharmaceutical mixture had higher HSI than the sleep aid and opiate agonist treatments. Male mixture fish HSI were significantly higher than those exposed to the muscle relaxant. Male aggression was significantly higher for the sleep aid and control relative to other treatments. Male field exposures using on site mini-mobile units resulted in significant declines in body condition factor, HSI, and secondary sex characteristics relative to a baseline control. Vitellogenin induction was significantly higher in field-exposed fish. The study merits the importance of endpoint selection spanning multiple levels of biological organization and development when assessing contaminant interactions.

437 Contribution of PPCPs and LAS to whole toxicity of effluent-dominant urban streams and toxicity characterization approach

H. Yamamoto, The University of Tokushima / Faculty of Integrated Art & Science; Y. Yasuda, University of Tokushima; N. Nakada, V. Kumar, Kyoto University / Research Center for Environmental Quality Management; Y. Kameda, Chiba Institute of Technology / Water Quality; K. Kimura, Institute of Health

Science and Research Saitama City; N. Tatarazako, National Institute for Environmental Studies / Endocrine Disrupter Research Laboratory

Over the last three years, we had conducted chemical analyses and toxicity tests for effluent-dominant urban streams in Tokushima, Kyoto, and Saitama to determine the contribution of PPCPs and surfactants on whole toxicity of the ambient waters. Total of approximately 100 pharmaceuticals and personal care products (PPCPs) and one of most popular anionic surfactants, linear alkylbenzene sulfonate (LAS), were chemically analyzed while short-term chronic toxicity tests using three aquatic species, green alga (*Pseudokirchneriella subcapitata*), daphnia (*Ceriodaphnia dubia*), and fish (*Danio rerio*) for total of over 30 samples as well as toxicity tests for selected PPCPs/LAS. As results, some antibiotic agents such as triclosan and clarithromycin were found to contribute 7 to 40% of the whole toxicity of the water for algae while the estimated contribution of LAS and the other PPCPs are far below these compounds. In contrast, some LAS congeners contributed 3 to over 100% and 6 to 53% of whole toxicity for daphnia and fish, respectively while the contribution of PPCPs were less than 3% for all the sampling points for these species. These percentages ranged widely and differed for different sampling points and seasons, so that some other hazardous chemicals may contribute much higher than PPCPs and LAS for most of the samples. Toxicity characterization of water samples was also conducted using solid phase extraction, activated carbon, and EDTA to identify the characteristics of the major source of the ecotoxicity. We found that the pre-treatment using Oasis HLB cartridge did not decrease algal toxicity and possibly other compounds contribute higher than moderately or highly hydrophobic organics or independent mode of action might be possible for the compounds trapped by the cartridge and the fraction untrapped by the cartridges.

438 Trophic level effects in an effluent-impacted system

M.O. Minor, St. Cloud State University; D.W. Kolpin, USGS; E.T. Furlong, US Geological Survey / National Water Quality Laboratory; S.M. Bartell, Cardno Entrix; L.E. Hubbard, USGS; H.I. Schoenfuss, St. Cloud State University

The effects of wastewater treatment plant (WWTP) discharge on effluent-impacted aquatic ecosystems have been demonstrated. Previous studies have documented an impact on the water-quality of Fourmile Creek (Iowa) by WWTP discharge. The goal of this study was to examine the effects of WWTP effluent on various organismal trophic levels in Fourmile Creek. Two reference sites were chosen above the effluent outfall (-1.7 km and –1.9 km), and three below the outfall (0.3 km, 2.9 km, and 8.4 km). Field work for this study was conducted during September, 2011 and May, 2012. Water samples for each site were analyzed for a suite of 110 pharmaceuticals. Analysis of trophic levels included macroinvertebrate diversity, behavior of aquatic organisms, fish vitellogenin concentrations, and fish histopathology. Water chemistry analysis documented an increase in occurrence and concentrations of pharmaceuticals between sites above and below the outfall. Biotic indices collected suggest water quality and macroinvertebrate diversity are highest above the outfall, with a sharp decline below the outfall and progressive improvement further downstream. In addition, larval fathead minnows (*Pimephales promelas*) were analyzed after 21 days daily static renewal exposure to an effluent dilution series with no significant difference in growth or escape performance. In contrast, when water boatmen (Family: *Corixidae*) were exposed to the same effluent dilution series over a 4-day period, boatmen resurfaced more frequently than controls across all effluent treatments. Increased respiration rates are a common sign of physiological stress associated with effluent exposure. Vitellogenin concentrations and histopathological changes were also analyzed in native fish populations and caged fathead minnows with few biomarkers suggesting impact at this trophic level. This study highlights the impacts of treated wastewater effluent on macroinvertebrate diversity and physiology, which may be detrimental to the nutrient cycling of aquatic systems.

Ensuring the Quality of Toxicity Test Results

439 The role of laboratory audits for ensuring the quality of toxicity testing results

J.T. Markwiese, Environmental Standards Inc. / Environmental Assessment Department; D. Thal, TestAmerica, Environmental Standards, Inc.; R.J. Vitale, Environmental Standards

A laboratory must actively ensure that its quality system is being properly implemented and that it is achieving the required standard of quality. Quality control measures, such as method-specific demonstrations of bioassay capability, can provide feedback on the quality system but in and of themselves are not enough to ensure quality results. Audits are designed to provide an objective and thorough evaluation of quality issues affecting a laboratory by covering the entirety of the quality system. Routine audits can detect actual or potential non-conformances before they impact data quality and can even anticipate future problems. Where there are no problems, the audit results in a record that the quality assurance system has been thoroughly evaluated and found to be acceptable. The quality assurance elements reviewed in a toxicity testing audit include: quality documents such as quality management plans, quality assurance project plans and standard operating procedures; records including technician training, chain of custody, equipment calibration, environmental conditions, toxicity test data and reports and corrective actions; and, test implementation and performance. The most important, and perhaps most difficult to gauge, aspect of the testing involves evaluating the competence of laboratory personnel for routine maintenance such as culturing test organisms. For confidence in bioassay results, it is imperative to demonstrate that the organism is responding to the test substance and/or conditions and not some confounding factor (lack of acclimation to test conditions, disease, etc.). Validity of results is evaluated on a test-specific basis and efforts are underway to gauge long-term performance of testing laboratories. The information presented in this talk will be useful to a laboratory's quality assurance staff for conducting internal audits and preparing for external (e.g., accreditation) audits and for external auditors to accredit toxicity testing laboratories or inspect such laboratories on behalf of a client. Although audits typically only last from one to several days, and therefore represent a narrow window for gauging year-round laboratory operations, they offer an unparalleled means for evaluating of a laboratory's implementation of its quality assurance system.

440 Toxicity Testing: The Good, The Bad, The Ugly

P.M. Chapman, Golder Associates Ltd.

Toxicity testing provides a powerful, but imperfect, line of evidence for assessing the potential harm from chemicals individually and as mixtures in different matrices (e.g., effluent, water, sediment, tissue) as well as other stressors (e.g., temperature). The 'Good' aspects of toxicity testing include: standardized laboratory tests and organisms; variety of end-points; ease of interpretation in the laboratory; -cosm and actual environmental testing; quality assurance/quality control (QA/QC). The 'Bad' components include: stakeholder belief that conservative laboratory testing (sensitive organisms and endpoints, worst case conditions) reflects reality; excessive standardization that reinforces this belief; resistance to using other than standardized tests; endpoints (e.g., NOEC/LOEC) that are not technically defensible; not reporting full details of testing and findings (i.e., lack of transparency, not repeatable); inadequate QA/QC. The 'Ugly' aspects include: confusing ecologically relevant and non-relevant endpoints; applying simplistic testing to real world situations without a "reality check"; conducting testing because it is possible, not because it will best provide necessary information / answer the questions that need answering (e.g., not applying data quality objectives); assuming similar sensitivities for different test organisms and using organisms interchangeably without confirmatory testing; failing to conduct useful / necessary experimental testing because it is not standardized, not easy, and/or too expensive; misleading QA/QC. Each of these aspects, including the above and other examples, will be examined; recommendations to eliminate the Bad and the Ugly in favor of the Good will be provided.

441 Continuing challenges in terrestrial ecotoxicology

L. Kapustka, LK Consultancy

Two decades ago, Peter Calow edited the *Handbook of Ecotoxicology*. In the years immediately before and since then a considerable number of test guidelines have been produced by government agencies and consensus-driven standards organizations. Substantive advances made during this time

include moving away from ANOVA study designs toward regression-based tests that allow for non-linear characterization of the concentration-response relationships. There has also been considerable expansion of test organisms used; particularly extending the diversity of plant species beyond the narrow suite of agronomic species that were used traditionally in safety testing protocols for pesticide registration. Despite these considerable improvements, the field remains in many ways in its infancy. Though test procedures for population-, community-, and systems-level tests exist, there are only a handful of examples where these higher-level tests have been done. Familiar memes prevail: "they are too expensive," … too hard to interpret," … "too difficult to do at a commercial scale." Though not unique to terrestrial systems, the following concerns define the current state of the practice: Consideration of statistical power seldom enters into the design of tests. This is especially so for bird, mammal, and herpetofaunal tests. Use of data quality objectives to guide test selection and design is rare. Measurement endpoints continue to emphasize organism-level responses and often are largely (even entirely) divorced from population-, community-, or systems-level determinants. Reliance on look-up tables for "safe" concentrations threatens to eliminate most testing of contaminated soils. The talk will conclude with straight-forward recommendations to improve the quality and utility of terrestrial toxicity tests.

442 Testing difficult substances for aquatic toxicity: strategies for poorly water soluble test items

C.V. Eickhoff, Maxxam Analytics / Ecotoxicology

To function correctly, industrial and commercial chemical formulations are often made to be poorly soluble in water. For example, inks and dyes, pesticides, pharmaceutical formulations, lubricants, oils, and petrochemical-based products are necessarily insoluble in water and this desirable property makes them effective. However, this same property makes aquatic toxicity testing challenging and can result in erroneous test endpoints if steps are not taken to analyze these test items properly. Regulatory authorities and risk assessors require reliability and accuracy in toxicity testing data in order to make decisions about the environmental safety of these products. Testing poorly soluble test items is not as simple as adding them to water in a fish tank and giving the solution a stir prior to adding fish. Strategies for dealing with poorly soluble test items to ensure reliable results include: a) testing to the limit of water solubility; b) preparing water accommodated or soluble fractions (WAF, WSF); c) using generator columns or other methods for introducing the test item to the test medium ; d) using confirmatory chemical analysis for determining measured concentrations of the soluble test item in test solutions (important for endpoint determination and ensuring data quality). Interpretation of toxicity test results for poorly soluble test items also requires special attention. For example, one might ask: "Were biological or toxicity effects caused by the chemical acting on the test organism after absorption or externally through physical processes?" This presentation will discuss many of these considerations that must be addressed by chemical test sponsors, laboratories conducting toxicity tests, regulators and risk assessors in order to ensure that toxicity analysis accurately reflects the toxic potential of poorly soluble test items.

443 Improving the Quality of Aquatic Toxicity Tests: Lessons Learned and Proficiency Needs

T.J. Norberg-King, USEPA / ORD, NHEERL, Mid-Continent Ecology Division; R.B. Naddy, AECOM Environment

Aquatic toxicity testing methodologies have been widely used to assess potential adverse effects of chemicals and wastewater discharges on aquatic life in the United States since the 1970s. Over the years, continued method modifications, increased training, and technical resources have abounded. Test organism responses integrate chemical, biological, and physical aspects of effluents, ambient waters, storm waters, produced waters, and other aqueous mixtures, while chemical analyses simply focus on select parameters. In whole effluent testing (WET), use of the toxicity test has become a valuable component of most water quality monitoring programs. Sediment testing has proliferated and relies on similar quality control parameters that originated in aqueous testing. While acute and sublethal test freshwater and marine methods have specific test acceptability criteria (TAC), data reviews of test results have shown that, when laboratories do not follow the methods as prescribed, that in turn, can affect the determination of acute and sublethal toxicity. Further when laboratories become more proficient, they can in turn have very tight data that may trigger a test falling out of the discharge monitoring report (DMR) for the 'wrong' reasons. Another example is that the method is performed with various small modifications that can affect the outcome; for instance, the *Ceriodaphnia dubia* test is a three brood test, yet some laboratories report it as a 7-d test. When reported young averages 45-55 young/female; most likely 4 broods were counted. In this paper, we discuss the progress that has been made in the conduct and application of the tests and discuss a variety of ways laboratories may not always meet the required TAC as required which may invalidate tests. We will also discuss the data reviews needed and the application of the performance requirements to each test result to ensure accurate interpretation of effluent toxicity. As the tests are more routinely used, some observations and misapplications that occur in the areas of effluent sampling and renewal, reference toxicant testing, counting of broods for test termination, endpoints (e.g., LC/IC) without EPAs preferred method of analysis, and more. We'll discuss the progress from our perspective, discuss some challenges, and provide some recommendations to improve the quality and utility and provide suggestions for laboratory proficiency guidelines. This abstract does not necessarily reflect EPA policy.

444 USEPA and ASTM sediment and water toxicity methods for H. azteca and C. dilutus: Draft guidance for control test acceptability and historic performance

C.G. Ingersoll, US Geological Survey / Columbia Environmental Research Center; D.R. Mount, USEPA / ORD; D.J. Soucek, Illinois Natural History Survey; R.J. Hockett, USEPA / Mid-Continent Ecology Division; T.J. Norberg-King, USEPA / ORD, NHEERL, Mid-Continent Ecology Division; N.E. Kemble, USGS – Biological Resources Division / Columbia Environmental Research Center; C.D. Ivey, USGS – CERC / Columbia Environmental Research Center, USGS / Toxicology; J.L. Kunz, US Geological Survey / Columbia Environmental Reseach Center; T. Valenti, Syngenta Crop Protection LLC; L.N. Taylor, Environment Canada

USEPA and ASTM developed the first standard methods for conducting laboratory sediment toxicity tests in the 1980s and 1990s. A work group is currently developing revised guidance to those sediment methods for conducting 10-d and long-term reproductive exposures with the amphipod *Hyalella azteca* and the midge *Chironomus dilutus*. The revisions focus on: (1) Increasing food rations provided during an exposure, (2) Chemical constituents of reconstituted water (for *H. azteca*), (3) Starting age or size of test organisms, (4) Use of a biomass endpoint, (5) Testing replicates for chemistry analyses, (6) Use of a sand control to establish quality of water and food; (7) Equilibration of sediments in the exposure beakers, (8) Water-only toxicity testing methods, (9) Updated test acceptability criteria (TAC) for an individual test, and (10) Proficiency guidelines for repeated testing by an individual laboratory. Draft revised TAC for controls include acceptable minimum values for: (1) Ending survival, (2) Proportional increase in ending weight (*H. azteca*), (3) Ending larval weight (*C. dilutus*), (4) Percent emergence of adults (*C. dilutus*), and (5) Various reproductive endpoints. Draft proficiency guidelines for repeated testing of historic controls are also being established for these same five values (e.g., nine tests conducted over the past three years with the same control material). The laboratory proficiency guidelines will likely be provided as "minimum" and "mean" performance levels. Minimum descriptors reflect performance that laboratories should only rarely fail to meet, while mean descriptors reflect performance that should be typical. Across multiple tests, control performance above TAC should be expected from a laboratory proficient with the methods. If the typical performance of a laboratory is not substantially above minimum TAC, it may be indicative of underlying problems with laboratory facilities, practices, or experience. Importantly, the proficiency guidelines are not intended for use in judging validity of an individual test. Instead, these proficiency guidelines should be used to demonstrate overall laboratory capability to repeatedly conduct a valid toxicity test.

445 Development of a Formulated Marine Sediment in Support of Chronic Sediment Testing with the Estuarine Amphipod Leptocheirus plumulosus

M.J. Bradley, Smithers Viscient / Environmental Toxicology

A recent increase in risk assessments requiring the chronic marine amphipod exposure with *L. plumulosus* (EPA 600-R-01-020, 2001) has prompted the need for developing a formulated marine sediment which can support survival of the amphipod, as well as promote growth and reproduction, over a 28-day exposure period. As the quality of a natural sediment as a control

or reference can vary geospatially and seasonally, development of a formulated sediment will allow for testing which can be more readily standardized, is more sustainable, poses fewer logistical challenges to the testing facility (e.g., sediment collection and processing) and can generate consistent control performance, thus increasing the value of this test method in a risk assessment. Early recommendations by the EPA suggested that OECD formulated freshwater sediment adjusted for a marine matrix would be suitable for *L. plumulosus*, however, pilot testing using the substrate was unsuccessful. A variety of formulations have since been tested using constituents readily available to consumers and previously explored in freshwater formulated sediment development. Critical variables identified during development were compaction of the sediment and the quality of organic matter as a source of nourishment, while also providing a source of organic carbon. Initial findings show that a formulated sediment is capable of supporting *L. plumulosus* survival, growth, and reproduction in a 28-day exposure and meeting test method performance criteria. Further research is necessary, however, to determine a proper nutritional amendment to either the sediment or diet, in order to consistently achieve acceptable growth and reproduction of the amphipod during the exposure.

446 Identifying Best Available Ecotoxicology Data for Assessing Potential Risks of Pesticides to Endangered and Threatened Species and Their Habitats

T.W. Valenti, Syngenta Crop Protection LLC / National Research Council / Environmental Safety; R. Brain, Syngenta Crop Protection, Inc. / Department of Environmental Risk Characterization; J.M. Giddings, Compliance Services International; K.S. Henry, Syngenta Crop Protection, LLC / Ecological Sciences; A.J. Hosmer, Syngenta Crop Protection, Inc.; A.K. Kabler, Syngenta Crop Protection, LLC / Ecological Risk Assessment; J. Overmyer, Syngenta Crop Protection, LLC. / Ecological Sciences, Americas

Accurately characterizing ecological effects is pivotal for assessing potential risk to endangered and threatened (listed) species from pesticide use. Such effects data are available from a variety of sources, and one of the greatest challenges for risk assessors is evaluating their overall quality. This task typically requires assessors to weigh the relative robustness and reliability of endpoints generated from a range of studies. Factors that contribute to this evaluation may include the comprehensiveness of information reported in the publication or study report (i.e., replicate data, test conditions, QA/QC measures, etc.), analytical confirmation of test treatments, adherence to Good Laboratory Practices (GLP) or standardized test guidelines, and the rigor of the review process prior to publication or regulatory acceptance of the study. For some data sets, it can be challenging to evaluate study validity if adequate data for dose-response analyses or sufficient details of methodological approaches are not reported. The National Research Council (NRC) of the National Academies of Science Committee on Ecological Risk Assessment under FIFRA and ESA recently released a report entitled "Assessing Risks to Endangered and Threatened Species from Pesticides." In this report, the NRC emphasized that there is little guidance on what constitutes "best data available." The conundrum lies not in the ability of assessors to evaluate the quality and relevance of data generated from individual ecotoxicology studies, as various frameworks for screening the data quality of studies already exist, but rather in the challenge of determining the relative importance or usefulness of each study in terms of the overall scope of the assessment. This latter aspect is essential for determining the prominence a given study should receive in a risk assessment (e.g., critical endpoint, useful for developing weight-of-evidence, or not acceptable and/or relevant). The purpose of this presentation is to briefly review some of the current frameworks used for evaluating the quality of data generated from ecotoxicology studies, while being mindful of the data characteristics highlighted in the recent NRC report. The conceptual approach for evaluating data relevance and quality in the NRC report is also leveraged to offer suggestions and discuss examples of how data with different levels of quality should be used for assessing risks to listed species from pesticides.

Applying Adverse Outcome Pathways to Wildlife Studies

447 Linking Adverse Outcome Pathways and Population Models: Current State of the Science and Future Directions

D.H. Miller, USEPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory

Analysis of population impacts of chemical stressors through the use of modeling provides a linkage between endpoints observed in the individual and ecological risk to the population as a whole. In this presentation, we describe the evolution of an approach developed in our laboratory to link chemically-induced alterations in molecular and biochemical endpoints to adverse outcomes in whole organisms and populations. Our approach employs a simple density dependent logistic matrix model linked to adverse outcome pathways (AOPs) for reproductive effects in fish of contaminants that impact different points within the hypothalamic-pituitary-gonadal axis. We provide two examples that illustrate the implementation of AOPs in conjunction with the population models to forecast chemical impacts. In the first example, quantitative relationships between estradiol, testosterone, and vitellogenin concentrations and fecundity established in fathead minnow *(Pimephales promelas)* 21-d reproduction studies with different HPG-active chemicals are used to forecast the effects on populations exposed to stressors that reduce vitellogenesis. The second example utilizes linked AOP and population models parameterized with long-term monitoring data for white sucker (*Catostomus commersoni*) collected from a study site at Jackfish Bay, Lake Superior to predict population trends over time, including after removal of chemical stressors. Together, these case studies demonstrate the practical utility of linking population models to AOPs to support ecological risk assessment of chemicals. In progressing beyond these studies, further exploratory research efforts include linking other types of AOPs to population models (e.g., AOPs for early developmental effects), examining cross-species applicability of linked AOP and population models, and incorporating multiple stressors (both chemical and non-chemical) and community interactions within a framework for ecological risk.

448 An adverse outcome pathway for secondary poisoning of non-target wildlife by anticoagulant rodenticides

B.A. Rattner, USGS-Patuxent Wildlife Research Ctr / USGS; R.S. Lazarus, USGS Patuxent Wildlife Research Center / USGS / Beltsville Laboratory; J.E. Elliott, Environment Canada; R.F. Shore, CEH Lancaster; N.W. Van den Brink, Alterra / Dept. of Ecology & Environment

Anticoagulant rodenticides (ARs) are used on a global scale (urban and suburban settings, agriculture, and island restoration) for the control of vertebrate pest species. The hazard of first and second generation ARs to children and companion animals has long been recognized, and based on ecological risk assessments and field observations, there are now growing concerns of exposure and adverse effects in non-target wildlife. We are applying existing data in a conceptual construct to link a molecular initiating event to an adverse outcome at a biological level of organization, with emphasis on responses at the individual and population level. For these economic poisons, a great deal is known about the toxicant, use pattern and bait formulation, molecular interactions (inhibition of vitamin K-2,3 epoxide reductase) and mechanism of toxicity (impaired post-translational carboxylation of serine protease coagulation factors II, VII, IX and X). Data are available that: (i) identify mutations on the VKORC1 gene that afford AR resistance in pest rodents, (ii) illustrate the toxicokinetics of exposure, and onset and duration of adverse effects in both target and non-target species, and (iii) describe inter-specific differences in AR metabolism that affect sensitivity of some species. Responses at the cellular (impaired clotting), organ system (microscopic and gross evidence of hemorrhage, anemia), organismal (mortality), and population level of organization (local eradication of pest species, potential risk to some non-target species) have been compiled for registration purposes, and through post-registration monitoring and eco-epidemiology. In general, exposure of non-target wildlife to ARs can cause reduced fitness, possibly making individuals more vulnerable to other stressors, and can also cause low-level mortality. In some exposure scenarios, local populations of raptorial birds, particularly threatened or endangered species (e.g., Hawaiian short-eared owl, *Asio flammeus sandwichensis*, Hawaiian hawk, *Buteo solitaries*, red kite, *Milvus milvus*), are at increased risk. However, there is no evidence of dramatic adverse population-level effects in raptors on a global scale.

449 Characterization of Polychlorinated Biphenyl Action on Novel Biochemical Pathways in Japanese Quail (Coturnix japonica)

M.E. Barton, University of Maryland, College Park / Animal and Avian Sciences; T. Porter, M. Ottinger, University of Maryland / Department of Animal and Avian Sciences

Polychlorinated biphenyls (PCBs) work in a dioxin-like manner to induce cytochrome P450 enzymes activated via the aryl hydrocarbon receptor (AhR); ultimately impacting biological pathways to cause physiological damage including oxidative stress and endocrine disruption. The purpose of this study was to characterize transcript levels in a suite of genes associated with AhR activation, endocrine metabolism, oxidative repair, and energy balance in quail exposed to PCBs. Eggs were dosed on ED3 via air cell injection with increasing concentrations of PCB 126, PCB 77, and two environmentally relevant PCB mixtures found in eggs at the Upper Hudson River. Whole liver was collected from hatchlings within 24 hours of hatch and snap frozen in liquid nitrogen. RNA was extracted using the Qiagen RNeasy extraction kit with Trizol; quantification was performed using Ribogreen (Invitrogen). cDNA synthesis was performed using SuperScript III (Invitrogen). An ethoxyresorufin-O-deethylase (EROD) enzyme activity study on the same samples revealed that EROD was induced with PCB 126 and the two mixtures, but not with PCB 77. This appears to be an example of cytochrome P450 enzyme induction via the AhR, since EROD is the enzyme coded by the cytochrome P450 1A4 (CYP1A4) avian gene, which has been shown to be highly differentially expressed with exposure to dioxin-like compounds. We hypothesize that CYP1A4 and other cytochrome P450 genes that are AhR-mediated (such as CYP1A5) will be differentially expressed with PCB 126 and the two mixtures. Furthermore, we hypothesize all four compounds will demonstrate transcriptional changes in hormonal metabolism, energy balance, and oxidative repair. These data provide a more comprehensive profile for the biological action of PCBs, point to connections and crosstalk between pathways, as well as inform future studies in the mechanisms of action of PCBs that extend past the AhR-mediated responses.

450 Gene Expression Changes Across Multiple Generations of Japanese Quail Exposed to the Endocrine Active Chemical 17ß-Trenbolone

N.K. Karouna-Renier, USGS Patuxent Wildlife Research Center / Beltsville Lab; Y. Chen, Department of Fisheries and Wildlife Sciences, USGS Patuxent Wildlife Research Center, Virginia Tech / Department of Fisheries and Wildlife Conservation; P.F. Henry, US Geological Survey / Patuxent Wildlife Research Center; C. Maddox, D. Sprague, USGS Patuxent Wildlife Research Center

17ß-Trenbolone, a known endocrine active compound used extensively as a growth promoter in livestock, has been found to interfere with gene expression in fish and other vertebrates. Its persistence in feedlot runoff, surface water and in soil fertilized with contaminated manure also presents a risk to avian species, which to date has been poorly characterized. This study presents the first analysis of the effects of 17ß-Trenbolone on gene expression in an avian species. We exposed three generations of Japanese quail (*Coturnix japonica*) to multiple doses of 17ß-trenbolone, through feed only (F0), feed and *in ovo* (F1) and *in ovo* only (F2) and investigated the expression of steroid-hormone responsive genes in the liver and gonads, and thyroid-hormone related genes in the liver of male and female adult quail using qPCR. Significant changes in gene expression were observed only in the F0 and F1 birds for select genes, including vitellogenin II (VTG), very low-density apolipoprotein II (apoVLDL II), ZP1, and thyroid receptors (TRα and TRβ) in liver, and P450 aromatase in the gonads. However, expression profiles differed substantially between generations. For example, VTG, while down-regulated in the F0 females, was up-regulated in F1 females, non-monotonically. A similar pattern was observed for thyroid receptor genes (TRα and TRβ) in F0 and F1 females. In males, testicular aromatase gene expression was unaffected in F0 birds but was significantly up-regulated at higher doses in F1 birds. TRα gene expression in F0 males was non-monotonic, exhibiting an inverted U shape, but showed a consistent dose-dependent decrease relative to controls in F1 males. F2 birds, which were exposed only *in ovo*, exhibited no significant changes in the expression of any monitored genes. We will discuss our findings and relate them to hormone levels and higher level effects also monitored in the quail.

451 Development and evaluation of adverse outcome pathways predicting adverse effects of conazole fungicides on avian species

C.A. LaLone, USEPA; D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; K.V. Garber, USEPA / Environmental Fate and Effects Division; C. Russom, M.A. Etterson, USEPA / Mid Continent Ecology Division; R.S. Bennett, USEPA / ORD, NHEERL, Mid-Continent Ecology Division; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory

Conazoles are a class of fungicides commonly used in agriculture and as pharmaceuticals to prevent the spread of fungus through inhibition of cytochrome P450 14α-demethylase (CYP51). However these fungicides are known to act promiscuously on other cytochrome P450 enzymes (CYPs), including those involved in steriodogenesis, leading to adverse reproductive effects in fish. Key events in the adverse outcome pathway (AOP) describing the effects of steriodogenesis-inhibiting conazoles on fish include depressed steroid synthesis, decreased vitellogenin production, impaired oocyte growth and maturation, and ultimately reductions in spawning and cumulative fecundity. As oviparous species, egg formation in birds involves tightly regulated steriodogenesis and vitellogenesis, making these species potentially susceptible to analogous effects associated with conazole exposure. Further, birds have been shown to experience dysregulation of non-steroidogenic CYPs upon exposure to various fungicides. Utilizing mammalian-oriented ToxCast data for 16 conazoles sorted by potency and positive hit rate in the screening assays, we identified additional molecular targets impacted by exposure to conozoles, including hepatic CYPs (multiple CYP2 and CYP3 isoforms) and PXR. Upon conducting several protein alignments comparing mammalian CYP2 or CYP3 isoforms to available aves protein sequences we identified CYP 2C45 and CYP 2H2 as potential analogous targets for the action of conazoles in bird species (e.g., great cormorant, zebra finch, chicken, and wild turkey). Among these, CYP2C45 is thought to be involved in the biotransformation of steroids in birds, suggesting it may be another important target to consider relative to reproductive and endocrine effects. By leveraging the defined AOP for the effects of conazole fungicides on fish, USEPA's ToxCast data to identify molecular targets subject to perturbation by conazoles, and analyses of CYP enzyme similarity among taxa we have begun to delineate a number of hypothesized AOPs relevant to the effects of conazoles on birds. The contents of this abstract neither constitute nor reflect official USEPA policy.

452 Impacts of Embryonic Exposure to PCBs on Heart Development in Japanese Quail

M. Ottinger, University of Maryland / Department of Animal and Avian Sciences; T. Diaz, University of Maryland; R. Sclarsky, University of Maryland / Animal and Avian Sciences; T. Carro, DuPont Crop Protection / Animal Science; K.D. Dean, University of Maryland / Neuroscience; L.A. Baltos, University of Maryland

Polychlorinated biphenyls (PCBs) remain ubiquitous in the environment and are linked with adverse health effects, such as endocrine disruption, decreased reproductive fitness, reduced immune responsiveness, and inhibition of neurological function. There is some evidence that PCBs adversely impact the cardiovascular system and our previous studies showed embryonic exposure to a coplanar PCB congener, PCB 77 in wild birds affected the development of the ventricular wall compact layer. Further, although this impairment to a critical morphological region of the heart, did not diminish hatching success. To study the effects of environmentally relevant PCB mixtures on heart development, Japanese quail (*Coturnix japonica*) and broiler chicken (*Gallus domesticus*) embryos were treated with graded doses of PCB mixtures, and selected doses of PCB 77, PCB 126, estradiol, or vehicle. Both mixtures and PCB 126 affected hatching success at higher concentrations. Hatchlings dosed with a 58-congener PCB mixture produced a variety of cardiomyopathies including ventricular hypertrophy, ventricular hypoplasia, abnormal septation, and the most common cardiomyopathy identified: noncompaction of the ventricular wall. Further analysis of the Japanese quail hatchling hearts is currently ongoing. The effects noted in these hatchling studies suggest that environmentally relevant PCB mixtures do adversely affect hatch success and cardiac morphology of hatchlings following exposure *in ovo*. These cardiomyopathies identified in PCB-treated hatchlings are reminiscent of congenital cardiovascular malformations identified in humans, which increase the risk of cardiovascular dysfunction, myocardial infarction, and ultimately contribute to overall heart failure. The conclusions and opinions presented here are those of the authors, they do not represent the official position of any of the funding agencies, the Hudson River Trustees, or the United States.

453 Effects of Aroclor 1254 on Migratory Behaviour in Juvenile European Starlings

L. Flahr, University of Saskatchewan / Toxicology Centre; A. Zahara, C.A. Morrissey, University of Saskatchewan / Biology

Onset of migratory restlessness, orientation, and navigation during avian migration are directly under neurological and hormonal control. Birds exposed to endocrine disrupting chemicals during development could develop neurological changes affecting migratory behaviour. We are investigating the potential of Aroclor 1254, a PCB mixture, as a thyroid hormone disruptor in juvenile European starlings (*Sturnus vulgaris*). 84 birds were orally administered 0, 0.35, 0.70, or 1.05 µg Aroclor 1254/g-bw from 1 to 18 days post-hatch. Birds were taken into captivity and later exposed to a 6-week photoperiod shift to simulate autumn migration. Morphological measurements were taken biweekly and plasma thyroid hormone measurements taken to measure thyroid function. Emlen funnel trials were used to assess migratory orientation and activity. There was a significant increase in mass, fat, and moult score over time. This, combined with a significant increase in activity as starlings were shifted from 13L:11D (light:dark) to 12L:12D photoperiod, demonstrates that migratory condition was induced in captive starlings. At 12L:12D, control birds showed a directional preference for 155.95° (South-southeast), while birds treated with 1.05 µg Aroclor 1254 exhibited a random distribution, indicating a lack of orientation ability. Instead, these birds showed a delayed directional preference for 197.48° (South-southwest) under 10L:14D, concomitant with delays in moult and fattening. These findings link alterations in avian behaviour to contaminant-specific mechanisms. We speculate that subtle alterations in thyroid hormone homeostasis during development could give rise to larger-scale effects, including changes in cognition and migratory behaviour, which could partly explain observed global declines in migratory species.

454 Whole genome-based molecular aquatic toxicology

J. Lee, Hanyang University / Dept of Chemistry, Hanyang University Graduate School

To elucidate the impacts of environmental stressors in aquatic organisms, we are building the whole genome and RNA-Seq databases for the copepod *Tigriopus japonicus*, the fishes (marine medaka *Oryzias melastigma* and killifish *Kryptolebias marmoratus*), and the rotifer *Brachionus* sp. These genome data currently used for multipurposes such as development of molecular and environmental omics (e.g., transcriptomics, proteomics), physiological omics and others. At the moment, I obtained the substantial amount (i.e., more than 100 x genome coverage) of data for whole genomes on these organisms. These databases will lead the great development of molecular and environmental omics using aquatic organisms on marine environmental pollution research. In this presentation, I will show some examples how to use such data to better understand the molecular mechanism upon environmental stressors.

Ecotoxicology and Risk Assessment of Soils: Part B

455 Sensitivity of Ecological Soil Screening Levels to Exposure Model Parameterization and Toxicity Reference Values

B.E. Sample, Ecological Risk, Inc; A. Fairbrother, A.A. Kaiser, Exponent, Inc. / EcoSciences; B.J. Adams, Rio Tinto / Product Stewardship

Eco-SSLs for wildlife represent a simplified dietary exposure model solved in terms of soil to produce exposure equal to a NOAEL. Parameters are chemical-specific (i.e., NOAELs and BAFs or log-linear regression models) and receptor-specific (i.e., food and soil ingestion rates, and diet composition), and selected to be conservative. While suitable for screening purposes, they may be overly conservative and are not suitable for higher-tier risk evaluation nor as remedial goals. Nominal-range sensitivity analyses were performed to investigate the relative sensitivity of the Eco-SSL wildlife model. Sensitivity analyses were performed for six model parameters (e.g., TRV, food ingestion, soil ingestion, bioaccumulation, and bioavailability from soil and food) for all six bird and mammal receptors and all 16 metals/metalloids for which Eco-SSLs have been developed. The relative influence of each parameter was expressed as the absolute value of the range of calculated soil concentrations equal to the TRV. Parameters with the greatest influence were those with the broadest calculated soil concentration range. Rank ANOVA was used to identify parameters with greatest influence on model output. Soil ingestion displayed the broadest range of variation of model output and bioavailability in food the least. Although soil ingestion displaying the broadest overall range, TRV consistently displayed the greatest influence on calculated soil concentrations, regardless of how the data were grouped. Food and soil ingestion rates, bioaccumulation, and soil bioavailability were the next most influential parameters. Their relative degree of influence varied by taxa, trophic group and analyte. Relative importance of parameters differed by trophic group. Whereas soil ingestion was the second most important parameter for carnivores and herbivores, it was the 5th most important parameter for insectivores. Statistically significant interactions for trophic group and model parameters were also observed cobalt, copper, lead, vanadium, and zinc. Different patterns were exhibited depending upon which parameter, trophic group, and analyte combination are considered. Reciprocal relationships were evident for groups of parameters. For example, as FIR, bioaccumulation, and food bioavailability increased in importance, the importance soil ingestion and soil bioavailability decreased. The importance of soil ingestion in carnivores was driven by low bioaccumulation rates in small mammal prey.

456 Updates to Ecological Preliminary Remediation Goals for Soils at the Los Alamos National Laboratory

R.T. Ryti, P.L. Wald-Hopkins, Neptune and Company, Inc.; R.J. Mirenda, Los Alamos National Laboratory / Environmental Restoration Project

Approximately 10 years ago Los Alamos National Laboratory (LANL) developed an approach for calculating ecological soil preliminary remediation goals (ECOPRGs) for wildlife. This initial approach was based on LANL's protocol for screening level ecological risk assessment, except that impacts on plants or soil invertebrates were not assessed quantitatively with these original ECOPRGs. Aspects of the ecological screening assessment were modified so that more representative toxicity and exposure parameters replaced the protective assumptions used in calculating screening levels. For example, exposure estimates for wildlife receptors were modified based on representative area-use factors and site-specific data. Updates to these ECOPRGs are possible because new information regarding toxicity and bioavailability to wildlife, plants, and soil invertebrates is available. For instance, the European Commission has published adjustments to toxicity to account for soil properties, using the terrestrial biotic ligand model (tBLM) or equivalent. Such studies are based on toxicity tests with standard soil biota, which may or may not be representative of species commonly found in arid and semiarid environments. This presentation focuses on refinements to the LANL ECOPRGs such that they are directly protective of impacts on plants and soil invertebrates. These updated ECOPRGs reflect ecotoxicity literature that is relevant to the LANL environment. . Refinements include separating studies based on the relevance of the test species to arid environments or matching/adjusting key soil properties like organic matter content to better reflect LANL soils. Los Alamos Unlimited Release LA-UR-13-23866

457 Linking variable spatial exposures of chemicals to individual and population level effects in soil invertebrates

V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; M. Meli, Roskilde University / Department of Environmental, Social and Spatial Change; A.S. Johnston, University of Reading / Biological Sciences; R.A. Pastorok, Integral Consulting, Inc.; F. Voss, Dr. Knoell Consult GmbH; S. Chelinho, IMAR – CMA / Department of Zoology; H. Sundberg, Swedish Chemicals Agency; T. Alvarez, EcoRisk Solutions Ltd / Dept. of Ecological Sciences, SRG / Syngenta / Product Safety; P. van Vliet, CTGB; M. Reed, , Chemicals Regulation Directorate, Chemicals Regulation Directorate, HSE

Current risk assessment methods for assessing the toxicity of plant protection products (PPPs) to soil invertebrates (e.g., under EC Regulation 1107/2009) use standardized laboratory conditions to determine acute effects on mortality and sublethal effects on reproduction. If an unacceptable risk is identified at the lower tier, population-level effects are assessed using semi-field and field trials at a higher tier. Yet, field trials are expensive, time-consuming and cannot be applied to variable landscape scenarios, whereas there is currently no way of extrapolating available lower tier information to population-level effects. Mechanistic modelling of chemical effects on individual responses to PPPs shows great potential in fulfilling such a need, aiding ecologically informed extrapolations from this basic information. Here, we introduce and evaluate two models, using ubiquitous soil invertebrates (collembolans and earthworms) as refinement options for higher tier risk assessment. Both are spatially explicit agent-based models (ABMs) that incorporate individual and landscape variability. These models were presented to interested stakeholders from academia, industry and regulatory

authorities at a two-part SETAC Europe technical workshop, MODELINK. A relevant case study scenario was developed for applying and testing the models. Initial discussions defined the necessary modelling criteria for the models to be considered for use in risk assessment. Stakeholders outlined a need for clear model documentation following available guidance documents and thorough validation of individual life cycle and population-level effects. For assessing chemical effects, stakeholders also pointed to the need for spatio-temporal exposure modelling to be incorporated within these effects models and a desire for flexible model outputs, highlighting the importance of changes in population density and biomass as indicators of population-level effects. Mechanistic effect models, such as these, can improve the scientific basis of ecological risk assessment and provide outputs that are more directly linked to protection goals than current outputs and that are therefore more relevant for informing risk management.

458 Assessing effects of spatial heterogeneity in soil contamination: an ecological modeling approach

M. Meli, Roskilde University / Department of Environmental, Social and Spatial Change; A. Palmqvist, Roskilde University / Dept.of Environmental, Social & Spatial Change; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences

The use of mechanistic effect models (MEMs) in ecological risk assessment (ERA) of pesticides has been gaining momentum. MEMs are useful tools for extrapolating effects from the individual to the population level and for adding ecological relevance to ERA. Despite increasing recognition of their potential, the use of ecological effect models in ERA is still limited. Among the main reasons are the lack of official guidance for developing and using MEMs and contradicting expectations of stakeholders involved in ERA. Here we show how, through the use of good modeling practice and standardized documentation formats, MEMs can improve ERA. More specifically, we present two population models of the collembolan *Folsomia candida* that we developed and used to investigate the effects of heterogeneous soil contamination on its population dynamics. A spatially-explicit individual-based population model (IBM), was developed and tested according to pattern-oriented modeling theory. Model rules and parameters are based on information on the species from published literature; for implementation of toxicity, data from standard laboratory tests (survival, reproduction and avoidance) were used. We present results of testing various hypotheses regarding effects on growth and recovery of *F. candida* populations assuming different patterns of habitat fragmentation and disturbance. The scenarios are designed to simulate interactions between natural stress factors and different agrochemical application regimes. To explore whether all the complexity included in the IBM is necessary to predict risk for a species with a relatively simple life cycle such as *F. candida*, we contrast the IBM with a simpler, more standardized model, based on the generic metapopulation matrix model RAMAS. From the comparison of results in terms of assessing population-level effects for different spatial distributions of a toxic chemical, we conclude that choosing the right model type for ERA depends on whether or not population-level effects of small-scale heterogeneity in exposure need to be detected. Overall, model results suggest that disregarding spatial heterogeneity in exposure, as is the case in current ERA procedures for terrestrial ecosystems, may lead to an overestimation of risk if homogeneous contamination is assumed when this is not the case.

459 Estimation of risks to terrestrial macroinvertebrates and plants from exposure to para-nonylphenol in biosolids-amended soil

C.A. Staples, Assessment Technologies, Inc.; K. Coady, The Dow Chemical Company; B. Losey, RegNet Environmental Services

A key by-product from municipal wastewater treatment plants (WWTP) is sludge. Sludge from WWTP is often treated further (e.g., dewatering, composting, drying, etc.) prior to disposal. In the US, approximately 60% of all final sewage sludge is applied to land as a soil amendment and is thus referred to as biosolids. Biosolids contain many conventional organic pollutants (e.g., PCBs and PAHs), heavy metals, and pathogens that are of concern from a public health standpoint; hence, their use and application to soil is closely regulated. Besides the conventional pollutants, biosolids also contain various biodegradation intermediates of the commercially important nonylphenol ethoxylates (e.g., NP9EO). The key biodegradation intermediates of NP9EO include constituents with carboxylated side chains (NPEC), a carboxyated nonyl group (CAPE) or both (CAPEC), low mole ethoxylates (NP1,2EO) and nonylphenol (NP).Also, after ring opening, various ring fragments may become incorporated into bacterial biomass. Biosolids may contain all of these constituents, however, since NP is the most toxic of the NPEO intermediates and monitoring data are available in sewage sludge and biosolids it provides the most conservative basis for an assessment of potential risks to terrestrial organisms and plants following application of biosolids to soil. Toxicity data exist for NP in soil macroinvertebrates, such as annelids and arthropods, as well as for several species of plants. Predicted no effect concentrations (PNECsoil) for soil dwelling macroinvertebrates and plants for NP range from 2.3 to 5.7 mg/kg-dw (differences due to regionally varying assumptions). Concentrations of NP in biosolids from North America and Europe have been compiled and range from non-detected to approximately 1500 mg/kg-dw, with an overall average of about 500 mg/kg-dw. Soil concentrations of NP were calculated after biosolids application following regional regulatory requirements. Ratios of derived soil concentrations of NP following biosolids application to soil PNEC are all below one or approach one, generally indicating a low potential for risk. These results will be presented and discussed with regards to regional-specific regulations for disposal of biosolids to land as a soil amendment.

460 An individual-based bioenergetic model to predict the effects of toxicity and fluctuating temperature on Folsomia candida exposed to heavy metals

D.M. Jevtic, Institute of Environmental Sciences, Jagiellonian Univeristy / Institute of Environmental Sciences; N. Galic, University of Nebraska-Lincoln / School of Biological Sciences; J.L. Maino, The University of Melbourne / Department of Zoology; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; R. Laskowski, Jagiellonian University

The importance of temperature in (eco)toxicological tests has been long recognized. Still, ecological risk assessment (ERA) protocols are based on tests conducted at one constant temperature. This approach lacks ecological realism and possibly leads to erroneous conclusions on effects of toxicants in natural environments. Relatively little research has been done on the effects of toxicants in thermally fluctuating environments, even though several authors have shown that extrapolation from studies conducted under artificial constant thermal conditions may poorly predict the performance of organisms at fluctuating temperatures. Taking into account the lack of empirical data on the effects of interactions between chemicals and fluctuating temperature, the use of mechanistic effect models may provide useful theoretical predictions. Furthermore, such models may guide the design of experimental approaches for testing and validation of model predictions. We developed an individual-based model (IBM) of the springtail *Folsomia candida*, a species routinely used in ecological risk assessment. We account for temperature-dependent energy budgets of individuals and implement rates of toxicity processes, focusing on effects of heavy metals. We analyze the effects of temperature fluctuations and chemical exposure on individual life history, but also on the dynamics of populations. Finally, we provide suggestions for ERA on how to make better use of mechanistic effect models that are able to combine effects of fluctuating temperatures with effects of chemicals.

461 In vivo jumping assay of Folsomia candida for metal ecotoxicity evaluation

S. Kim; Y. An, Konkuk University / Department of Environmental Sciences

This study attempted evaluation of the jumping ability of the springtail *Folsomia candida* in order to assess the soil toxicity. The results were compared with reproduction and survival data. The jumping frequency, distance, and direction were observed for evaluation of jumping ability of the springtail *Folsomia candida* exposed to metal-contaminated soil. The jumping assay was performed using survival individuals after a chronic-toxicity soil test, and jumping being induced by chemical stress. Reproduction and survival rates show the expected toxic effects on chronic-toxicity soil test. In the jumping assay, the control group exhibited high jumping frequency and variable escape maneuvers. In contrast, a significant decrease in jumping ability was observed on exposed group. The escape maneuvers were simplified, with some individuals being unable to jump. The toxic values of the jumping assay were comparable to reproduction data and were more sensitive than the survival data. To the best of our knowledge, this is the first study to evaluate the jumping ability of *F. candida* on metal-contaminated soil. The jumping assay may represent a viable field screening method for soil ecotoxicity. This subject is supported by Korea Ministry of Environment as the GAIA project (2012000540011).

462 A sensitive method for earthworm cytotoxicity: Calcein acetoxymethyl ester (Calcein-AM) staining

J. Kwak; S. Kim; Y. An, Konkuk University / Department of Environmental Sciences

We investigated *in vivo* and *in vitro* toxicity of copper on earthworm. Copper was selected as a model chemical. *Eisenia andrei* and *Perionyx excavates* were exposed in the OECD standard soil for 7 days in *in vivo* test. Extracted coelomocytes of each earthworm were exposed to copper in the LBSS medium for 1 hour in *in vitro* test. Cytotoxicities were assessed with flow cytometry after calcein acetoxymethyl ester (Calcein-AM) staining. After the exposure duration, relatively reduced calcein intensities were observed at the lowest exposure concentration (100 mg Cu/kg soil) while mortality and abnormality (mucous excretion, bleeding, swelling, thinning, and fragmentation) were significantly increased from the 400-600 mg Cu/kg soil. Also, *in vitro* cytotoxicies induced by copper were observed. We suggest that flow cytometry with Calcein-AM staining is a simple and sensitive method to assess the cytotoxicities of earthworm coelomocytes. This subject is supported by Korea Ministry of Environment as the GAIA project (2012000540011).

Fate and Effects of Metals: Aquatic Biological Perspective: Dietary and Bioconcentration

463 Development of Site-Specific Uptake Factors for Metals

M.A. Beauchemin, ARCADIS / Environmental Sciences Assessment and Planning; S.C. Selden, ARCADIS

In ecological risk assessment, bioaccumulation factors (BAFs) are often used to estimate the concentration of constituents in endpoint receptors and prey tissue items. Standard literature-based BAFs and biota-sediment accumulation factors (BSAFs) are limited. Accurate prediction of metals uptake is difficult since metals are not lipophilic, persistent organic pollutants that will readily bind to lipid content in an organism. Rather, the accumulation of metals and the resulting tissue concentrations are based on metal uptake and elimination by the organism. Metal uptake is dependent on the concentration and availability of metals in the environment as well as the binding and transport properties into tissue. To identify uptake factors for metals, co-located soil or sediment and tissue (plant and invertebrate) datasets from several sites are examined. Regression plots, using log-transformed data, attempt to estimate a relationship between the soil or sediment and biota tissue datasets. Physical parameters in soil or sediment (e.g., pH, clay content, and organic matter) introduce variability in uptake rates which makes it difficult to establish a regression relationship. Uncertainty regarding the appropriateness of regressions was addressed by rejecting plots that do not possess adequate sample size, goodness of fit, and significant relationships ($p < 0.05$). When regression plots were rejected, uptake rates were based upon the median BAFs calculated using site-specific data. These BAFs assume that accumulation is linear and constant across all soil or sediment concentrations; therefore they do not account for variability in uptake rates across different soil or sediment concentrations, which is common for many species. This uncertainty is widely documented, which is why regression models are generally recommended over median BAFs for estimating bioaccumulation. However, individual BAFs are considered the next best uptake estimation tool when regressions are deemed inappropriate for use. Uptake factors were calculated for individual sites to allow for a comparison and observation of any trends. In addition, they were also calculated using the pooled dataset and compared to the standard literature-based BAFs and BSAFs to evaluate their accuracy in predicting tissue concentrations.

464 Adult insect emergence declines disproportionately to larval density along a stream metals gradient

T.S. Schmidt, USGS / Water Resouces Div; J.M. Kraus, USGS; D.M. Walters, USGS / Fort Collins Science Center; R.B. Wanty, USGS / Crustal Geophysics and Geochemistry Science Center

Effects of contaminants on adult aquatic insect emergence are less well understood than effects on insect larvae. We compared responses of larval density and adult emergence along a metal contamination gradient. Nonlinear threshold responses were generally observed for larvae and emergers. Larval densities decreased significantly at low metal concentrations but precipitously at concentrations of metal mixtures above aquatic life criteria (Cumulative Criterion Accumulation Ratio (CCAR) ≥ 1). In contrast, adult emergence declined precipitously at low metal concentrations (CCAR ≤ 1), followed by a modest decline above this threshold. Adult emergence was a more sensitive indicator of the effect of low metals concentrations on aquatic insect communities compared to larvae, presumably because emergence is limited by a combination of larval survival and other factors limiting successful emergence. Thus effects of exposure to larvae are not manifest until later in life (during metamorphosis and emergence). This loss in emergence reduces prey subsidies to riparian communities at concentrations considered safe for aquatic life. Our results also challenge the widely held assumption that adult emergence is a constant proportion of larval densities in all streams.

465 Nickel toxicity in sediments: bioavailability, organism feeding behaviour and exposure for the oligochaete Lumbriculus variegatus

L.T. Nguyen, Ghent University / Applied Ecology and Environmental Biology; M. van Gheluwe, ARCHE; M.B. Vandegehuchte, Ghent University / Applied Ecology & Environment Bio; J. Garrevoet, B. Vekemans, L. Vincze, Ghent University / X-Ray Microspectroscopy and Imaging group; E.R. Garman, NiPERA / Ecotoxicologist; C.E. Schlekat, NiPERA; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology

Lumbriculus variegatus were exposed for 28 days to control – and 320 mg Ni/kg spiked sediments. Two natural sediments were used: one with low (1-3 mmol AVS/kg dw and 1.5% TOC – S1) and one with high (25-30 mmol AVS/kg dw and 4.5% TOC – S2) binding capacities. All experiments were conducted after a 4-month pre-equilibration period to ensure a stable partitioning of Ni between sediment, pore water and overlying water. Feeding regimes used in the toxicity tests were (i) food (*Urtica*+cellulose) spiked into the sediment with no additional feeding during the exposure (sed+) and (ii) daily feeding with TetraMin (TM). This 2x2x2 design experiment was set up to test the hypothesis that toxicant bioavailability, feeding characteristics and exposure route can affect the performance, response to toxicants and internal toxicant distribution of *L. variegatus*. For the two sediments, no adverse effects were noted in the TM feeding regime. The total number of worms and biomass, however, were significantly reduced under the sed+ feeding mode. The lack of toxicity in the TM treatment was attributed to selective feeding on clean TetraMin and on the daily feeding regime, which promoted the organisms to spend less time in the sediment reducing overall Ni exposure, as Ni concentrations in the overlying water were < 52 µg/L (USEPA chronic water quality criteria for Ni). The results also show that in the TM feeding regime, Ni body burdens were significantly increased only in worms exposed to S1 (SEM_{Ni}-AVS>0). In the sed+ feeding treatment, however, Ni body burdens were significantly increased in worms exposed to S1 (SEM_{Ni}-AVS>0) as well as in the worms exposed to S2 (SEM_{Ni}-AVS< 0), suggesting the occurrence of dietary effects. The internal Ni distributions were analysed using micro X-ray fluorescence (XRF). The data show that Ni was predominantly accumulated in the epidermis of *L. variegatus* fed clean food (TM) and exposed to S1. This demonstrates the role of dissolved Ni as the predominant exposure route at SEM_{Ni}-AVS>0. The results also show that for both sediments, Ni was predominantly presentin the gut area of worms fed via sediment (sed+). This highlights the importance of diet-born Ni when contaminated sediment was the only dietary source. Analysis of Ni concentrations in different sub-cellular compartments will provide additional information on the contribution and the toxicological relevance of the different exposure routes under different scenarios of Ni bioavailability.

466 Evolutionary patterns of metal (Cd, Zn) bioaccumulation in aquatic insects

M.D. Poteat, North Carolina State University / Department of Environmental and Molecular Toxicology; D.B. Buchwalter, North Carolina State University / Department of Environmental and Molecular Toxicology

Aquatic insects are used extensively in biomonitoring and bioassessment programs due to their predominance in freshwater systems and differential sensitivities to pollutants (e.g., metals). Here, we measured physiological traits (uptake and efflux rate constants, k_u and k_e, respectively) using 65-Zn and 109-Cd in 18 aquatic insect species representing two species-rich families generalized to be sensitive (Ephemerellidae (Order: Ephemeroptera)) and tolerant (Hydropsychidae (Order: Trichoptera)) to metals. Our goals are to better understand the mechanistic basis for differences in metal bioaccumulation between the two families and to better understand variance among closely related species within each family. Our results reveal that on average k_u and k_e values for Zn and Cd explain large bioconcentration differences between families. However within families, both k_u and k_e measurements varied widely. For example, in Ephemerellidae, Zn and Cd k_us varied from

0.44 to 1.58 L g^{-1} d^{-1} and 0.30 to 1.96 L g^{-1} d^{-1}, respectively, whereas in Hydropsychidae, Zn and Cd k_us varied from 0.010 to 0.17 L g^{-1} d^{-1} and 0.0098 to 0.37 L g^{-1} d^{-1}, respectively. In Ephemerellidae, Zn and Cd k_es varied from 0.013 to 0.15 d^{-1} and 0.010 to 0.16 d^{-1}, respectively, whereas in Hydropsychidae, Zn and Cd k_es varied from 0.028 to 0.13 d^{-1} and 0.042 to 0.17 d^{-1}, respectively. Analysis of this growing dataset shows Zn and Cd k_us and k_es are strongly correlated (r = 0.96, r = 0.93, respectively). Phylogenetic statistics were utilized in order to better understand how metal bioaccumulation parameters vary within and among taxonomic groups. Blomberg's K statistic was calculated to quantify phylogenetic signal (the tendency for related species to resemble each other), and their randomization test based on the mean squared error was used to test for the significance of phylogenetic signal. Across species, Zn and Cd k_us both exhibited a strong significant phylogenetic signal (K = 0.97, K = 1.58, respectively). Cd k_es also displayed a significant phylogenetic signal (K = 0.86). While Zn k_es did not exhibit a significant signal (p = 0.078), this was likely an artifact of the small sample size. This work represents a novel attempt at examining physiological traits phylogenetically within a set of closely related species. Future work will focus on adding species to our unique dataset as well as using phylogenetic methods to predict metal bioaccumulation parameters for untested species.

467 Development of a multi-metric index for metal contamination assessment in lotic systems

C. Fortin, I. Lavoie, University of Quebec / INRS-ETE; J.L. Levy, Sydney Water Corporation / INRS-ETE; S. Leguay, University of Quebec / INRS-ETE

The total metal concentration in solution does not necessarily reflect its bioavailability. Metal concentrations also fluctuate over time and numerous water samples may be necessary to adequately estimate the level of contamination. Water quality assessment would therefore benefit from an approach including bioindicators due to their potential for integrating changes in water conditions as well as providing information on the response to metal-stress. The aim of our project is to develop a biological tool to estimate metal pollution in lotic systems. Two biomonitoring approaches have been tested in the field to assess the response of biofilms to chronic metal contamination from mine tailings: (i) intracellular metal concentrations as an indicator of exposure, (ii) the response of diatom assemblages as a sign of stress related to metal contamination. The results from a study conducted in 2010 showed a clear link between free metal concentrations in the water and operationally defined intracellular metal concentrations in the biofilm, suggesting that metal content in biofilm cells represents a robust indicator of metal exposure and is a good proxy for free metal concentrations in the water. Additional samples were collected in 2012 at new sites to evaluate if the relationships between free metal ions and intracellular metal concentrations were similar for mining sites with different water chemistry (pH, DOC, alkalinity, hardness). The strong dominance of certain diatom species known to be tolerant to metals is a good indicator of metal contamination, as well as the low diversity observed at our most contaminated sites. Although cell deformities were observed, a direct association between the percentage of abnormal diatoms and the concentrations of metal was not possible. The abundance of abnormal diatoms reflects a response to pollution related to mining, despite the fact that it is difficult to differentiate between the stress caused by high metal concentrations and low water pH. Additional experiments under controlled conditions should allow for a better understanding of the link between diatom deformities and metal bioavailability. Ultimately, we hope to provide a multi-metric index allowing water managers to better assess the levels of metal contamination at which effects can be observed on the biota. This biomonitoring tool would also be of great use in assessing the recovery of lotic systems after restoration.

468 Explaining Selenium Concentrations in Lacustrine Food Webs

D. Ponton, Institut National de la Recherche Scientifique / Eau, Terre et Environnement, INRS-ETE; L. Hare, INRS-ETE

We measured selenium (Se) in zooplankton, zoobenthos and yellow perch (*Perca flavescans*) collected from lakes in mining areas in eastern Canada. To explain Se concentrations in these animals, we also measured Se speciation in lakewater and sulfur stable isotopes in animals. Selenium speciation varied widely among lakes such that any one of three main Se species (selenite, selenate and organo-Se) could dominate in a given lake. Selenium concentrations in zooplankton were best predicted by the sum of the organo-Se and selenate concentrations in lakewater. These field results were confirmed in the laboratory by exposing the green alga *Chlamydomonas reinhardtii* to the various Se species, as well as by measuring the influence of sulfate and pH on Se bioaccumulation. In the field, the ratio of Se concentrations in a predatory zooplankter to those of its planktonic prey was about 0.9, whereas the comparable ratios for young-of-the-year yellow perch eating zooplankton and older perch eating benthos were higher (1.6). The sulphur stable isotope signatures ($\delta^{34}S$) of the animals involved suggest that the more that fish depend on benthos for food, the higher will be their Se concentrations. The results of our study should be useful for evaluating Se exposure and risk assessment strategies for Se-contaminated lacustrine food webs.

469 Interactions and relative contributions of chronic waterborne and dietary lead exposure to toxicity and accumulation in rainbow trout

C.M. Wood, McMaster University / Department of Biology; T. Ng, McMaster University; D.H. Alsop, McMaster University / Biology; M.J. Chowdhury, International Lead Zinc Research Organization

Effects of waterborne or dietary Pb exposures have been well studied in freshwater fish, but little known about the effects of combined exposure, and the potential interactions of the two pathways, as likely occurs under natural environmental conditions. Our aim is to understand the interactions and relative contributions of waterborne and dietary Pb to toxicity and accumulation in the juvenile rainbow trout, *Oncorhynchus mykiss*. The 96 LC50 was 487 mg l^{-1} and the 7 week LC 10 and LC20 were 56 and 97 mg l^{-1} respectively for waterborne Pb in moderately hard water (from Lake Ontario), with no effects on growth (SGR). In a subsequent test, trout were exposed to waterborne Pb alone (0, 8.5, 20, 60, 110 mg l^{-1}); dietborne Pb alone (oligochaete worms, *Lumbriculus variegates* pre-exposed to the same waterborne concentrations of Pb for 28 d); and combined waterborne and dietborne Pb for 7 weeks. Again, waterborne Pb did not affect SGR, but dietborne Pb reduced it by up to 32 %. This effect was not observed in the combined exposure. Trout accumulated higher Pb from the water than from the diet. Tissue-specific distribution from the waterborne exposure was gill > carcass > liver ~ gut, whereas distribution from the dietborne exposure was gill ~ gut > liver ~ carcass. Gill and gut accumulation in the combined exposure were mostly determined by the waterborne exposure but liver and carcass actually accumulated less Pb in the combined than in the waterborne exposure. Pb did not affect the concentration of Ca in the carcass, but it reduced the Na concentration in the blood at 110 mg l^{-1} in both the waterborne and the dietborne exposure, but not in the combined exposure. The effect of waterborne Pb may relate to reductions of Na/K ATPase activity observed in the gill (52 %) and gut (62 %) observed in the waterborne exposure only. Ion analysis of the worms demonstrated no effect of Pb on Ca, but a higher Na concentration at 110 mg l^{-1}. Nutrient measurements (protein, lipid, carbohydrate) on the worms are presently underway and may help to understand the mechanism of the effects on growth. In brief, Pb exposure from the two pathways produced different toxicity and accumulation patterns. Co- exposure to dietary and waterborne Pb produced antagonistic effects and therefore was protective against endpoints measured in this study. (ILZRO).

470 Dietborne-metal toxicity to aquatic organisms: A literature review

J.S. Meyer, Arcadis US, Inc., ARCADIS; D.K. DeForest, Windward Environmental LLC

Recently published studies of dietborne-metal toxicity to aquatic biota provide increased insight into the relative importance of dietborne-metal versus dissolved-metal exposure. To provide an updated synthesis of the literature, we reviewed the state of the science about dietborne-metal toxicity to aquatic biota, with a focus on 12 metals: Ag, Al, As, B, Cd, Co, Cu, Mo, Ni, Pb, V, and Zn. Although Hg and Se are also of concern for dietborne-metal toxicity, they have recently been reviewed extensively. Of the metals we reviewed, limited or no dietborne toxicity data were identified for B, Co, Mo, and V. Additionally, little or no data were available about the toxicity of dietborne Al and Pb to aquatic invertebrates; however, available data suggest toxicity of dietborne Al and Pb to fish is unlikely to be of concern. In contrast, Ag, As, Cd, Cu, Ni, and Zn have been demonstrated to cause dietborne toxicity to aquatic organisms in laboratory exposures. For Ag, Cd, and Zn, dietborne-metal toxicity occurred at potentially environmentally-relevant concentrations (i.e., some of the waterborne concentrations to which the food was exposed were at or near the US Environmental Protection Agency's existing waterborne criteria for Ag, Cd, and Zn, sometimes resulting in dietborne concentrations that contributed to effects reported for the most sensitive species [usually filter-feeding herbivores like freshwater

daphnids and saltwater copepods] beyond the toxicity caused by waterborne exposure alone). Additionally, although generally ignored in the past, dietborne As has recently been demonstrated to be toxic to rainbow trout (*Oncorhynchus mykiss*). These results indicate that dietborne metal can be more toxic than waterborne metal under some exposure scenarios; however, these results might also simply indicate that some water quality criteria are outdated and do not adequately incorporate the same sensitive species or endpoints that are most sensitive to dietborne metals. To develop an understanding of the relationships between waterborne and dietborne exposures in natural systems, aqueous and algal concentrations of metals should be surveyed in a variety of real-world freshwater and saltwater systems to determine dietborne:waterborne metal ratios and the chemical forms in which the metals occur in various food items – thus allowing linkage of exposure and effects in more refined risk assessments of dietborne metals.

Canadian Oil Sands Part II: Advancements in Ecotoxicology of Process-Affected Materials and Analytical Detection

471 Toxicity of naphthenic acids to the freshwater amphipod Hyalella azteca: Commercial mixtures versus extracts from oil sands process-affected water

A.J. Bartlett, Environment Canada; R.A. Frank, Environment Canada / AEPRD; L.R. Brown, Environment Canada; P.L. Gillis, Environment Canada / Aquatic Contaminants Research Division; M. Hewitt, Environment Canada / National Water Research Institute; J.L. Parrott, Environment Canada / Water Science and Technology Directorate

Naphthenic acid (NA) mixtures are concentrated in oil sands process-affected water (OSPW) through the extraction of bitumen from oil sands deposits in Alberta, Canada, and have been identified among the primary toxic components of OSPW. Previous research has shown that changes in the composition of complex NA mixtures impact the observed toxicity of these mixtures. The objective of this study was to compare the toxicity of commercially available NA mixtures to that of NA mixtures extracted from a fresh source of OSPW using *Hyalella azteca*, a freshwater amphipod, as a representative invertebrate species reflective of general ecosystem health. Acute toxicity tests (24 h, 48 h, 7 d) were conducted initially to determine effects on survival for three NA commercial mixtures (Acros, Aldrich, and Merichem) as well as an NA extract prepared from OSPW. Toxicity of the commercial mixtures was similar, with LC50s of 3.5-4.1 mg/L, 1.4-1.9 mg/L, and 0.8-1.3 mg/L for 24 h, 48 h, and 7 d, respectively. However, the toxicity of the NA extract was more than 10-fold lower than that of the commercial mixtures, with LC50s of 69 mg/L, 28 mg/L, and 15 mg/L for 24 h, 48 h, and 7 d, respectively. Chronic toxicity tests were then conducted with the NA extract to determine effects on survival (14 d, 21 d, 28 d) and growth (28 d). LC50s remained stable at 11 mg/L from 14-28 d, and growth was a less sensitive measure of toxicity than survival, with a 28-d EC50 of 18 mg/L. Our results indicate that not all NA mixtures are equal: NA commercial mixtures derived from petroleum sources are not accurate predictors of the NAs that are present in oil sands mixtures, and this should be taken into account when assessing toxicity of these compounds. In addition, the toxicity of the NA extract increases with duration of exposure, with LC50s dropping almost 7-fold between 24-h and 14-d exposures before stabilizing at 11 mg/L. These results suggest that 14-d exposures are sufficient to assess the effects of NA extracts on survival of *Hyalella*, but because NAs are environmentally persistent, the potential for longer-term effects on reproduction should also be considered when assessing ecological impacts.

472 An effects directed analysis to identify toxic constituents of OSPW

S.B. Wiseman, University of Saskatchewan / Toxicology Centre; N. Remenda, Y. He, R. Mankidy, H. Alharbi, University of Saskatchewan; A. Dos Santos Pereira, University of Alberta; J.W. Martin, University of Alberta / Laboratory Medicine and Pathology; J. Giesy, University of Saskatchewan

Oil sands process affected water (OSPW) is toxic to aquatic organisms. However, acute toxicity of OSPW is short-lived due to microbial biodegradation. The view that toxicity is caused by naphthenic acids is not proven and evidence to date only allows for the conclusion that toxicity is caused by organic chemicals dissolved in OSPW. As the first step in an EDA to identify toxic constituents in OSPW, and to elucidate critical mechanism(s) of toxicity, effects of fractions of organic chemicals dissolved in OSPW were assessed in a battery of bioassays and the analysis was complemented by characterization of fractions by use of Orbitrap LC-MS. Fresh OSPW from the west-in-pit (WIP), which is destined to be the first end-pit-lake for the remediation of OSPW, was fractionated at pH 7, pH 2, and pH 11 into neutral-, acid-, and base-extractable fractions, respectively. For toxicity assays, fractions were resuspended in DCM to produce stock solutions that had concentrations 1000x greater than in the original sample. There was no differences in the EC_{50} of the neutral-, acid-, and base extractable fractions, which were 7.32x, 8.36x, and 7.78x, respectively. Toxicity of fractions to embryos of fathead minnows was assayed and the neutral fraction had significantly greater effects. At concentrations of 2.5x the percent survival of embryos exposed to the neutral-, acid-, and base extractable fractions were 0.0%, 56.7%, and 53.7%, respectively. However, at concentrations of 1x the percent survival of embryos exposed to the neutral-, acid-, and base extractable fractions were 68.8%, 88.1%, and 93.1%, respectively. Impairment of hatching and incidences of pericardial edema were significantly greater in embryos exposed to the neutral-extractable fraction, but not the acid- or base-extractable fractions relative to embryos exposed to control solutions. Endocrine-disrupting effects of fractions were assessed in the T47D kbluc assay to determine if fractions activate the estrogen receptor. Compared to controls, only the neutral extractable fraction had estrogenic activity. Overall, results suggest that the neutral extractable fraction of OSPW has the greatest toxic effects *in vitro* and *in vivo*. Additional fraction of the neutral-, acid-, and base-extractable fractions is being performed to refine the identification of toxic constituents.

473 Estimating the bioaccumulation potential of organic compounds in oil sands process-affected water by PDMS-coated stir bar sorptive extraction

Z. Kun, University of Alberta; A. dos Santos Pereira, University of Alberta / Division of Analytical and Environmental Toxicology; J.W. Martin, University of Alberta / Laboratory Medicine and Pathology

Bitumen extraction by the surface mining oil sands industry in Northern Alberta, Canada, produces large volumes of toxic oil sands process-affected water (OSPW) containing a complex mixture of dissolved organic compounds. Several thousand chemical species can now be identified in OSPW by ultra-high resolution mass spectrometry, but the relative toxicity of each remains uncertain. To some extent, the toxicity of each species will depend on their propensity to cross biological membranes, and to bioaccumulate. However, information on the bioaccumulation potential of any compound class in OSPW is very limited. In this study, the bioconcentration of all detectable organic compounds in OSPW was estimated in vitro by partitioning to polydimethylsiloxane (PDMS). Biomimetic extraction was conducted with GERSTEL-Twister™ bars coated with a 1.0 mm film of PDMS. After exposure to OSPW for 144 hours, the partitioning of compounds was measured by reversed-phase liquid chromatography / ultrahigh resolution linear ion trap-orbitrap mass spectrometry in electrospray positive and negative ionization modes. Many compound classes showed negligible accumulation potential, but others showed very high accumulation potential and are highlighted here. In positive mode, the logarithm of the partition coefficient between the PDMS coating and OSPW (Kt) ranged from 0.4 to 1.6 for mono-oxygenated compounds (O group), from 0.2 to 4.2 for NO compounds, and from 1.1 to 4.2 for SO compounds. In negative mode, Kt ranged from -0.75 to 1.83 for SO_2 compounds, from -0.4 to 2.1 for naphthenic acids (i.e., O_2 compounds). In general, for all accumulating compound classes a positive relationship was found between Kt and carbon number, and a negative relationship was found between Kt and "double bond equivalents". The results of this study clearly demonstrate that OSPW contain numerous compounds of high bioaccumulation potential, and that compounds besides naphthenic acids must be considered.

474 Measurement of Polynuclear Aromatic Hydrocarbons (PAHs) in Epiphytic Lichens for Receptor Modeling in the Alberta Oil Sands Region

W. Studabaker, RTI International; S. Krupa, University of Minnesota / Department of Plant Pathology; R. Jayanty, J. Raymer, RTI International

As mining and refinery operations in the Alberta Oil Sands Region (AOSR) have expanded, concern has grown over the impacts of air pollution generated by those operations on human and ecosystem health. The inaccessibility of much of the AOSR makes it difficult to establish enough conventional air-quality monitoring stations to model long-range impacts of emissions from the AOSR operations. Epiphytic lichens are important markers of

ecosystem health, are well-established bioaccumulators of trace metals, and are potentially useful biomonitors of air pollution. However, their ability to take up organic pollutants has not been extensively explored, and only recently have they been used for biomonitoring of pollution by polynuclear aromatic hydrocarbons (PAHs). Here we describe the determination of PAHs in lichens, collected from sites throughout the AOSR, for modeling emissions associated with mining operations. Lichens (*Hypogymnia physodes*) were collected on two separate occasions. During the summer of 2009, single samples were taken from 200 sites in the AOSR; a subset of 20 of these was selected for determination of PAHs. During the summer of 2011, triplicate samples (from separate trees within a site) were collected from 20 sites representing similar locations to the 2008 sites. Milled lichens (0.2 g) were extracted with cyclohexane. Extracts were cleaned on silica using automated solid-phase extraction and analyzed by gas chromatography with mass selective detection. Method detection limits for individual PAHs were 2-4 ng/g. Total PAHs in the samples from both collection events ranged from 50 ng/g to 350 ng/g and declined with increasing distance from the mining operations. Total variability, including analytical and within-site sampling variability, was approximately 12%. The relative contribution of low ring number PAHs to total PAHs increased with increasing distance. Total PAHs correlated strongly ($R^2 > 0.80$, $p < 0.05$) with crustal elements, suggesting similar transport mechanisms. PAH profiles of samples collected near the mining operations are similar to PAH profiles of mine mineral samples. The results are consistent with PAH transport close to the mines being more influenced by particulate matter transport mechanisms, with PAHs in samples collected from remote areas more reflective of vapor phase transport mechanisms.

475 Assessing effects of dibenzothiophnes and related compounds on Aryl-hydrocarbon receptor antagonism and embryotoxicity to fathead minnow

Y. He, S.B. Wiseman, R. Mankidy, University of Saskatchewan / Toxicology Centre; H. Alharbi, Carleton University / Department of Biology, University of Saskatchewan / Toxicology Centre; J.P. Giesy, University of Saskatchewan / Toxicology Centre and Department of Veterinary Biomedical Sciences

Heterocyclic derivatives of polycyclic aromatic hydrocarbons (PAHs) heterocycles, in which one or more of the carbons within the aromatic structure is substituted by a nitrogen, sulfur, or oxygen atom, are significant components of PAH mixtures in many contaminated environment samples such as sediments from oil sands tailing ponds. Toxicity of PAH heterocycles, including dibenzothiophene (DBT) are poorly understood. It is hypothesized that toxicity of dibenzothiophene might be related to antagonistic activity mediated by the Aryl-hydrocarbon receptor (AhR), which could lead to developmental deformities in embryos of fishes. The objective of this study was to assess effects of dibenzothiophenes and six related compounds on antagonism of the AhR and mortality and teratogenicity of fathead minnow embryos. Co-exposure of H4IIE;*luc* cells to 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and dibenzothiophene or it's derivatives, demonstrated significant reduction in signaling viaAhR. Magnitudes of an AhR-mediated response of a transactivation reporter gene assay based on H4IIE cells when exposed to 0.01, 0.1, 1 or 10 µg /L DBT in the presence of 1.85 pM TCDD were 85.2±6.4%, 69.6±8.8%, 68.5±4.8%, and 0.428±0.109, respectively, compared to that of 1.85 pM TCDD alone which was 100%. Exposure of embryos to 100 µg /L DBT for 96 hr resulted in significantly lesser survival (60.7±8.1%) and greater incidence of malformations of the spine (28.2±4.0% curved spine) of fathead minnow embryos. Exposure to 4' methyl-dibenzothiophene (4-Me-DBT) and 4'6' methyl-dibenzothiophene (4,6-Me-DBT) resulted in similar expression of the reporter gene in H4IIE cells and mortality and deformities of fathead minnow embryos. Exposure to thianaphthene (TNP) and 3' methyl-benzothiophene (3-Me-BT) also resulted in similar but lesser extent of expression of the reporter gene in in H4IIE cells as well as mortality and deformities of fathead minnow embryos. This study demonstrated a link between the AhR antagonistic activity of DBT and derivatives and their toxic effects on embryo deformity, which is potentially structure-related. The presence of these potential AhR antagonists in environmental samples such as sediments from oil sands tailing ponds should be considered when assessing the embryotoxicity of these samples.

476 Larval Fish Toxicity of Sediments, Waters, Groundwaters, and Snow Melt Waters from Oil Sands Areas

J.L. Parrott, Environment Canada / Water Science and Technology Directorate; J.R. Marentette, Environment Canada / Psychology, Neuroscience and Behaviour, AEPRD; M.E. McMaster, Environment Canada / National Water Research Institute; W.P. Norwood, P.L. Gillis, Environment Canada / Aquatic Contaminants Research Division; J.V. Headley, Environment Canada / AEPRD; A.J. Bartlett, G. Bickerton, J.W. Roy, C. Yang, Z. Wang, Environment Canada; M. Hewitt, Environment Canada / National Water Research Institute; R.A. Frank, Environment Canada / AEPRD

As part of the Joint Canada-Alberta Oil Sands Monitoring Plan, the toxicology of natural and oil sands related environmental samples was studied. One of the goals of the toxicity tests is to examine pathways and sources of contaminants that may be causing effects in wild fish and invertebrates. Samples were collected from sites where wild fish health assessments and invertebrate communities were assessed. In this way, linkages could be made between wild invertebrates and fish, in comparison to controlled studies of lab fish exposed to certain components of the environment (sediment, water, groundwater, and snowmelt). Embryo-larval fathead minnows were used to assess the chronic toxicity of the following environmental samples: river sediments, river waters, groundwaters, snow melt waters, spring freshet waters, and suspended sediments collected in the vicinity of the Canadian oil sands. Samples were collected in 2009- 2013 from rivers near oil sands processing facilities along the Athabasca River and tributaries in areas of oil sands development, and compared to samples collected far from sites of oil sands mining and processing. Fertilized fathead minnow eggs were exposed for 21 days (through hatch to 7-15 days post-hatch) to samples in dose-response gradients. Most environmental samples caused no effects in larval fish in 21 day assays. Samples that caused effects in larval fathead minnows were several snow melt samples, several groundwaters, sediments from the Steepbank and Ells Rivers, and waters from the Muskeg River. Some of the toxic samples were from sites close to industry (Steepbank River sediments, snow samples close to stacks, Muskeg River waters). Other samples (groundwaters, Ells River waters) showed toxicity far from oil sands activities, with effects in lab fish seen at "background' sites where natural oil sands weathering or water movement thru bitumen occurs. Samples were analyzed for naphthenic acids, PAHs, C1-C4 alkylates PAHs, and metals. Sites where sediments and waters were toxic in lab fish bioassays are being assessed to determine whether wild young-of-year fish are abundant and growing normally in these areas of potentially toxic sediments or waters. The results of this work will help guide future studies and locations to sample wild fish and invertebrates to fully assess environmental health in the oil sands area.

477 Liquid Chromatography Tandem Mass Spectrometry Method for Measurement of Naphthenic Acids in Tissue

M.B. Woudneh, AXYS Analytical Services Ltd; J.P. Benskin, Axys Analytical Services Ltd.; C. Hamilton, Axys Analytical Services Ltd.; G. Wang, Axys Analytical Services Ltd.; J.R. Cosgrove, AXYS Analytical Services Ltd

Naphthenic acids (NAs) are complex mixtures of naturally occurring carboxylic acids which are common in and around oil sands deposits. Of particular concern is the potential for increased exposure of wildlife (in particular aquatic species) to NAs due to ongoing industrial activities and regional development. To support risk assessment of NAs in Northern Alberta, new analytical tools are required which are capable of extracting and quantifying NAs from tissue samples. In this work, a new analytical methodology for extraction and quantification of NAs from tissue is reported. Particular challenges that arise due to saturated and unsaturated fatty acids as well as resin acids in tissue analysis are discussed. Potential marker NAs, that are not impacted by endogenous fatty aids, are identified for monitoring NAs in exposed aquatic organisms.Method accuracy (50 to 150%, n=5) and precision (< 20% RSD, *n*=5) were achieved during method validation using a refined Merichem spiked tissue. The method specificity was evaluated using dicarboxylic acids, hetero atomic carboxylic acids, and hydroxy acids that are isobaric to the target NAs. Application of the method to tissues from a wider variety of aquatic organisms is necessary to properly demonstrate applicability of scope.

478 Chemical profiling of waters associated with mining operations and the McMurray Formation for source identification in the Alberta oil sands region

R.A. Frank, Environment Canada / AEPRD; J. Roy, G. Bickerton, Environment Canada; S. Rowland, University of Plymouth; J.V. Headley, Environment Canada / AEPRD; A. Scarlett, University of Plymouth / School of Geography, Earth and Environmental Sciences; C. West, University of Plymouth; K. Peru, A. De Silva, C. Gagnon, Z. Wang, Environment Canada; W.P. Norwood, Environment Canada / Aquatic Contaminants Research Division; C. Milestone, Environment Canada; A. Bauer, University of Waterloo; J.L. Parrott, Environment Canada / Water Science and Technology Directorate; M. Conly, Environment Canada; M. Hewitt, Environment Canada / National Water Research Institute

Extensive research in support of the Canada-Alberta Joint Oil Sands Monitoring Program (JOSMP) is focussed on identifying a chemical profile of oil sands components that will enable the differentiation of groundwater affected by natural oil sands materials from that affected by OSPW. Presented is an overview of the program that has focussed on compound classes to facilitate source identification including polar organic compounds (i.e., oil sands naphthenic acids), metals, geochemistry and industrial and anthropogenic additives. Sampling has been integrated with other initiatives under JOSMP that include effects-based assessments of in situ biota, laboratory bioassays, water quality monitoring and atmospheric deposition. Work thus far on polar organics in groundwater and OSPW has exhibited consistent trends in the diagnostic ability of O2:O4 ion classes, as determined by ESI-HRMS, and ions for two classes of mono-aromatic acids, as determined by GC´GC-TOF/MS, to differentiate natural from industrial sources.

Remedy Effectiveness Assessments and Monitoring Contaminated Sediment Remediation: Part B

479 Evaluation of Cap Effectiveness for Reducing Aquatic Biota Exposure to PAHs using PEDs and HDs on the West Branch of the Grand Calumet River, IN

J.M. Lazorchak, USEPA / National Exposure Research Laboratory; R. Ford, B. Lien, USEPA Office of Research and Development; M.A. Mills, USEPA, ORD / Remediation and Pollution Control Division, NRMRL; H. Williams, USEPA Great Lakes Program Office Region 5

The Grand Calumet River (GCR), located in Lake County in northwestern Indiana has been designated under the Great Lakes Legacy Act (GLLA) as an Area of Concern (AOC). One of the GCR's branches is the West Branch of the Grand Calumet River (WBGCR) which has heavy metals and polycyclic aromatic hydrocarbons (PAHs) as the primary contaminants of concern. The Great Lakes Program Office (GLNPO) initiated two phases to address these contaminants. This presentation addresses Phase II of the remediation project which is located in the WBGCR in Hammond and East Chicago, Indiana (an approximately 1.3-mile stretch of the WBGCR). Phase II involved partial removal of the existing river bed sediments followed by installation of a cap with a reactive barrier incorporated into the design. Within the Phase II area two fixed transect locations were established across the river at stations approximately 260 feet upstream of the Hammond Sewer District Outfall (ORD West Transect) and approximately 480 feet upstream of the Indianapolis Boulevard overpass (ORD East Transect). A temporary un-remediated reference location (URRL) was established approximately 100 feet downstream from the Indianapolis Boulevard overpass. Five 5X5' Hester-Dendy (HD) artificial benthic samplers and one Polyethylene Diffusion (PED) sampler were deployed for 6 weeks above individual transect screen locations and URRL. After 6 weeks both HDs and PEDs were collected, processed, and analyzed for PAHs. The total PAH (16) concentrations were highest in the URRL. The range of measured total PAH concentration measured in macrobenthos and PEDs respectively, were for the West Transect, 118-450 mg/kg and 23-33 mg, East Transect, 749-1413 mg/kg and 60-69 mg, and URRL Transect, 1247-5002 mg/kg and 112-143 mg. The results indicate that the cap with reactive barrier reduced exposure to aquatic biota as compared to the URRL by 12 fold in the West Transect and 3 fold in the East Transect. The trends in reductions were also reflected in the PEDs in the West and East Transects. The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the US Environmental Protection Agency.

480 Monitoring Effectiveness of In situ Treatment with Reactive Amendments of Contaminated Sediments in an Active DoD Harbor

V.J. Kirtay, SPAWARSYSCEN San Diego; B. Chadwick, SPAWAR Systems Center San Diego; G.H. Rosen, SPAWAR Systems Center Pacific / Environmental; R. Johnston, US Navy / Marine Environmental Support Office-NW; J.M. Conder, M.M. Grover, ENVIRON International Corp; J.D. Germano, Germano & Associates, Inc.

A demonstration project at the Puget Sound Naval Shipyard and Intermediate Maintenance Facility located in Sinclair Inlet, Puget Sound, WA is being conducted to demonstrate and validate placement, stability and performance of reactive amendments for the treatment of contaminated sediments in an area with elevated polychlorinated biphenyl (PCB) and Hg contamination. A ½-acre contaminated area underneath a pier and into the berthing area was amended with powdered activated carbon (PAC) using a composite aggregate system to decrease the bioavailability of PCBs in contaminated sediment. Pre-installation monitoring was conducted in August 2012, the amendment was installed in October 2012, and post-installation activities are currently underway. The pre- and post-monitoring events are utilizing a suite of innovative and traditional monitoring tools including a 14-day in-situ bioassay using a bent-nosed clam and polychaete worm and chemical characterization using the sediment ecotoxicity assessment (SEA) Ring protocol and an in situ solid phase micro-extraction (SPME) technique. Physical and biological conditions are being documented with the sediment profile imaging (SPI) camera, measurement of black carbon and total organic carbon content and traditional benthic infauna sampling. The monitoring will establish pre-placement conditions, verify that the amendment placement meets minimum specifications and evaluate performance after approximately one, two and three years of amendment placement. Results from the pre-installation and year one post-installation monitoring events will be presented.

481 Residual Seafood Consumption Risks in the Lower Duwamish Waterway and Comparison to Background

K.L. Godtfredsen, Windward Environmental LLC; S. Replinger, Windward Environmental, LLC; J. Stern, King County-DNRP / Dept. of Natural Resources, King County Dept of Natural Resources

The Lower Duwamish Waterway (LDW), a highly modified estuary located in the industrial core of Seattle, Washington, was listed as a Superfund site in September 2001 and as a Washington State Model Toxics Control Act (MTCA) site in February 2002. Since its listing, human health and ecological risks and risk-based sediment cleanup goals have been estimated in the remedial investigation (RI) using empirical data from the site, residual risks for the various remedial alternatives have been estimated in the feasibility study (FS) using model-predicted concentrations, and background seafood consumption risks have been approximated using a non-urban tissue dataset from Puget Sound. EPA's Proposed Plan has put forth a Preferred Alternative that would remove approximately 790,000 cubic yards (cy) of contaminated sediment from the LDW, by dredging approximately 84 acres of the LDW. EPA's proposed plan also presents tissue preliminary remediation goals (PRGs) that cannot be achieved in an urban waterway, such as the LDW. The analyses performed in the RI/FS show that a reduced dredging footprint would be just as effective at reducing risks in this waterway with a large upstream sediment load, and greatly reduce the costs and construction-related impacts of remediation. In addition, rather than establishing tissue cleanup levels in the record of decision, target tissue concentrations would be more useful if they were established for risk communication purposes and to track the remedy's progress toward achieving the remedial action objectives.

482 Monitoring of Contaminated Sediments Before and After Dam Removal on 12 Mile River; Pickens County, SC

C. Zeller, USEPA – Region 4 / Superfund Division

In 1994, the Region 4 Office of the US Environmental Protection Agency (EPA) issued a Monitored Natural Recovery (MNR) Record of Decision (ROD) for the Sangamo Weston/12 Mile River/Lake Hartwell Superfund Site located in Upstate South Carolina. Annual monitoring conducted since the ROD was issued indicates a steady decline of PCB concentrations in surface sediments of the 12 Mile Arm of Lake Hartwell that is attributed to physical processes such as burial, mixing, and dispersion. PCB concentrations in surface sediments have been below the 1 mg/kg cleanup level for the past several years. PCB levels in *corbicula* tissue have shown a 90% decline since source control efforts were largely completed in the mid-1990s,

although additional studies have shown the need for further source control work. However, declines of PCB concentrations in fish tissue have not kept pace with those observed in surface sediments and *corbicula*. A Federal Consent Decree was issued in 2006 to settle Natural Resource Damage claims. A major component of the Trustee agreement involved the removal of two, turn of the century textile mill dams from 12 Mile River. This work was completed in 2011 and generally included hydraulic dredging/mechanical excavation of approximately 450,000 cubic yards (CYs) of sediment entrained behind the dams, sediment processing, water treatment, material disposal in a 10 acre Sediment Management Unit (SMU), and stream restoration. Concerns regarding PCB concentrations in residual sediments that remained in the 12 Mile River channel post dam removal required EPA to initiate a Supplemental Remedial Investigation 18 years after the ROD was issued. This presentation will discuss the 12 Mile River dam removal project and how the monitoring program for the Sangamo Weston/12 Mile River/Lake Hartwell Superfund Site has been modified pre- and post-dam removal.

483 Monitoring Cap Effectiveness Over Time Using Profiling SPME: A Case Study of the West Branch Grand Calumet River

C.L. Thomas, The University of Texas at Austin / Civil, Architectural and Environmental Engineering; D.D. Reible, The University of Texas-Austin, University of Texas / Environmental and Water Resources / Dept. of Civil & Environmental Eng.; K. Gustavson, P. Schroeder, US Army Corps of Engineers

In May 2012 and September 2013, solid-phase microextraction (SPME) fibers were deployed in an effort to characterize the performance of a sediment cap. The cap consisting of approximately 6 inches of organoclay and 12 inches of sand covering 345,000 cubic yards of contaminated sediment was completed in May 2012. The SPME fibers used in this study were made of a 36.4 µm polydimethylsiloxane layer surrounding a glass core with a diameter of 486 µm. Rapid uptake of PAHs in the fiber occurs without interference of colloidally-bound contaminants, and this provides an improved measure of dissolved COC concentrations in porewater. Porewater provides a direct measure of bioavailable contaminants in sediment, and indicates potential exposure for benthos. The primary goal of the May 2012 study was to evaluate the initial conditions as defined immediately after cap placement and therefore provide a baseline for evaluating the long-term performance of the cap. Trends of high concentrations within the underlying native sediment and low concentrations within the capped and near surface regions or relatively low concentrations at both depth and near the surface were seen at most of the twenty-one sampling locations. The clear differences between the cap layer and the underlying native sediment porewater concentrations can be compared to such differences measured in the September 2013 study to determine the effectiveness of the cap approximately a year after cap placement and the effect of any consolidation induced migration. Sampling locations where migration trends through the cap and/or exceedances of surface water quality criteria are noted will be presented and compared between the two studies.

484 Storing contaminated marine sediments in a fresh water sludge deposit, benefits and risks

A. Wijdeveld, DELTARES

In the Netherlands, a large part of the contaminated sediments have a marine origin. At the same time, the Dutch government has invested in a large (20 mln. m^3) fresh water deposit for the storage of mainly Rhine related contaminated sediments. With the improvements in the fresh water and sediment quality of the river Rhine since the 80 ties, the need for a fresh water deposit for contaminated sediments is dwindling. An option is to use the deposit for the storage of marine contaminated sediments. To estimate the impact of the storage of marine sediments in this deposit, we have sampled contaminated marine sediments in a small (2 mln. m^3) marine sediment deposit. We analyzed the total concentration and the pore water concentration of the salts and metals in the sediment. We also measured supportive sediment and pore water parameters, like the CEC (measured for Na^+, K^+, Mg^{2+} and Ca^{2+}), redox potential, pH, EC, DOC, OC and TIC. With this input we calibrated a geochemical speciation model (CHARON). This model was used together with a MODFLOW schematization of the deposit for reactive transport in the sediment. The reactive transport model could then be used to predict the emission to the surface water for salts for the coming 100 years. The deposit discharges into a large fresh water reservoir (Lake IJsselmeer). While the contribution of the deposit discharge on the overall salinity of Lake Ijsselmeer is limited (with a maximum of 1,5% of the total allowed dissolved salt concentration during a dry summer), the current salinity during dry summers is already close to the allowed limit. We also calculated the emission of metals from the sediment to the surface water for both saline and fresh surface water for a period of 40 years. The overall emission of metals is reduced, mainly due to the decrease in the chloride metal ligand concentration. For most metals the impact of fresh surface water on the emission is minimal. All emissions are well below (< 1%) the MAC for surface water. The relative low metal emission might be Rhine water specific, due to the high pH and alkalinity of the river Rhine. Based on the measured concentrations and the calculated emissions for the coming decades, the Dutch authorities can judge if the storage of marine sediments in a fresh water lake is beneficial with regard to the cost and overall emission, or poses a too high threat for the salinity of the fresh water reservoir.

485 Great Lakes Legacy Act approach to sediment remedy effectiveness – results of a multi-Agency workgroup effort

A.P. Mucha, USEPA / Great Lakes National Program Office; M.A. Mills, USEPA, ORD, USEPA / Remediation and Pollution Control Division, NRMRL; D.M. Walters, USGS / Fort Collins Science Center; B.R. Jones, USEPA GLNPO / Great Lakes National Program Office; M. Loomis, USEPA GLNPO

The Great Lakes legacy act (GLLA) is housed in the Great Lakes national program office of USEPA and is a relatively new program specifically focused on sediment remediation at Great Lakes areas of concern. It is a voluntary non-enforcement program that requires a non-Federal cost share to implement its work. There are some process similarities to Superfund but differs in several key aspects including not utilizing a risk-based paradigm. Therefore defining and measuring a successful sediment remediation for GLLA will overlap with remedy effectiveness (RE) at Superfund sites but will also require some unique considerations. Building upon existing collaborations with other EPA programs and outside agencies, a GLLA RE workgroup was formed. The workgroup reviewed the current state of the science in RE methods and outlined the key factors in selecting these methods. An approach emerged that calls for collecting data not only along the chemical and physical lines of evidence (e.g., SWAC and volume remediated) but also biological lines which more closely align with the ultimate goal of the remediation. In addition the approach outlines a framework to understanding the site which ties the conceptual site model to the RE data collected in an iterative fashion. The framework's holistic approach will most clearly make the case for a remedy's effectiveness. The GLLA RE approach will be discussed describing both the recommended RE method selection process as well as the decision framework.

486 Metal Fate and Transport Simulation Using SWAT in the Tri-State Mining District

M. Niazi, Office of Research and Development; J.P. Schubauer-Berigan, M. Hantush, B. Dyson, K. Bailey, USEPA

The Tri-State Mining District (TSMD) has been the center of mining activities to extract ores containing lead, zinc, cadmium and other metals for decades. Transport of remaining debris from mining activities via overland runoff led to the listing of Spring River as an impaired surface water. For the first time, the Soil and Water Assessment Tool (SWAT) model was developed and calibrated as part of an integrated modeling system to investigate fate and transport of heavy metals in the overland flow and stream network for The Spring River Watershed comprising approximately 6,000 km^2. The primary goals of developing the SWAT model were a) to identify hotspots for heavy metals where concentration of the metals exceeds the standard levels, and b) to estimate annual average mass flux for sediment and heavy metals for the Spring River and its tributaries in order to determine how many years will be required for natural recovery to achieve reduced toxicant level in the sediments and water column necessary to minimize the risks to human health and the environment. To do this, two USGS gages, Spring River and Shoal Creek, were selected to calibrate the SWAT model for flow, total suspended sediment (TSS) and heavy metals (lead, cadmium and zinc) on yearly and monthly bases from 2003 to 2010. While the results shows a satisfying goodness of fit (R^2 = 0.95 yearly and R^2 = 0.73 monthly) for flow, the results for sediment is fair (R^2 = 0.68 yearly and R^2 = 0.35 monthly) and the calibration for heavy metals is ongoing. Karst formation of geology in the watershed could be the primary reason for achieving the lower R^2 values.

Nanotechnology: Part C

487 Toxicogenomic responses of Caenorhabditis elegans exposed to silver nanomaterials

D.L. Starnes, University of Kentucky / Plant and Soil Sciences; C. Starnes, University of Kentucky / Biostatistics, Epidemiology, and Research Design; Center for Clinical and Translational Science; J. Smith, University of Kentucky; E.K. Oostveen, University of Kentucky / Department of Plant and Soil Sciences; J.M. Unrine, University of Kentucky; B. Collin, P. Bertsch, University of Kentucky / Plant and Soil Sciences; O.V. Tsyusko, University of Kentucky / Department of Plant & Soil Sciences

There are over 2000 consumer products containing manufactured nanomaterials (MNMs) available today. One of the MNMs of major concern is silver nanoparticles (AgNPs). During wastewater treatment, AgNPs can undergo transformations resulting in partially or fully sulfidized AgNPs. We aim to understand the bioavailability and toxicity of polyvinylpyrrolidone (PVP) coated AgNPs (Ag-PVP) and fully sulfidated AgNPs (Ag-S) to a model organism *Caenorhabditis elegans* using a toxicogenomic approach. Since AgNP toxicity can also be determined by dissolution and release of Ag+, AgNO3 was included as an additional treatment. Our objectives were to examine the particle and ion specific toxicogenomic effects of Ag-PVP, Ag-S, and Ag+ as well as their mechanisms of toxicity. Our results showed that for AgNPs, Ag-PVP are more toxic to *C. elegans* than Ag-S due in part to a greater bioavailability and uptake as evidenced by synchrotron-based x-ray microscopy. Evidence suggests that the observed toxicity is partially particle specific for both Ag-PVP and Ag-S because nematodes exposed to particle free supernatants showed very low mortality. Among endpoints screened, reproduction was the most sensitive and used for the microarray study. The transcriptomic data indicate that each treatment produced a distinct genomic response. Ag+ had the largest number of differentially expressed genes (312) followed by Ag-S (223), and lastly by Ag-PVP (136). Of the total number of significant differentially expressed genes only 3% were shared among all of the treatments and 86% were uniquely expressed for the respective treatments. The genomic data support the hypothesis that the observed responses are partially particle specific, since both Ag-PVP and Ag-S have a unique set of genes that are not shared with the Ag+ treatment. In addition, our results demonstrate that C. elegans transcriptomic response to the Ag-S are distinct from those to Ag-PVP resulting in their different toxicities.

488 Influence of natural organic matter on the toxicity and bioaccumulation of functionalized ceria nanoparticles in Caenorhabditis elegans

B. Collin, University of Kentucky / Plant and Soil Sciences; E.K. Oostveen, University of Kentucky / Department of Plant and Soil Sciences; O.V. Tsyusko, University of Kentucky / Department of Plant and Soil Sciences; J.M. Unrine, University of Kentucky

Ceria nanoparticles (CeO_2 NPs) are increasingly being used for a variety of applications including fuel catalysts and mechanical planarization media; however, little is known about their fate, transport and possible toxicity in the environment. Once released in the environment, CeO_2 NPs will interact with abundant organic ligands, and particularly natural organic matter (NOM) known to strongly influence surface properties and aggregation behavior. Our objective is to investigate the role of nanoparticle surface charge and interactions with NOM in determining their bioavailability and toxicity to a model organism (*Caenorhabditis elegans*). CeO_2 NPs with a mean geometric diameter of 4 nm were synthesized and coated with 10 kDa dextran (DEX). This coating was then functionalized to confer either a net positive charge (diethylaminoethyl dextran; DEAE), or a negative charge (carboxymethyl dextran; CM). The positively charged DEAE CeO_2 NPs were far more toxic to *C. elegans* and were bioaccumulated to a greater extend than the neutral and negatively charged DEX and CM CeO_2 NPs. Spatially resolved XANES demonstrated that while Ce in the particles as synthesized was mainly in the +IV valence state in the exposure media, Ce associated with nematode was present as a mixture of +III and +IV valence state. Within *C. elegans*, the surface charge of CeO_2 NPs significantly influenced the proportion of the two valence states, with a higher proportion of Ce +IV in DEAE CeO_2 NP exposed *C. elegans* than in DEX and CM CeO_2 exposed animals. These results suggest that the coating surface charge influenced the Ce speciation in *C. elegans* and that the mechanism of toxicity may involve oxidation of biomolecules coupled with reduction of Ce in the particles. The addition of NOM, a humic acid standard, to the more toxic NPs, DEAE CeO_2 drastically decreased the toxicity even at low NOM concentrations. NOM caused DEAE NPs to aggregate in the exposure media, but the size and stability of the aggregates were dependent of the ratio between NOM and NPs. The synchrotron based X-ray fluorescence measurements showed that NOM influence the bioaccumulation: by increasing the Ce content in *C. elegans* at a low ratio of NOM to NPs and by decreasing Ce content for a higher ratio i.e. a higher NOM concentration than NPs. These results show that NOM decreased NPs toxicity but the Ce bioaccumulation depends on the nature of the aggregates formed between NOM and CeO_2 NPs.

489 Intracellular trafficking and toxicity of silver nanoparticles in Caenorhabditis elegans

X. Yang, Nicholas School of the Environment; A.R. Badireddy, R. Taggart, Duke University / Civil and Environmental Engineering; H. Hsu-Kim, Duke University / Department of Civil and Environmental Engineering; M. Wiesner, Duke University / Civil and Environmental Engineering; D.E. Hinton, Duke University; J.N. Meyer, Duke University / Nicholas School of the Environment

Nanoparticles (NPs) have unique physical properties, and may also exhibit unique mechanisms of organismal transport and toxicity. We used *Caenorhabditis elegans* to study the role of intracellular trafficking in silver NP uptake and subsequent toxicity. The intracellular trafficking of silver NPs includes endocytosis, endolysosomal and lysosomal pathways. Because we wanted to focus on NP uptake and effects, we used citrate-coated silver NPs (CIT), which dissolve the least of all the silver NPs we have tested, reducing the importance of ionic silver in their toxicity. Electron microscopy demonstrated that silver ions cause toxicity in a fashion distinct from that caused by silver NPs. Two pharmacological clathrin-mediated endocytosis inhibitors, phenothiazine and Chlorpromazine, reduced silver NP toxicity. We also tested three endocytosis-deficient mutants (*rme-1*, *rme-6* and *rme-8*) and two lysosome- deficient mutants (*cup-5* and *glo-1*). *rme-6* mutants showed less CIT uptake than N2s and were also more resistant to CIT than N2s. *glo-1* mutants were more resistant to CIT acute toxicity than N2s, but demonstrated much higher sensitivity to reproductive toxicity. The reproductive toxicity was mediated by a dramatic lack-of-vulva phenotype that was never observed in silver NP-exposed N2s. These results highlight the critical role that intracellular trafficking plays in silver NP toxicity *in vivo*.

490 Gene Expression and Membrane Disruption of Daphnia magna Gut Exposed to Functionalized Nanomaterials

G.A. Dominguez, School of Freshwater Sciences – University of Wisconsin Milwaukee; S. Lohse, University of Illinois / Department of Chemistry; M. Torelli, University of Wisconsin-Madison / Department of Chemistry; C. Murphy, University of Illinois / Department of Chemistry; R. Hamers, University of Wisconsin-Madison / Department of Chemistry; R. Klaper, University of Wisconsin-Milwaukee / School of Freshwater Sciences

Environmental concern has been raised due to the potential presence of nanomaterials entering aquatic environments from industry and other sources. However, the impacts of surface chemistry of nanoparticles on their potential environmental impact have been minimally explored. In many aquatic species the main point of interaction of nanomaterials with the organism is the gut as they ingest particulates as they would food particles. The goal of this project was to evaluate the effects of functionalized nanomaterials with varying properties within the gut of *Daphnia magna*. Daphnia were exposed to a range of sublethal concentrations of gold and diamond nanoparticles carrying different surface functional groups: CTAB (cetyltrimethylammonium bromide), CIT (citrate), polyethylene glycol (PEG), and PAH (polyallylamine hydrochloride) for 24 and 48 hours. The impact of nanoparticle exposure in the dissected guts was measured using gene expression associated with oxidative stress and particle uptake, and membrane destabilization accompanied by subsequent oxidative stress. We determined expression of oxidative stress and particle uptake associated genes changed with surface chemistry, nanoparticle concentration, and longer time exposure. The production of reactive oxygen species was examined by staining dissected guts with indicators of oxidative stress: dichlorodihydrofluorescein diacetate (H2DCFDA) or dihydroethidium bromide (DHE), and observing stained tissues under fluorescence microscopy. Membrane permeability and production of reactive oxygen species also increased with higher nanoparticle concentration and longer time exposure. The outcome of this study reveals that it is possible to evaluate gene expression and membrane disruption along with production of reactive oxygen species at a fine scale in the gut

of *D. magna*. In addition, these findings may contribute to expand the knowledge-base on the likely impact of functionalized nanoparticles on the aquatic fauna using an *in vivo* study.

491 Sex Specific Nanomaterial Protein Coronas Following Exposure to Fish Plasma

C.M. Lavelle, Center for Environmental Health and Toxicology; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology

Toxicity screening of namomaterials (NMs) relies on the ability to link high-throughput assessments with whole organism level effects. Interactions of NMs with the proteins in biological environments, including culture media and plasma, allows for the formation of a protein corona that binds proteins with different affinities. It has been shown that the identity of proteins in the corona surrounding the NMs surface can direct bio-distribution *in vivo.* Identification of preferentially bound proteins may elucidate which biological processes or tissues are most susceptible to toxicity by NM exposure. Additionally, studies have shown that NM characteristics (including surface hydrophobicity and size) dictate the protein composition of the corona. The protein composition of fish plasma is sexually distinct, with the plasma of reproductively mature female fish containing high levels (10-20 mg/mL) of the egg yolk pre-cursor protein, vitellogenin (VTG). In oviparous fish, VTG is synthesized in the liver before being transported through the blood for incorporation into developing oocytes. VTG serves an important function as a carrier of essential minerals to developing oocytes and this mechanism of transfer has also been exploited by hydrophobic contaminants. It is possible that NMs may also be transferred to developing oocytes in this manner as well. A series of in vivo studies found that quantum dot (QD; or constituent) uptake into ovaries to be significant following multiple oral doses over 2 weeks as opposed to a larger single oral dose. This supports the idea that VTG can act as a transporter into developing oocytes, which occurs in only a fraction of oocytes at a given time in the fish species studied, the fathead minnow. When QDs with three different surface chemistries were incubated in cell culture media with male plasma or estrogenized male plasma (17ug VTG/mL media) an increase in the hydrodynamic radius was seen in the QDs that had been surface functionalized with PEG terminated with amino groups. Recent studies are using isobaric tagging for relative and absolute quantification (iTRAQ) and mass spectrometry to identify and quantify proteins in the corona of different NMs. Specifically, we are analyzing samples for sex specific protein corona differences of differentially surface modified NMs. Quantitative proteomics enables a better understanding of the protein corona on NMs and how these proteins may influence their effects *in vivo*.

492 Nanoceria biotransform in root epidermis of Kidney bean (Phaseolus vulgaris) and demonstrate antioxidant properties in the tissues

S. Majumdar, UTEP / Chemistry; J.R. Peralta-Videa, The University of Texas at El Paso; J. Gardea-Torresdey, The University of Texas-El Paso

As reported in previous literatures, nanoceria can be taken up by different edible plants with variable physiological responses depending on the plant species. But there is scarce information on the interaction of the nanoceria with the plant at the biochemical level. Kidney beans (*Phaseolus vulgaris)* are an important legume crop and one of the major sources of proteins worldwide. In this study, 15 day-old kidney bean plants were exposed to nanoceria suspensions (0-500 mg/L) for 15 days in hydroponic culture. Upon harvest, quadruplicate plant tissue samples were analyzed for cerium accumulation and stress enzyme activities. Samples were also processed for micro-X-ray absorption spectroscopy (μ-XAS) and scanning electron microscopy (SEM) analyses. Using ICP-OES, we found that Ce was taken up passively by the roots in the first 24 h of exposure. With prolonged exposure of 7 and 15 days, there was saturation in the Ce concentration in the roots exposed to 125-500 mg/L nanoceria. Cerium concentration in the aerial tissues did not increase with exposure period. SEM confirmed the presence of Ce in the tissues, while μ-XAS of roots showed that a fraction of CeO_2 in the epidermis was biotransformed to $Ce(SO_4)_2$ and $Ce(CH_3COO)_3$. $nCeO_2$ did not affect the antioxidant system upon 7 day exposure, whereas on prolonged exposure for 15 days, the antioxidant enzyme activities decreased in the roots, which can be attributed to the ROS scavenging ability of $nCeO_2$. Whereas in the leaves, GPOX was the main enzyme associated with the stress resulting from $nCeO_2$ exposure. It seems that the nanoceria utilize their unique property of reversible conversion of oxidation states to cope with oxidative stress in kidney beans.

493 Nanomicex – Ecotoxicity of pristine and modified pigment nanoparticles

T.F. Fernandes, Edinburgh Napier University / School of Life Sciences, Heriot-Watt University

A variety of nanoparticles are used as pigments in paints and dyes and for medical applications. Exposure to these particles can be experienced in an occupational setting during their production and incorporation into the finished product, application and refinement (such as sanding), as well as by the end user. Nanoparticulate pigments can also reach the environment through waste, spills, abrasion and wash-off. Nanomicex is a recently launched FP7 project combining partners from industry and academia, in which nano-sized pigments produced by the industrial partners are investigated for their toxic effects in the environment and on human cell lines. The particles used include silver (Ag), titanium dioxide (TiO_2), aluminium oxide (Al_2O_3), cobalt aluminate spinels ($CoAl_2O_4$), cadmium selenide core quantum dots with a zinc sulfate shell (CdSe/ZnS), iron oxide (magnetite, Fe_3O_4) and zinc oxide (ZnO). The environmental test organisms are *Daphnia magna* (water flea), *Pseudokirchnerialla subcapitata* (microalga) and *Lumbriculus variegatus* (blackworm). Endpoints investigated on the environmental level include mortality, growth and reproduction for *D. magna* and *L. variegatus*, biomass for *P. subcapitata,* particle bioaccumulation, thorough characterisation of particles in the test media, and where possible mechanistic endpoints such as oxidative and metal stress. Initially, pristine nanoparticles will be investigated. Toxic particles will then be modified by the project partners to reduce adverse effects in the model organisms and human cells while preserving the properties which make them valuable as paints and dyes. In addition, the project seeks to establish structure-activity relationships and to investigate realistic exposure scenarios. The collaboration with the industrial partners, the combined information on human and environmental hazards, as well as the facilities to modify particles to keep them suitable for their intended application while minimising the hazard to environmental and public health make Nanomicex a very promising project for investigating realistic steps towards safe use of nanomaterials.

494 Preconcentration of silver nanoparticles using carbon nanotubes as an adsorbant for solid phase extraction

E. Hoque, Environmental & Resource Studies; C.D. Metcalfe, Trent University / Environmental & Resource Studies

Because of its antibacterial properties, silver nanoparticles or nanosilver (nAg) are widely used in consumer products, such as textiles, personal care products, food containers, laundry detergents and home appliances. It is likely that nAg is discharged with wastewater from municipal and industrial sources into aquatic ecosystems. However, modelled estimates of concentrations of nAg in treated wastewater and surface waters indicate that concentrations of nAg will be in the part per trillion range; below the limits of detection for analysis by inductively coupled plasma emission mass spectrometry (ICP-MS). Hence, it is important to develop analytical methods to preconcentrate nAg in samples of water and wastewater. In this study, we used carbon nanotubes (CNT) as a sorbent for solid phase extraction (SPE) of nAg and other Ag species, and then analyzed the extracts by ICP-MS. The CNT surface has a strong interaction with other molecules or atoms because of its hexagonal arrays of carbon atoms in a graphene sheet structure. Different types of CNTs, including NH_2 and COOH functionalized material are being investigated as the solid phase. Experiments conducted with suspensions of PVP (polyvinylpyrrolidone*)* and citrate capped nAg (50 nm) purchased from NanoComposix (CA, USA) spiked at 10 ppb into 50 mL of Milli-Q water showed that SPEs composed of CNT-NH_2 efficiently extracted nAg. Good recoveries (~80 to 88%) were achieved through a single 10 mL elution of the column with 20% HNO_3. The amount of Ag extracted increased linearly with the concentration in suspension over column loadings of 0.50 to 167 ng Ag per mg CNT. The method is currently being optimized to maximize the degree of preconcentration. However, the CNT columns extract both nAg and dissolved Ag (dAg), so other available separation or preconcentration techniques are being explored to differentiate between nAg and dAg in water. These methods will be used to investigate the fate of nAg released into aquatic ecosystems, including surface waters downstream of wastewater treatment plants.

Pyrethroid Pesticides in Aquatic Environments: Exposure, Effects, andMitigations: Part B

495 The Effect of Embryonic Exposure to Deltamethrin on Brain-derived Neurotrophic Factor Expression and Aggression in the Zebrafish

T.S. Kung, Rutgers, The State University of NJ; K.R. Cooper, J.R. Richardson, Rutgers University; L.A. White, Rutgers University / Department of Biochemistry and Microbiology

Pyrethroids are commonly used insecticides that are generally considered to pose little risk to human and environmental health. However, there is increasing concern that children are more susceptible to the adverse effects of pesticides. Pyrethroid primarily bind to and hold open the voltage gated sodium channels, thereby prolonging membrane depolarization. Brain-derived neurotrophic factor (BDNF) is an activity dependent neurotrophic factor that is involved in regulating synaptic plasticity and long-term potentiation. BDNF binds to the TrkB tyrosine kinase receptor and initiates MAPK and CAMKII signal transduction. Because of its role in neurodevelopment and association with neurological and behavioral disorders, including activity and aggression, we hypothesize that developmental exposure to pyrethroid pesticide deltamethrin leads to persistent behavioral alterations via BDNF upregulation. Zebrafish embryos were treated with deltamethrin at a dose below the LOAEL (0.5 µg/L) and a dose that elicited acute poisoning symptoms (25 ug/L), during the embryonic period (3-72hpf) using a static non-renewal water exposure. After 72hpf, embryos were either harvested for RT-qPCR or reared in pesticide free water until adulthood for behavioral analysis. At 72hpf, embryos exposed to deltamethrin exhibited a statistically significant 3.5-fold increase in BDNF transcript levels. To determine whether developmental deltamethrin exposure altered swimming behavior, we quantified the swim activity of 2-week old zebrafish (larval stage) and 1-year old zebrafish (adult stage) using the Noldus Ethovision system. We observed significant increases in swim activity as early as 2 weeks after removal from treatment which persist into adulthood. Also, during adulthood, dominant male fish exhibited statistically significant increases in aggressive behavior, quantified by counting the number of times they attacked their own image in a mirror. Taken together, this suggests that neuronal overstimulation during development due to pyrethroid exposure may increase BDNF expression and result in permanent behavioral disorders. NIEHS R56ES018863, T32ES007148, R01ES015991

496 First report of pyrethroids in river fish: a case study in Iberian river basins (Spain)

C. Corcellas, IDAEA / Química Ambiental; E. Eljarrat, IDAEA-CSIC; D. Barcelo, IIQAB-CSIC, ICRA / Environmental Chemistry, ICRA / Catalan Institute for Water Research, IDAEA-CSIC / Environmental Chemistry Department

Lately, it seems to be clear that the presence of pyrethroids in the environment is almost ubiquitous. Some authors determined levels of pyrethroid in rivers (water and sediment). Knowing that, our study involves for the first time the determination of pyrethroid in fishes of different Iberian rivers, e.g., the Ebro, the Llobregat, the Júcar and the Guadalquivir. 35 samples of different fish species and different size were collected at selected sampling points along each basin river. The analyzed species were barbels (*Luciobarbus sclateri*, *L. graellsii*, *Barbus guiraonis*), carps (*Cyprinus carpio*), and trouts (*Salmo trutta*). Whole fish were analyzed. Each individual was homogenized, freeze-dried and stored until analyses at -20ºC. Sample preparation consisted in an extraction with hexane:dichloromethane (2:1) and a tandem SPE cleanup with alumina and C18 cartridges. Finally, pyrethroids were determined by a GC-MS-MS system working with negative chemical ionization. The analytical method included 12 different pyrethroids: bifentrhin, cifluthrin, cypermethrin, cyhalothrin, deltamethrin, fluvalinate, fenvalerate, permethrin, phenothrin, resmethrin, tetramethrin and tralomethrin. The first remarkable result was that pyrethroids were detected in all the tested samples, indicating the bioavailability and bioaccumulation of these pesticides by river fish. Concentrations of pyrethroids in fishes ranged between 10 and 590 ng/g lipid weight (lw), with the highest levels being found in areas with greater agricultural impact. The most abundant pyrethroids were cypermethrin and permethrin, with a contribution of 60 and 23% of the total pyrethroid contamination. In contrast, fluvalinate, phenothrin and resmethrin were never detected. Finally, it should be pointed out that pyrethroid isomerism could affect toxicology. For this reason, isomeric and enantiomeric studies have been also carried out in order to determine potential isomeric- and/or enantiomeric-selective accumulation processes by fish. For this purpose, an additional analysis through a chiral GC column was performed.

497 Pyrethroid concentrations and biological effects in the lower American River

S.L. Clark, R. Ogle, A.S. Gantner, Pacific EcoRisk; C.M. Harbourt, Waterborne Environmental, Inc.; G.A. Hancock, Waterborne Environmental, Inc. / Ecotoxicology; T. Albertson, Caltest Analytical Laboratories; J.M. Giddings, Compliance Services International; G. Mitchell, FMC Agricultural Solutions; A.C. Barefoot, DuPont Crop Protection / Crop Protection Products; D. Tessier, DuPont Crop Protection; M.G. Dobbs, Bayer CropScience / Ecotoxicology Group; K.S. Henry, Syngenta Crop Protection, LLC / Ecological Sciences

The American River is considered to be a high quality water source for the Sacramento metropolitan area. Previous studies of the lower American River reported that pyrethroid insecticides were detected in water samples collected over a 30-km reach of the lower American River at concentrations that were reported to be toxic to the amphipod *Hyalella azteca*. Beginning in October 2011, we initiated a monitoring study with the goal of providing a robust picture of pyrethroids in the lower American River. Water samples were collected during 3 rain events and 3 dry events along 7 cross-river transects, with 5 horizontal stations per transect and 3 depths per station, resulting in a maximum of 105 samples per event. All water samples were analyzed for bifenthrin, cyfluthrin, lambda-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, and permethrin; reporting limits for these pesticides were 1.5 ng/L, except for deltamethrin and esfenvalerate (3 ng/L) and permethrin (15 ng/L). All pyrethroids were

498 Compilation and Evaluation of Environmental Residue Monitoring Data for Synthetic Pyrethroids

J.M. Giddings, J.R. Wirtz, Compliance Services International

In recent years, there has been an increasing amount of chemical monitoring for synthetic pyrethroid residues in sediment and water column samples from urban and agricultural streams. These data provide an important resource for understanding the significance of exposure assessments based on modeling using conservative screening-level models and scenarios. Compliance Services International collated reported measurements of pyrethroid residue concentrations in water and sediment samples from US sites on behalf of the Pyrethroid Working Group as part of an ecological risk assessment of synthetic pyrethroids. Open literature publications, unpublished reports, and databases (e.g., the National Water Quality Assessment Program and the Washington State Surface Water Monitoring Program) were searched and evaluated, and relevant and reliable data were compiled in an Access® database. The database contains more than 20,000 individual analytical results for the nine pyrethroid active ingredients of interest (bifenthrin, cyfluthrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, lambda-cyhalothrin, permethrin, and tefluthrin). Key metrics recorded include sample location and date, sample type, water body type, watershed use, analytical method and reporting limit, and Quality Assurance/Quality Control approach. The data are being analyzed to determine the distribution and co-occurrence of pyrethroid residues in sediment and surface water as a function of sample type, water body type, watershed use, and geographic area. The results will be compared with output from modeling of agricultural and urban residential exposure scenarios, and with acute and chronic toxicity data for fish and aquatic invertebrates.

499 Fate and transport of the pyrethroid, tefluthrin, within an agricultural ecosystem

S. Mueting, Southern Illinois University Carbondale / Department of Zoology; M.J. Lydy, Southern Illinois University Carbondale / Fisheries and Illinois Aquaculture Center and Department of Zoology

Agricultural insecticides are used to increase yields and minimize economic loss from crop damage due to pests. Plant-incorporated protectants like transgenic insecticidal proteins (e.g., Bt maize) are commonly used in the US and farmers can also apply chemical insecticides for added protection. It is becoming increasingly popular for farmers in the Midwestern US to use the synthetic pyrethroid tefluthrin with Bt maize to control insect pests in agricultural settings. However, the environmental fate of tefluthrin needs further investigation as this information is critical to understanding exposures to non-target species. Therefore, the fate and transport of tefluthrin was investigated throughout an 80-acre maize field in Central Illinois over

three field seasons from 2010 to 2012. Concentrations of tefluthrin were measured in soil, runoff water, runoff sediment, groundwater, and soil pore water before, during, and after maize planting and tefluthrin application. Tefluthrin was detected at the highest concentrations in soil samples and was not found to be transported by soil pore water or groundwater. Runoff water often contained tefluthrin (38% of samples), with 394 ng/L as the highest measured concentration. Runoff sediment almost always contained tefluthrin with a mean concentration of 133 ± 57 ng/g dw in 2012. Tefluthrin concentrations were low or near the reporting limit before planting indicating that most of the compound was metabolized or broken down in the environment before the next growing season. Some risk may be present for non-target soil-dwelling organisms from exposure to tefluthrin due to soil concentrations often detected at or above documented earthworm LC50 literature values during the growing season.

500 Exploring and standardizing the methods used with Tenax extractions

S. Nutile, A.D. Harwood, M.J. Lydy, Southern Illinois University Carbondale / Center for Fisheries, Aquaculture, and Aquatic Sciences and Department of Zoology; P.F. Landrum, N/A

Traditionally, the toxicity of hydrophobic organic compounds (HOCs) in aquatic environments has been evaluated using organic carbon normalized chemical concentrations, which can lead to the overestimation of sediment toxicity to benthic invertebrates due to changes in bioavailability of HOCs across sediments. An alternative is a chemical technique (e.g., Tenax extraction), which should provide a more accurate estimate of toxicity for HOCs in aquatic environments than organic carbon normalization. The current study was designed to better define the experimental parameters for use during Tenax extractions of pyrethroids and characterize the recovery of compounds from Tenax to determine the applicability of Tenax extractions in evaluating sediment toxicity for this compound class. The average sampling rate of Tenax estimated from water using 0.010 g of Tenax was 12.1 L/g/h, suggesting that both the sampling rate of Tenax and the bioavailable concentration of pyrethroids from sediment was limited by desorption. Furthermore, the partition coefficient of Tenax for bifenthrin was lower than that of organic carbon, suggesting that Tenax will not affect desorption of pyrethroids. Also, the capacity of 0.010 g of Tenax for bifenthrin was significantly higher than any recorded field concentration, suggesting the capacity of Tenax will not be limiting for evaluating toxicity. In conclusion, Tenax extractions should provide an accurate estimate of bioavailable concentrations of pyrethroids in aquatic environments by providing a measure of desorption of these compounds from sediment, and therefore, an estimate of the bioavailable concentration in the sediment.

501 Analysis of a Baseline Survey of Pyrethroid Pesticides in Publicly-owned Treatment Work Facilities in California

J.C. Markle; B.H. Van Buuren, van Buuren Consulting / Quality Assurance Research Group; K.D. Moran, TDC Environmental, LLC; A.C. Barefoot, DuPont Crop Protection / Crop Protection Products

In response to the 2006 California Department of Pesticide Regulation data re-evaluation, the Pyrethroid Working Group and Tri-TAC, representing Publicly Owned Treatment Works (POTWs) developed a monitoring project, for eight pyrethroid pesticides in influent, effluent and biosolids. The project surveyed 32 POTWs between January and March 2013. At each location, samples of influent and effluent were collected as consecutive grab samples and at the 19 sites that accommodated sampling of biosolids, this matrix was collected and composited. Two laboratories were used to analyze the biosolids samples as replicates and the effluent and influent samples as consecutive grabs (distinct samples). Pyrethroids were observed in the influents of all the POTWs in the survey. Effluent concentrations were less than 10% of the influent concentrations and approached the detection limit for 8 of the POTWs. The concentration of pyrethroids in the biosolids confirmed the expectation that sorption to solids would be a significant removal process; however, a mass accounting was not attempted.

Quantitative Approaches to Mega-scale Risk Assessment

502 Quantitative risk assessment at mega-scales: lessons learned from case studies

W.G. Landis, K.K. Ayre, A.J. Markiewicz, J. Stinson, Western Washington University / Institute of Environmental Toxicology

Ecological risk assessment has evolved from estimating risk at contaminated sites at scale from a few kilometers to scales that cover large geographic areas of countries and continents. The relative risk model and its Bayesian network derivative has been applied successfully by Bartolo, Van Dam and Bayliss to the tropical watersheds across Northern Australia. Our group has estimated risks at the scales of the Willamette and McKenzie watershed in Oregon, the Mid-Atlantic States and the American Southwest. The relative risk model can be used to estimate risk at large geographical scales. In our case studies the most straightforward large-scale assessments have been when one regulatory agency was involved and the endpoints and management actions were clearly defined. Standardized and intensive monitoring programs reduce uncertainty in the risk analysis. However, a number of factors increase the uncertainty. At very large scales a number of endpoints are of interest by a variety of managers with little coordination. Datasets vary widely in sampling frequency, method, analytical accuracy and statistical interpretation. Data on fate and transport often are not of the scale of the study. Different standards of coding for landscape use, habitat types and mapping projections are used hampering effective amalgamation of the information into GIS. Data taken over short periods of time also provide little information on trends and the inherent variability of the system. Finally much of the information is based upon an outdated design of comparing a selected reference area to an area of interest, eliminating data on other gradients within the landscape. We present a number of suggestions for improving large-scale risk assessments and the types of tools required for long term landscape management.

503 The Great Lakes Region as a Meta-mega PCB Contaminated Sediment Site

J.W. Kern, Kern Statistical Services, Inc.

PCBs in sediment and water adversely impact ecological and commercial resources of the great lakes region. Consumption advisories are in place for all great lakes states, reducing the economic value of commercial and sport fisheries. The sources of PCBs to the great lakes are relatively well known including large PCB contaminated sediment sites associated with carbonless copy paper recycling, hydraulic fluids and electrical equipment. Several of these rivers are individually superfund mega sites which combined could be termed a meta-mega site. In spite of obvious commonalities (issues are usually not site specific), individual mega-sites are managed independently with, at times, contentious power struggles between state and federal agencies and limited communication between project managers among sites. Often, the technical issues are miniscule relative to the uncertainties in each, and the paucity of data to resolve them. These arguments transpire over decades, during which the opportunity to develop the key data necessary to support the carefully agreed to methods is usually lost. This presentation brings together results from, Fox, Kalamazoo, Tittabawassee, Hudson Rivers and the Lower Duwamish and other superfund mega sites to illustrate a data driven approach to site management decisions that is technically defensible, easily explained, highly likely to succeed and consistent across the meta-mega scale. The primary components of this approach require managers to: 1) Balance site specific remedial goals with meta-scale objectives to reduce loads to the great lakes. 2) Recognize that evaluation and selection of a remedy takes one to two decades providing the opportunity to collect fish water and sediment time series needed to accurately forecast remedial effectiveness. 3) Recognize that mega-site contamination is extensive and dispersed—surgical removal of individual hotspots rarely elicits risk reduction. 4)Rather risk reduction requires reduction of PCB concentrations over relatively large exposure areas. 5)Conversely, localized source control, remains necessary to reduce loads to the great lakes. 6)Accurate remedy selection requires unambiguous empirical description of spatial and temporal trends in PCBs in fish, water and sediment. Sampling designs which anticipate these predictable stages and provide coherent data that can be scaled with the temporally intensifying needs are required—these are standard sample survey problems.

504 The prevalence of order in mega-scale models and ways to estimate uncertainty from them

B. Brooks, Saint Francis Xavier University / Earth Sciences

Our planet is hierarchically structured from interactions at atomic and molecular levels through synoptic and global scale circulation. Regional and global climate models (RCMs and GCMs) acknowledge this by representing interaction and circulation through a hierarchy of mathematical simplifications. RCMs and GCMs provide important inputs for understanding environmental problems. Their model systems are studied to understand patterns and causality. However, despite progress in probing ever smaller components of the Earth system and dramatic improvement in capturing climatological means there has been a notable lack of symmetry of improvement in capturing climate change and extremes. At present these mega-scale models provide more useful information about large-scale trends and constraints than about uncertainty and risk. In this presentation I show examples of determinism, regularity, and order from large-scale biosphere-atmosphere models in contrast to variability, diminished regularity, and patchiness in site level measurements. An empirical Bayesian approach is presented for mapping uncertainty in air sample surveying and for improved detection of fugitive emissions. Trends and boundary conditions from large-scale models and data sets are used to inform this probability model about time varying conditions that affect the spatial distribution of uncertainty.

505 Human Extinction and the Challenges of a Global Assessment

S.M. Bartell, Cardno Entrix

Estimating the probability of human extinction is arguably the most important risk assessment ever undertaken. The first systematic and formal mathematical evaluation of the existential risk resulted in the 1972 prognostications of Meadows *et al.* in the now famous book *Limits to Growth*. These investigators simulated the longer term (1900-2100) consequences of world population growth constrained by finite world resources. Using the World3 model, Meadows and her colleagues examined the interactions and feedback among population growth, industrialization, pollution, food production, and resource depletion. Two of the model scenarios projected global "overshoot and collapse" on the timeframe of 2020-2040. The *Limits to Growth* projections were resoundingly discredited by neoclassical economists and some systems analysts. Importantly, Turner (2008) compared the results of the original World3 model to 30 years of data and concluded that the model projections appeared alarmingly accurate. Given impending global collapse and attendant human consequences, the presentation addresses the challenges of such a global scale risk assessment. These challenges include (1) underlying structure of the World3 model, (2) critical modeling assumptions, (3) parameter estimation, and (4) data available for model development and model:data comparisons. The presentation will focus on the relative contribution of modeled pollution to simulated global collapse and conclude with refined speculation concerning the potential demise of *Homo sapiens* by 2100.

506 Ecological Modeling for Mega-Scale Risk Assessment – Can the Gestalt Conquer Chaos?

D.V. Preziosi, R.A. Pastorok, Integral Consulting, Inc.

Applying ecosystem and landscape models to large spatial-scale risk assessments poses challenges with choice of a modeling approach (e.g., agent-based model vs aggregate populations), model complexity (e.g., taxonomic resolution and spatial-temporal heterogeneity in model system structure), and interpretation of potentially chaotic behavior. In particular, the role of chaos in models of natural systems is an unresolved issue. Indeterminacy is an accepted and observable phenomenon in natural systems comprised of multiple, variable components. At the mega-scale, the interactions among many variable components can lead to highly complex dynamics – perhaps so complex as some authors postulate (e.g., Progogine and Stengers, 1984) – that precise predictions are not possible. This means that mega-scale systems that experience some form of perturbation, whether chemical, biological, or physical, are very unlikely to revert to a precise and identical baseline configuration after cessation of the perturbation. Moreover, different initial conditions may cause model output to vary substantially in response to the same perturbation. In the face of these challenges, we nevertheless contend that process-based modeling of such systems can provide critical information required to support decision-making. For example, process-based models can be used to predict both direct and indirect effects within individual food webs or even integrated, multiple food webs in a spatially explicit model. Process-based models can also be used to predict long-term trajectories towards recovery, whether the "recovered" state is defined as comparable baseline conditions or as alternately configured states of approximately equal functioning. Most importantly, process-based models can be developed to not only accept chaotic behavior intrinsic in nature, but also test the role of constraints (e.g., temperature in the ocean, competition among species) which can reduce the blur of chaos so otherwise imperceptible changes can be more readily recognized. We provide some examples of observed chaotic behavior in natural systems, how process models can be developed to provide useful information in the face of chaos, and how pattern-oriented modeling can be used to reveal dynamic equilibrium in systems while increasing the credibility of models.

507 Results of EPA's Assessment of Fish Tissue from US Rivers for Persistent Organic Compounds with implications for Aquatic Life and Human Health

J.B. Wathen, USEPA / Office of Science and Technology; L.L. Stahl, USEPA / Office of Science and Technology; J.M. Lazorchak, USEPA / National Exposure Research Laboratory; A.L. Batt, USEPA Office of Research and Development / National Exposure Research Laboratory; B.D. Snyder, Tetra Tech, Inc. / Center for Ecological Sciences; H.B. McCarty, CSC

Mercury and persistent halogenated organic compounds occur in fish tissue in US lakes, rivers, and streams. Mercury is the leading cause of fish consumption advisories, but advisories based on PCBs and DDT are also widespread. These and other persistent organo-halogen compounds both of a legacy nature and compounds of more recent manufacture and introduction into the environment occur in fish tissue. In a continuing effort to characterize the extent of contamination in the Nation's waters, EPA's Office of Water and Office of Research and Development collaborated to conduct the first statistically based survey of persistent and bio-accumulative contaminants in fish from US rivers. This national fish survey was conducted under the framework of EPA's National Rivers and Streams Assessment (NRSA), a probability-based survey designed to assess the condition of the Nation's streams and rivers. In 2008 and 2009, field teams applied consistent methods nationwide to collect samples of fish commonly consumed by humans at 542 randomly selected river locations (≥ 5th order based on 1:100,000-scale Strahler order) in the lower 48 states during June through October. They collected one fish composite sample at every sampling location, and each composite sample consisted of five similarly sized adult fish of the same species. Largemouth and smallmouth bass were the primary species collected for the study, accounting for 34% and 24% of all fish composites, respectively. Homogenate samples were analyzed for a suite of ~50 organo-halogen compounds including PCBs (21 congeners), PBDEs (8 congeners), chlordane and DDT compounds and degradation products, and other pesticides, (Aldrin Dieldrin, Mirex, and Endosulfan) by GC-ECD. Samples were collected from both non-urban (379 sites) and urban locations (163 sites). Study data indicate that PCBs, PBDEs, chlordane, and DDT compounds occur at quantifiable levels in almost every fish sample collected for the study. Ongoing analysis of the data from this study will apply both human health and aquatic life thresholds to fish tissue concentrations of these four organo-halogen contaminant groups from both urban and non-urban sites. In addition, we are examining the percentages of co-occurrence of these four compound groups at concentrations above the respective contaminant group medians.

508 Geospatial Modeling: Don't Take Your GIS Statistics Software For Granted

W.J. Warren-Hicks, Cardno Entrix / CEO; T. Bohrmann, Cardno Entrix

Almost always, geospatial models typically found in modern GIS packages are used for larger scale statistical analysis of spatial data. In most cases, the software will provide the user with a wide range of model choices, including kriging, nearest neighbor, natural neighbor, inverse distance, and many others. Users untrained in the probability theory underlying geospatial statistical methods can make naïve choices. Often, assumptions that underlay the typical available geospatial models are violated, including assumptions of isotropy and normality. Accounting for temporal effects is similarly overlooked. This talk will emphasize the need to examine geospatial data prior to choice of model structure. Examples where typical geospatial model assumptions are violated, leading to incorrect results, will be presented. Alternative

approaches, such as Bayesian hierarchical model structures that work well with messy geospatial monitoring programs will be presented and explained through case studies.

509 Updating the EPA National Guidelines for Developing Water Quality Criteria for Toxics

W. Lehmann, USEPA / Health and Ecological Criteria Division, OST, OW; K. Gallagher, USEPA / Office of the Science Advisor, Risk Assessment Forum; C.G. Delos, USEPA / Mail Code 4304; D.R. Mount, USEPA / ORD

EPA's Office of Water derives water quality criteria for the protection of aquatic life based on the *1985 Guidelines* based on a National approach. This approach contains methodologies that are being re-evaluated and updated to reflect current scientific practices and lessons learned from the application of the *Guidelines* in recent criteria development and prior Science Advisory Panel guidance. In addition to updating current practices, this effort will consider newer scientific approaches for *Guidelines* inclusion such as: developing criteria with less than the required eight Minimum Data Requirements (taxa); partitioning of sensitivity distributions (SDs) for plants, invertebrates, fish, as appropriate based on mode of action (MOA) and empirical data showing evidence of specific taxon-targeted effects; consideration of MOAs and Adverse Outcome Pathways (AOPs) in problem formulation and effects analysis; developing approaches for quantifying variability and uncertainty (probabilistic risk assessment and weight of evidence). Discussion of analyses related to these specific areas will be presented to inform further method refinement on the National scale assessment.

Ecotoxicology in Latin America: Issues and Perspectives

510 Zebrafish embryo-toxicity test in the Atoyac River, Mexico

S. Solis-Angeles, Institute for Biomedical Research. Universidad Nacional Autónoma de México / Department of Environmental Toxicology; O.A. Aguilar, Universidad Nacional Autonoma de Mexico / Medicina Genómica y Toxicología Ambiental, Institute for Biomedical Research / Department of Environmental Toxicology; R. Montero Montoya, Institute for Biomedical Research. Universidad Nacional Autónoma de México / Department of Environmental Toxicology

Industrial discharges incorporate thousands of different chemical substances on rivers and ocean, representing a hazard for aquatic ecosystems and health. The Puebla-Tlaxcala Industrial corridor in Mexico has a history of wastewater discharges from at least 23 factories, including a petrochemical plant, on the Atoyac river since the late 1980s. For this reason the Atoyac river has been considered as the most polluted river in the Central Mexican Plateau. In order to contribute with a proposal to biomonitoring program, we used the Fish embryo-toxicity test (FET) to determine the risk of the pollution in the river. In addition, our aim is to identify endpoints in the zebrafish embryonic development to improve the FET for monitoring. We collected wastewater samples from three industrial discharges and five river water samples from upstream and downstream sites with respect to the discharges. We measured 26 physicochemical parameters, including heavy metals in each sample. Seven perceptual dilutions (0.2% to 20%) of the samples were used to obtain the LC50 at 72 hours post fertilization and teratological effects in three points of embryonic development; as control group, we used the water from aquarium tanks. The wastewater samples were toxic at sample dilutions below 1%; meanwhile the LC50 of river water samples were obtained between 2 and 11% of sample dilution. All dilutions of river samples induced some type of malformation in eyes, head, symmetrical body axes and developmental delay. Yolk sac edema, somites and caudal malformations were the most common effects observed in this study. Beside, the average of heart rate was affected by both wastewater and river samples. We observed that only two wastewater samples induced embryonic arrest, a phenomenon reported in other works in zebrafish embryo exposed to pesticides or flame retardants. The Atoyac river receives discharges from textile factories, petrochemical and paper milk industries. Therefore, a complex mix of chemicals has caused an environmental collapse in this region. Our proposal is to apply the FET in this polluted site as a tool in biomonitoring programs. In addition, embryonic arrest observed in this study should be exploredand evaluatedin the future studies.

511 Environmental-health risk assessment: a case study in México

O.A. Aguilar, Universidad Nacional Autonoma de Mexico / Medicina Genómica y Toxicología Ambiental, Institute for Biomedical Research / Department of Environmental Toxicology; P. Gesundheit Montero, L. Ortega Elorza, Union of Concerned Scientists to Society (UCSS)

Currently called the Mexican Silicon Valley, El Salto and Juanacatlán are rural municipalities located in the Upper Santiago River Basin near to Guadalajara City. At the end of the 1950s, metallurgic, electronics, pharmaceutics, agrochemicals, paintings, food and textileindustriesarrived to El Salto. In 1990, the industrial corridor reached an amount of 16,000 factories. A faster industrialization and urbanization process brought environmental degradation due to the high pollution in the Santiago River and land use change. At the beginning of the 2000's, hundreds of people demanded government actions to resolve the environmental problem. However, the social conflict increased after the people perceived their own health failure. Although the local government recognized the environmental problem, few health actions have been taken. How to attend the society demand? According to the framework of environmental risk assessment (ERA), this work is a proposal to integrate health and environment in order to find possible ways to resolve environmental-social-health conflicts in countries like Mexico where legislation and environmental policies are weak in relation to pollutant control. We used the free access data base from the National Health Secretary, the Pollutant Release and Transfer Registry from the Environmental Secretary and Monthly Records of Water Quality in the Santiago River from the Water Commission of Jalisco State. We analyzed the period since 1990 to 2012 and crossed this information to determine the hotspots associated with health, water quality and pollutant sources data to determine the polluted site and possible risk exposure. In addition to data analysis, ERA literature was consulted to complete an health-environmental risk-map.

512 Assessment of risks in the jungle: Case example of the use of herbicide to control coca

K.R. Solomon, University of Guelph / School of Environmental Sciences

Conducting risk assessments in remote locations is logistically challenging. To be successful, one must engage local scientists and others to provide location-specific data. However, this exercise also can be useful as an educational and learning tool. To address identified data needs for amphibians, laboratory studies with 8 species of tadpoles of frogs from Colombia were conducted. This involved using local facilities as well as residue analysis with ELISA in place of the more widely used LC-MS. These studies showed that, under laboratory conditions, LC50 values ranged from 1,200 to 2,780 µg glyphosate a.e./L. However, under realistic field conditions in sediment-bottomed microcosms, LC50s ranged from 5,963 to 7,303 µg glyphosate a.e./L when treated with an overspray to mimic aerial application. Bioassays with terrestrial stages of frogs (juveniles and adults) were conducted with simple equipment obtained from local farm and garden suppliers. Spraying was conducted with a modified atomizer to apply consistent volumes of spray solution. These studies, carried out under conditions that mimicked the field, produced LC50 values between 4.5 and >22.8 kg a.e./ha. This study was linked with an analysis of the spatial distribution of frogs in relation to areas where coca was being produced and sprayed. Records of collections of frogs from local museums were digitized and then overlaid on areas of spraying of coca. These maps showed that species of frogs most likely to be exposed were widely distributed and commonly found in the region. Rarer species were found at altitudes where coca is not grown and were at *de minimis* risk of exposure. In another example, a study on humans was carried out with volunteers from five regions of Colombia, which were characterized by different exposure to glyphosate and other pesticides. The study assessed the effects of exposure to the coca-spray and other activities on the frequency of binucleated lymphocytes with micronuclei (BNMN). This study was conducted through the use of local medical health workers from regional clinics who were familiar to the volunteers and were trusted by the populace as impartial. Blood was collected before spraying, 4-5 d and 4 mths post-spraying. The samples were processed in a local laboratory and prepared for assessing microscopic assessment in the EU. These results showed no significant association between self-reported direct contact with eradication sprays and frequency of BNMN.

513 The environmental consequences of illicit crop cultivation in Colombia: A large scale overview of risks and challenges

A.C. Bejarano, University of South Carolina / Environmental Health Sciences; L. Correa, H. Bernal, Sistema Integrado de Monitoreo de Cultivos Ilícitos- SIMCI-UNODC

Illicit coca crops have been present in the Colombian landscape for several decades. Although the total area of these crops has declined over the last few years, their ecological and environmental damages have not been fully studied. Quantifying the environmental effects of illicit crop cultivation is not an easy task as many of these areas remain inaccessible to the scientific community. Consequently, one the most reliable and readily accessible sources of information available to addressing these potential damages come from aerial monitoring of coca crops and limited on-ground observations. In this research, we evaluated the potential environmental consequences of illicit crop cultivation through the different stages of the crop season, including potential damages associated with land clearing and soil preparation, coca crop maintenance, and alkaloid purification. Preliminary results indicate that coca crops have remained relatively stable in areas of the country lacking governmental presence. Surveys with coca growers also revealed regional differences in chemical use, partially driven by site-specific characteristics. During both, the coca growing season and during the extraction and purification of the alkaloid, contaminant residues and waste are dumped into soils and water bodies without any treatment, potentially causing localized impacts to these resources. Our analyses also show that national parks and natural reserves are not immune to the potential damages caused directly by illicit coca crops, or indirectly by the extraction and purification of the alkaloid. An additional source of concern is the spatial correlation between the presence of coca crops and the increased incidence of illegal mining, which we suspect is no coincidence. Coca crops and associated activities, in combination with other sources of landscape change such as rural urbanization, are also concerning as these activities threaten many biotic resources in Colombia. National and international awareness on the different issues

arising from illicit crop cultivation is critical to help shape better policies. These are urgently needed to protect the landscapes and biological resources of one of the 12 mega-diverse countries in the word.

514 Assessing Chemical Pollution in the Gulf of Guayaquil, Ecuador

K.P. Calle Delgado, Escuela Superior Politecnica del Litoral / Facultad de Ingeniería Marítima Ciencias Biologicas, Oceanicas y Recursos Naturales; J. Alava, School of Resource and Environmental Management; M. Montano, Escuela Superior Politecnica del Litoral / Facultad de Ciencias Naturales y Matematicas

The Gulf of Guayaquil is the largest estuary of the Pacific coast in South America (i.e., area of approximately 13,701 km^2, 230 km length). The entrance of the Gulf is located at 3ºS of Ecuador. This estuary rests on the edge of the Guayas river and the city of Guayaquil, containing about 81% of the total area of Ecuadorian mangroves, which is an ideal zone for artesanal and industrial fisheries. Urban sprawl and human development of the land associated with estuaries have degradated water and sediment quality, i.e. higher input of trace metals and organic contaminants (PAHs, PCBs, DDT), lower dissolved oxygen (DO) conditions, and changes in salinity. Several river tributaries drain into the Gulf Estuary, receiving point and non point sources of contamination. Some studies from the Municipality of Guayaquil, National Fisheries Institute, and the Escuela Superior Politécnica del Litoral have reported DO levels from normoxic (>60% saturation) to severe hypoxic (< 10% saturation) conditions, changes in salinity from 0.0 ups in the interior up to 25.0 ups at the exterior Gulf, and pH as low as 5.7 in the surface sediment. The negative effects of chemical pollution on the coastal-estuarine environment have been scarcely characterized. Based on the review of these studies, relatively high metal concentrations in sediment were reported for Hg (2.89 ppm), Pb (112 ppm), Cu (250 ppm), and Zn (550 ppm) exceeding the Effects Range Low (ERL) and the Effects Range Medium (ERM) sediment quality guidelines for Hg (0.71) and for ZN (410). Furthermore, some persistent organic pollutants (POPs) such as PCBs and DDT were still used in Ecuador after they were banned in the 1970s in developed countries, and released to soil and water bodies. åDDT concentrations were reported on the Taura River Basin, Gulf of Guayaquil, in sediments (1.36 mg/kg ww) and aquatic organisms (2.87 mg/kg ww). The DDE/åDDT ratio for these samples showed signs of relatively recent contamination by DDT-parental compound in sediment (ratio DDE/åDDTs = 0.66) and fish (ratio DDE/åDDTs=0.14) in the Taura River. Characterization of chemical pollutants and ecological risk assessments of agricultural activities and pesticide usage in Ecuador are needed to establish the proper environmental management strategies to mitigate and control the pollution problem. Future directions are also needed to understand the fate, transport and exposure in the environment of these contaminants.

515 The artificial nitrogen of agriculture: Top ecotoxicological threat for the humanity

M. Montaño, ESPOL / Departamento de Ciencias Químicas y Ambientales; P. Calle, P. Calle, ESPOL / Facultad de Ingenieria Maritima Ciencias Bioloógicas Oceánicas y Recursos Naturales

The activities of all kinds, such as agriculture, aquaculture, health care, environmental protection and resource exploitation, perfect their exercise when they are based on knowledge and fulfill a specific technology. In the perspective of Guayas Ecosystem and Tropical Knowledge these thoughts represent a challenge and a unique opportunity. It is known with certainty that cancer comes from a "bad expression" of at least four groups of genes: those activated uncontrollably (oncogenes), which inhibit cancer (suppressor genes), those which control the damage caused by toxic agents (genes repairmen) and the suicide genes of cells that function as a last defense line and determine the death of those will be carcinogenic (apoptosis). The imbalance between these genes, initiates, promotes and spreads cancer. Such "bad expression ", in hypothesis of this paper, emerges of the configuration of the nitrogen of urea impacted by the Bosch-Haber process. By the discoveries at CERN it states that the inseparable entanglement of the electron and photon gives memory to matter, suggesting that nitrogen of urea in our body remembers the stress happened during its manufacture. Health must be correlated with the chemical composition of the organism to establish its proper management. The human body is mainly composed of oxygen (65 %), carbon (18 %), hydrogen (10 %), nitrogen (3 %), calcium (1.4 %) and phosphorus (1 %). All these elements are natural because they arise from natural processes, except nitrogen, that could be artificial via the urea fertilizer industry that supports agriculture which provides food to the population. It is possible that nitrogen, due to the manufacturing process of urea, is eroding the health of this civilization. The life of every human being depends on the nitrogen. Amino acids, peptides, proteins, genes and other biomolecules are made of this element, and make up the structure, engine and consciousness of existence. For these reasons the agro food products containing natural nitrogen must be distinguished with "biogenic" term, as life generators.

516 Environmental quality of the Parana River Basing (Argentina): whole sediment toxicity test with fish

P.V. Gonzalez, Y. Elorriaga, Centro de Investigaciones del Medio Ambiente, Dto. Química, Facultad de Ciencias Exactas, UNLP, CONICET; M. Marinkovic, University of Amsterdam; D. Marino, A.E. Ronco, Centro de Investigaciones del Medio Ambiente, Dto. Química, Facultad de Ciencias Exactas, UNLP, CONICET; P. Carriquiriborde, Faculty of Exacts Sciences, Universidad Nacional de La Plata

Survival, growth, condition factor (K), hepatosomatic (HSI) and gonadosomatic (GSI) index together with a suit of biomarkers were evaluated in *Cyprinus carpio* juveniles exposed during 30 d to whole sediments samples collected at the mouth of nine tributaries along the Parana River Basin. Sediments from the upper – Pilcomayo (PI), Bermejo (BE), Negro (NE), Corrientes (CO), Feliciano (FE) – and the lower – Carcaraña (CA), San Lorenzo (SL), Arrecifes (AR) and Lujan (LU) – river basin tributaries were tested under laboratory conditions plus a none-sediment control group. Survival, K, HSI or GSI were significantly affected by the different sediments. Standard growth rate was reduced more than 80% in fish exposed to SL, AR and LU. EROD activity was increase above 40% in LU and CA and inhibit below 30% in AR. GST activity was increased more than 50% in PI and Fe. CAT and GPx were only increased above 60% and 50% in Ne and Fe, respectively. AChE was not statistically affected by sediment source either in the brain or the muscle. One intersex was found in Fe. Association among some biological variables were observed and also with some physicochemical, geochemical and chemical parameters. Sectors of the river basing with different degree of pollution could be discriminated. Acknowledgements: To the field team of the CIMA and the sampling support of Prefectura Naval Argentina. UNLP, PIT-AP-2010, CONICET PIP2010-2012 N0723.

517 Ecotoxicological risks in salmon farming in Southern Chile: The case of antiparasitaries

F. Tucca, EULA Chile Environmental Sciences Centre, University of Concepcion, Chile / Aquatic Systems Research Unit; H. Moya, EULA Chile Environmental Sciences Centre, University of Concepcion / Aquatic Systems Research Unit; E. Bay- Smith, University of Concepcion, Faculty of Natural and Oceanographic Sciences / Zoology, Bioassays Laboratory; D. Mauricio, Ecotoxicology and environmental chemistry Laboratory, National University of Mar del Plata; R. Barra, EULA Chile Environmental Science Centre, University of Concepcion, Chile / Aquatic Systems Research Unit

Salmon farming in southern Chile is an active area of economic growth being the second world producer of farmed salmon behind Norway. One of the challenges facing by this activity is the presence of diseases and parasites affecting the salmon production such as the copepod *Caligus rogercresseyi,* commonly known as "sea lice". For combating sea lice, a series of pesticide chemicals are used such as emamectin benzoate, diflubenzuron,cypermetrhyn and deltamethrin, among others. These chemicals are added as *in feed* or as baths, being introduced directly in to the marine environment.The registration of such chemicals for allowing their use in Salmon farming must proceed through a risk assessment procedure based on international guidelines (VICH). This procedure must account for the determination of a Risk Quotient between predicted environmental concentrations (PECs) and Non observed effect concentrations (NOECs) for local marine species. Based on these requirements, it is needed a comprehensive approach adapted to the Chilean conditions for an adequate risk assessment of such chemicals. A fugacity multimedia mass balance model was developed to predict PECs in a typical salmon cage under a realistic environmental scenario. In addition, bioassays with local species, in particular invertebrates, were performed with a series of such antiparasitaries, with the species *Monocorophium insidiosum*, we develop acute and chronic bioassays using also oxidative stress biomarkers as endpoints. As expected invertebrates resulted in the more sensitive species, at levels of ng g^{-1} in sediment samples for pyrethroids pesticides. Model predictions were compared with field studies, that in general gave a better fit between modeled and predicted data for

those chemicals added as *in feed,* both in water and sediment compartment. The model resulted to be sensitive to the organic carbon fraction in sediments and water velocity. This methodology resulted in a good approach to address in a preliminary way the environmental risk of such kind of chemicals in to the marine environment. Supported by FONDECYT 1110719.

Animal Alternative Methods for Evaluating Toxicity: Methods, Endpoints, and New Testing Strategies

518 Cell-based Metabolomics for Monitoring Adverse Impacts to Environmental Surface Waters

Q. Teng, USEPA / National Exposure Research Lab; D.R. Ekman, USEPA / National Exposure Research Laboratory; D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; J.E. Cavallin, USEPA, ORISE / National Health and Environmental Effects Research Laboratory; D. Skelton, USEPA; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; T.W. Collette, USEPA / National Exposure Research Laboratory,

Numerous surface waters are adversely impacted by contaminants released from sources such as WWTPs, CAFOs, mining activities, and agricultural operations. Ideally, an assessment strategy for these applications would include both chemical identification and effects-based monitoring. However, traditional effects testing methods are resource and time intensive and may not be sustainable in the long term, given the large number of ecosystems that require monitoring. Moreover, conventional effects testing often utilize large numbers of animals, making its continued use unsustainable given existing efforts to reduced animal use. Finally, only a handful of endpoints are typically measured using conventional approaches, limiting the availability of potentially critical toxicity information. A promising approach for addressing all of these concerns is cell culture-based metabolomics. By combining optimized cell-culturing techniques with NMR-based metabolomics, we have developed a relatively low cost, high throughput approach for obtaining multi-endpoint, molecular level responses using ambient water from environmentally impacted sites. Here we will describe the results of applying these techniques to assess the impacts of field-collected water samples from contaminated and reference sites on biological processes of Zebrafish (*Danio rerio*) liver cells (ZFL). These data will be interpreted in the context of an extensive set of site and chemical characterization data that will also be measured on these waters.

519 In vitro Assessment of the Disruption of Steroidogenesis in Three North American Fish Species

S. Beitel, University of Saskatchewan – Toxicology Centre; J.A. Doering, University of Saskatchewan; S. Patterson, University of Saskatchewan / Toxicology Centre; H. Prodahl, University of Saskatchewan; M. Hecker, University of Saskatchewan / School of the Environment & Sustainability

There is concern regarding exposure of aquatic organisms to chemicals that interfere with the endocrine system. One critical mechanism of endocrine disruption is impairment of steroidogenesis that can lead to altered hormone levels, altered or delayed sexual development, and ultimately reproductive failure. There is also a need to address the large number of animals required by current toxicity testing approaches, particularly when working with species native to environments of concern. The aim of this study was to develop an *in vitro* gonadal explant assay enabling the assessment of endocrine disrupting chemicals on sex-steroid production in wild fish species native to North America. Northern pike (*Esox lucius*), walleye (*Sander vitreus*), and white sucker (*Catostomus commeroni*) were sampled from Lake Diefenbaker, Saskatchewan, Canada at pre-spawn and multiple post-spawn time-points. Blood was taken and plasma was analyzed for 11-ketotestosterone (11-KT) and estradiol (E2). Gonads were excised and exposed for 24 hours to a model inducer (forskolin) and inhibitor (prochloraz) of steroidogenesis. Hormone concentrations in plasma and media were quantified using ELISA. The seasonal profile of plasma hormones had a similar trend to the seasonal profile of basal hormone production in gonadal tissue exposed to the solvent control. Tissues exposed to forskolin showed a concentration dependent increase in 11-KT and E2. Exposure to prochloraz resulted in a decrease of 11-KTand E2. These results illustrate that the gonadal tissue is undergoing steroidogenesis in an *in vitro* setting that is reflecting reproductive seasonality, and is also responsive to chemical exposure in a concentration-dependent manner. White sucker were found to be the most responsive species, with the seasonal time-point of greatest sensitivity differing between sexes. When exposed to 3 μM forskolin, white sucker males and females produced up to 15-fold greater 11-KT and 15-fold greater E2, respectively, when compared to the solvent controls. In conclusion, seasonality of reproductive function represented a critical factor that needs to be considered when using this here-established *in vitro* explant assay to assess responses of native species to disruptors of steroidogenesis. Further characterization of molecular and biochemical endpoints in the gonads and liver, and comparisons to *in vivo* effects are ongoing in an effort to validate these *in vitro* methods as a predictive ecotoxicological tool.

520 Quantitative toxicogenomics evaluation of DEHP, MEHP and the metabolites mixture using a yeast stress pathway ensemble library

J. Lan, Civil & Environmental Engineering; C. Gao, N. Gou, Northeastern University / Civil and Environmental Engineering; R. Loch-Caruso, University of Michigan; A.N. Alshawabkeh, Northeastern University, College of Engineering / Department of Civil and Environmental Engineering; A. Gu, Northeastern University / Department of Civil & Env Engineering, Biotechnology Initiative Program

Bis (2-ethylhexyl) phthalate (DEHP) is a widely used plasticizer with high mobility, and a ubiquitous environmental contaminant. It is rapidly hydrolyzed to mono (2-ethylhexyl) phthalate (MEHP) and subsequent metabolites *in vivo*. The toxicity mechanism of DEHP and its metabolites are still not fully understood yet. In this study, DHEP, MEHP and the DEHP/MEHP metabolite mixture generated by rat S9 fraction metabolism were evaluated using a high-throughput toxicogenomics approach, a GFP-tagged yeast stress pathway ensemble library across six-log concentrations below cytotoxic concentrations. The distinct concentration-response curves based on PELI index (protein expression level index) illustrated different levels and nature of toxicity among exposures. MEHP was confirmed to be the main toxic metabolite of DEHP, with higher $PELI_{max}$ (the maximal stress effect) and lower PELI50 (concentration for 50% of $PELI_{max}$) than DEHP. DEHP metabolite mixture exhibited $PELI_{max}$ and PELI50 between those values for DEHP and MEHP, and DEHP pre-incubation with S9 did not significantly changed the stress response. The latter results suggest that DEHP was rapidly metabolized to MEHP and subsequently to less toxic metabolites. Principal component analysis (PCA) of profiles indicated different modes of action of exposures. DEHP metabolite mixture showed response signatures similar to DEHP and MEHP, it also had some responses distinctive from the former two compounds. Pre-incubation with S9 did not significantly affect the response profiles of MEHP, suggesting that the distinctive response of the DEHP metabolite mixture likely is due to metabolites other than MEHP and its metabolites. Detailed analysis suggested that MEHP seemed to alter protein function directly, with significant activation of chemical and general stress responses too. Oxidative stress seemed to be the main effect of DEHP. Metabolite(s) of DEHP other than MEHP generated by S9 also induced strong DNA damage, which need further investigation. Although oxidative stress activation was statistically significant for MEHP and DEHP metabolites, the level was below the activation threshold, suggesting it may not be their main toxic mechanism. In summary, this is the first comparative toxicogenomics study of DEHP and its metabolites that revealed their quantitative stress response profiles. The study confirmed MEHP to be a major toxic metabolite of DEHP; its toxic mode related to direct protein stress is also reported for the first time.

521 Toxicity Evolution Along Oxidative Degradation of Environmental Micropollutants

N. Gou, Northeastern University / Civil and Environmental Engineering; S. Yuan, China University of Geoscience / State Key Lab of Biology and Environmental Geology; A.N. Alshawabkeh, Northeastern University, College of Engineering / Department of Civil and Environmental Engineering; A. Gu, Northeastern University / Department of Civil & Env Engineering, Biotechnology Initiative Program

Advanced oxidation has been shown to be the most promising technologies to remove contaminants of emerging concern (CECs) from water. However, studies that investigate the temporal course of intermediates associated with toxicity evolution during oxidation process are rare, mostly due to the time and labor intensiveness of the conventional toxicity assessment procedure. In addition, little mechanistic information can be obtained from single non-specific phenotypic assay to gain insight on intermediates and toxicity relationship. In this study we demonstrated the application of a toxicogenomic-based approach for evaluation of the toxicity evolution as the results of intermediates production during the course of degradation of three

CECs, bisphenol A, triclosan and ibuprofen. Conventional toxicity test as luminescence inhibition test using *V.fisheri* (Microtox) was also performed. The overall toxicity level changes were indicated by both Microtox results and TELI (transcriptional Effect Level Index) values, and the changes in the various stress response (indicating toxicity mechanisms) were obtained. For all the three CECs tested, the overall toxicity level did not always decrease proportionally and concomitantly with the disappearance of the parent contaminant. For example, bisphenol A was degraded around 70% after 15-minutes, but the overall toxicity level was reduced only 15%. The results from the Microtox assay and toxicogenomic approach are consistent, which may indicate that our quantitative index TELI is a good qualifier of potential toxicity as we have shown in our previous work. It was observed that not only the toxicity level, but also the mode of action (MOA), shift along the degradation process of each CEC. Furthermore, these types of shift are also correlated with the transformation intermediates identification. Taking bisphenol A for example, sustained DNA stress and even a higher level of membrane stress after a 15-minute process may be attributed to the corresponding intermediates 1,4-benzoquinone and hydroquinone. The mechanistic toxicogenomics-based toxicity assay used in this study could be potentially applied as a feasible method for quantitative water toxicity assessment, also provided a more comprehensive mode of action (MOA) information, which can be linked with the intermediates, thereby improving the understanding of underlying toxicity changes for a degradation process.

522 Chemical toxicity to fish – linking cellular responses to whole organism effects via mechanistic models

J. Stadnicka-Michalak, Eawag,Swiss Federal Inst. of Aquatic Science&Techn; K. Schirmer, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; R. Ashauer, University of York / Dept of Environmental Toxicology

Man-made chemical compounds play an important role in human life; however, they may negatively impact human wellbeing as well as environmental health. One method to help achieve safe and sustainable use of chemicals is environmental risk assessment. For aquatic environmental risk assessment, fish play a very important role, being the most frequently applied vertebrate representative for freshwater systems. Fish Early Life Stage (FELS) tests are often required by various environmental protection agencies because fish at these stages are more sensitive to chemicals than adult fish. On the other hand, fish toxicity tests are not only controversial from the ethical point of view but they also areresource intensive (time, material, man-power, etc.). For these reasons, methods other than *in vivo* fish toxicity testing should be developed to be included in an integrated testing framework. *In vitro* fishcell assays are considered as a promising step toward reduction or replacement of fish bioassays. Therefore, in our study, we explored how toxicological information obtained *in vitro* can be used to predict organism outcomes. Specifically, we used information on survival and proliferation of a fish cell line to conclude on impact on fish lethality and growth influenced by two pesticides: Cyproconazole and Propiconazole. Based on Toxicokinetic-Toxicodynamic (TKTD) models, we compared the predicted reduction of the cells' weight, based on reduced proliferation on chemical exposure, with the reduction of measured fish weight in Fish Early Life Stages (FELS) and found a very good correlation. The highest tested concentration of Cyproconazole reduced fish weight by 49% and our model based on *in vitro* data predicted a 47% reduction. The highest tested concentration of Propiconazole reduced fish weight by 77% and our model predicted a 70% reduction. Thus, the modeled inhibition of cell population growth under chemical stress could indeed be taken as a proxy for chemical effects on fish growth. This finding could form a part of alternatives to *in vivo* toxicity experiments on fish or constitute a first tier in chemical testing in order to prioritize for additional testing requirements.

523 High-content screening assay for identification of chemicals impacting cardiovascular function in zebrafish embryos

K.L. Yozzo, G.M. Isales, T.D. Raftery, D.C. Volz, University of South Carolina / Environmental Health Sciences

Targeted zebrafish-based assays are needed to better evaluate chemical effects on organogenesis and begin classifying chemicals by toxicologically relevant modes-of-action. Using transgenic zebrafish (*fli1:egfp*) that stably express eGFP within vascular endothelial cells, we have developed and optimized a 384-well-based high-content screening (HCS) assay that enables us to screen and identify chemicals that affect cardiovascular function at sub-lethal, non-teratogenic concentrations. Following static exposure of one embryo per well from 5-72 hours post-fertilization (hpf), automated image acquisition procedures and custom image analysis protocols are used to quantify body length, circulation, heart rate, pericardial area (a biomarker for cardiac looping defects), and intersegmental vessel area within freshly hatched live embryos. After optimizing 72-hpf anesthetization procedures, we evaluated each endpoint across four independent control plates containing 384 initial embryos per plate. Survival and imaging success rates across these four control plates ranged from 93-99% and 42-70%, respectively. Criteria were then defined for assay success and analysis of treatments and, based on these criteria, 10 reference chemicals were screened for targeted effects on cardiovascular function. Based on this small screen, we found that exposure to non-teratogenic concentrations of the halogenated aromatic hydrocarbon 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) significantly impacted arterial circulation and pericardial area in the absence of effects on heart rate and intersegmental vessel area, whereas exposure to the organophosphate paraoxon significantly decreased heart rate in the absence of effects on circulation, pericardial area, and intersegmental vessel area. Compared to existing zebrafish-based assays, this method provides a comprehensive discovery platform with 1) increased sample sizes (32 per treatment); 2) broad concentration-response format (vehicle control + 11 chemical concentrations per plate); and, most importantly, 3) the ability to identify chemicals that target cardiovascular function at non-teratogenic concentrations.

524 The development of alternative strategies and additional endpoints for whole effluent toxicity testing in fishes

M.K. Sellin Jeffries, Texas Christian University / Department of Biology; A. Stultz, Miami University / Department of Zoology; S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization; J.M. Rawlings, The Procter & Gamble Company / Central Product Safety; J.T. Oris, Miami University / Department of Biology

In the United States (US), any facility discharging effluent directly to surface waters must conduct routine evaluations of whole effluent toxicity (WET). One method commonly used to obtain WET data is the 7-day larval growth and survival test (LGS) in which newly hatched fathead minnows (FHMs, *Pimephales promelas*) are exposed to effluents for 7 days with mortality as an endpoint. Given the increasing emphasis on animal welfare in toxicity testing, the goal of the current study was to identify an alternative test method capable of fulfilling the regulatory requirements for WET data, while minimizing the use of protected organisms or life stages. One such alternative to the LGS is the fish embryo toxicity test (FET), which has been sanctioned for WET testing in the European Union using embryonic zebrafish (ZF, *Danio rerio*). In order for the FET to be adopted in the United States, it would ideally be applicable to species commonly used in contract laboratories (i.e., FHMs) and demonstrate comparable sensitivity to the LGS. Thus, the main objective of this study was to compare the sensitivity of the FET and the LGS using both FHMs and ZF. In addition, the inclusion of otolith growth rings and mRNA expression as endpoints for WET testing was evaluated. To compare the sensitivity of FET and LGS testing strategies in FHMs and ZF, lethal (e.g., LC50) and sublethal (e.g., length, weight) endpoints were evaluated following each test type in each species using two reference toxicants (3,4-dichloroaniline and ammonia) and two simulated effluents (one to serve as a surrogate for wastewater treatment plant effluent and one as a surrogate for oil refinery effluent). Differences in the sensitivity of each testing regime were identified and in general, the FHM LGS test offered the most sensitivity, while the FHM FET offered the least. While the inclusion of otolith growth rings as a test endpoint did not improve the sensitivity of tests, there was evidence to suggest that inclusion of gene expression as an endpoint could improve test sensitivity. The results of this study highlight some of the challenges associated with alternatives strategies for WET testing, but also offer potential solutions to overcome those challenges.

525 Best practices for the overall process of validation and regulatory acceptance of alternative test methods in ecotoxicology

T. Braunbeck, University of Heidelberg / Department of Zoology; S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization; J. Guinea, E. Sela, ZF Biolabs

This presentation attempts to trace potential pathways for the development of alternative methods in ecotoxicology, to summarize the current procedure for the validation of alternative methods and to describe ways towards the

acceptance of alternative methods. At each level of this three-step procedure, there are bottlenecks and barriers. EUROECOTOX, a European 7th Framework Program, was developed as a network to promote cooperation between research centers, industry and other stakeholders in Europe devoted to the R&D and application of alternative ecotoxicity methods. One of the major goals of EUROECOTOX was to identify barriers to the development, validation and implementation of alternative methods, and to provide perspective on how to overcome them. Two expert meetings, held in Leipzig, Germany, and in Zurich, Switzerland, included detailed discussions on the bottlenecks to progress in development and eventual adoption of animal alternative methods in regulatory applications. Some bottlenecks are in common with method development in human safety disciplines, whereas others are confined to environmental safety sciences (with a shorter history and different regulatory needs). Rate-limiting steps during the early phases of research and development, (lack of) funding provided to methods development and validation, scientific issues regarding experimental design needs for validation, quality of accepted standard "*in vivo*" data, and rate-limiting steps in formal validation were identified as the key bottlenecks. Solutions to these issues range from increased funding provided to promising development and validation programs, alignment of reward systems for participants from academia, government and industry, and improved to development of high quality conventional ecotoxicity data will be discussed.

Communities, Ecology, and Health: Making the Connection – Part A

526 Community Gardens on Brownfields –Should you be Scared?

S. Martin, G. Hettiarachchi, C. Attanayake, P. Defoe, Kansas State University / Department of Agronomy

Growing of local crops, especially in urban areas is on the increase and many community gardens are or will be located on land that may be impacted by previous use. These kinds of properties, i.e. vacant or abandoned properties with real or perceived contamination issues are called "brownfields". Tens of thousands of brownfields can be found across the US This presentation will highlight the latest Kansas State University research data on contaminant uptake by food crops grown on several brownfield sites across the US slated for community gardens. Three urban community garden sites located in Kansas City, Missouri; Tacoma, Washington; and Indianapolis, IN will be used as examples. Contaminants associated with these sites are lead, arsenic and polycyclic aromatic hydrocarbons (PAHs). Suitable safety/corrective measures were suggested and implemented after thorough evaluation of soil properties. Field test plots were established within the community gardens and three vegetable crop types with three very different growth and contaminant uptake patterns were planted over two growing seasons. Effectiveness of selected site-specific soil amendments to reduce bioavailability of Pb, As and/or PAHs was evaluated. Associated best management practices focusing on reduction of both direct (soil-human) and indirect (soil-plant-human) exposure to the gardeners and their children and potential human health risks will be discussed.

527 Urban Chicken Eggs: Evaluating a Potential Human Exposure Pathway for Lead (Pb) in Urban Community Gardens

R. Mitchell, H. Spliethoff, L. Ribaudo, NYS Department of Health / Bureau of Toxic Substance Assessment; O. Taylor, Just Food; H. Shayler, Cornell University / Department of Crop and Soil Sciences; V. Greene, D. Oglesby, NYS Department of Agriculture & Markets

Urban community gardens can have great benefits, especially in inner-city communities with limited access to fresh food and green space. Chief among these benefits is that gardens are a convenient source of healthy, nutritious foods including fruits, vegetables, and, in some cities, chicken eggs. However, some urban soils can be contaminated, and gardening can create pathways for exposure to lead (Pb) and other soil contaminants. The growing popularity of urban chicken keeping raises the question of whether chickens exposed to Pb-contaminated soil produce eggs with elevated Pb concentrations, creating a potential human exposure pathway for those who eat the eggs. To evaluate this pathway, we measured Pb concentrations in eggs, soil, and chickens' feed, water and dietary supplements from seven New York City community gardens. Pb concentrations in eggs ranged from < 10 ppb to 167 ppb (fresh weight), with about half of the 58 eggs exceeding market-basket levels (max: 13 ppb). Pb concentrations in eggs were significantly associated ($p < 0.005$) with Pb in garden soils (max: 558 ppm). Egg Pb levels were not associated with Pb levels in feed or water, but data suggested a connection between the availability of dietary calcium supplements and reduced transfer of Pb from soil to eggs. Soil-to-egg transfer efficiency was considerably lower than that found in a recent study in Belgium, demonstrating that there can be important geographic differences in this transfer. Comparison to guidance values and examination of IEUBK model output suggest that the increased health risk is low from consuming eggs with the Pb concentrations we measured. However, as it is prudent to minimize Pb exposure, and as higher Pb concentrations in soil appear to increase the potential for Pb exposure via egg consumption, urban chicken keepers should consider efforts to reduce Pb transfer from soil to chicken eggs. Suggested mitigation measures for include limiting the foraging areas of free-range chickens, providing clean surface soil in chicken runs, and providing dietary calcium supplements.

528 Characterization of Lead, Arsenic and PAHs in Community Gardens and Municipal Compost: Using Science to Guide Risk Management

W. Heiger-Bernays, Boston University School of Public / Department of Environmental Health; J. Tabony, P. Barrett, R. Kim, Boston University School of Public Health; J. Spargo, University of Massachusetts / Soil and Plant Tissue Testing Laboratory

Community gardens provide people with opportunities to grow nutritious and affordable fresh foods, enhance community building and increase productive use of abandoned land. The soil in these gardens is frequently contaminated with arsenic (As), lead (Pb) and polycyclic aromatic hydrocarbons (PAHs) from decades of anthropogenic activities. By partnering with Boston Natural Areas Network, a non-profit organization that helps coordinate activities related to all of the Boston area's community and school gardens, involving greater than 10,000 individuals and families, many of them low-income, we participated in renovation of three gardens. Using a combination of historical records, conversations with neighbors, and literature, we focused our efforts to characterize the Pb, As and PAH concentrations in the soil, determining the horizontal and vertical extent of contamination. Data are analyzed spatially, allowing us to document a predictable pattern of contamination. The major contributor to lead in the soils is lead paint, not aerial deposition nor distance from roadway. Likewise, distance from roadway is not a reliable predictor of PAH concentration. In efforts to decease the concentrations of these contaminants and to decrease bioavailability, compost is often added. We have examined the nutrient content, lead, arsenic and PAH concentration in municipal compost, tracking its composition since 2008. Increasing concentrations of lead over time indicate modification of feedstock and processing. In an effort to provide compost that improves the quality of the soil and decreases the total and bioaccessible lead concentrations, new processes for municipal composting began in 2012 and data indicate lower lead concentrations: less than 150 mg/kg, similar to those achieved in 2008 have been attained. This work is the first to integrate a systematic testing of gardens for common contaminants with a risk-based approach to recommendations that are practical, timely, cost effective and easily implemented, protective of human health.

529 Assessing and Communicating Benefits and Risks of Eating Fish with Subsistence-Fishing Ethnic Communities

M.J. Carvan, University of Wisconsin-Milwaukee / School of Freshwater Sciences; K. Gajeski, University of Wisconsin-Milwaukee / Zilber School of Public Health; R. Hutz, University of Wisconsin-Milwaukee / Biological Sciences; J. Hewitt, University of Wisconsin-Milwaukee / Children's Environmental Health Sciences Core Center

Subsistence fishing is a common strategy for ethnic communities to obtain high quality protein that partially compensates for economic inequalities that they experience. At the same time, subsistence fishing provides a way to transmit cultural traditions across generations. While fish is a source of high quality, low fat protein and health-promoting omega-3-fatty acids, fish also contain varying concentrations of environmental contaminants. Our Children's Environmental Health Sciences Core Center has conducted several fish risk/benefit communication projects with ethnic communities in Milwaukee and Anishinaabe tribal members. For our current project, we collaborated with community partners to understand patterns of fishing, cooking, and consumption of fish (by species, size, and specific waterways) through key informants and focus group participants in the urban African American community. We used these data together with existing

published data to estimate the concentration of omega-3-fatty acids and data from creel surveys, provided by the Department of Natural Resources, to estimate the uptake of mercury and other contaminants for men, women of childbearing age, and children. We describe the process and outcomes of estimating benefits and risks of eating locally caught fish for community members. In addition, we discuss multiple methods that we have employed to develop culturally sensitive communication processes and tools that involve community members, scientists, communication and outreach specialists, and videographers.

530 A rural, community-based study of mercury exposure among consumers of local fish and the challenge of explaining fish consumption guidelines

L.A. Schaider, Harvard School of Public Health / Department of Environmental Health; A. Backus, Harvard School of Public Health; Z. Dong, Harvard School of Public Health / Environmental Health; E. Hatley, R. Jim, L.E.A.D. Agency; R. Lynch, University of Oklahoma Health Sciences Center / Occupational and Environmental Health; K. Maymi, L.E.A.D. Agency; J.P. Shine, J. Spengler, Harvard School of Public Health / Department of Environmental Health

Mercury (Hg) bioaccumulation is a common reason for fish consumption advisories in the US, and the extent of Hg bioaccumulation in fish depends on proximity to emissions sources, ecology, and biogeochemistry. Grand Lake, a 190 km^2 reservoir in northeastern Oklahoma, USA., is located in the vicinity of six coal-fired power plants and is a popular fishing destination for seasonal and year-round populations, including members of local American Indian tribes. Our community-based research study addressed community concerns that consumers of local fish are exposed to elevated Hg in their diets. We analyzed over 1,100 samples of commonly-consumed fish collected throughout the watershed, primarily through donations from community members and through the local wildlife conservation department. We also recruited a cohort of 150 local fish consumers to complete five food frequency questionnaires and provide five hair samples for Hg analysis (as a biomarker of exposure) seasonally over the course of one year. In general, our findings did not show high levels of Hg in fish or high Hg exposure among local fish consumers, creating the need for more nuanced outreach messages. Our outreach materials aimed to balance benefits of fish consumption and concerns about several specific types of local fish. Only about 2% of fish samples, primarily flathead catfish and drum, were above USEPA's guideline value of 300 ppb (wet wt), which is based on an assumption of 2-3 fish meals per month. Rates of fish consumption were around twice the USaverage (including non-fish consumers), but around half the average for US fish consumers. Anecdotally, we expected members of local American Indian tribes and the local Micronesian community to rely heavily on local fish. However, Tribal members in our study reported similar rates of fish consumption as our Caucasian participants, and Micronesians rarely ate local fish, preferring saltwater fish instead. Only 5% of participants had hair Hg levels above 1 ppm, the guideline for children and women of childbearing age, consistent with regional averages. In developing outreach materials for community members, we learned that comparing species-specific average Hg concentrations to the EPA guideline was less effective than reporting the average mercury content in a typical fish meal relative to a mercury "allowance" per month to account for both species-specific mercury concentrations and varying rates of fish consumption.

531 Popular and Scientific Knowledge Translation to Reduce Occupational Risks among Female Farmworkers

J. Tovar, Farmworker Association of Florida; J.R. Runkle, Emory University; J. Economos, Farmworker Association of Florida; J. Flocks, University of Florida; L. McCauley, Emory University

There are few studies focused on the effects of agricultural work on the health of female farm workers or the impact on their pregnancy outcomes, and even less of this research is made available to the workers themselves. This presentation focuses on a training for female farmworkers developed with the results of community based participatory research study. The study in question explored farmworker knowledge and perceptions of risks to health and pregnancy; awareness of chemical exposures; and relationship to biomedical services. Guided by a community advisory board, a top priority was translation of results into an accessible final product, yielding an evidence-based training combining the workers' knowledge with medical and ergonomic information, occupational best practices, accessible literature, and study results. Study findings on biomarkers; narratives of pregnancy and work interactions; common complaints; problem-solving strategies; and patient-doctor relations were incorporated into the training. The training uses popular education techniques, web-based interactive visual presentation, and video vignettes to reach low-literacy populations. The initial training was piloted; after the training, it was evaluated on two levels: first, through role-play to test content assimilation; second, focus groups were used to test the delivery process. Focus group participants reported receiving valuable information and learning new content; pesticide information was rated the most useful. Participants complained about the length of the training, so trainers revised and reduced it from 3.5 hours to just under 2.5 hours. Role-plays showed a good level of content assimilation and the women pooled knowledge and resources to devise action plans, implement injury-prevention strategies, and provide assistance to workers in need. This training, available online, is an ideal strategy as distribution is easy, there are low costs associated with it, and it can be fairly easily modified to fit other fields. The training is available in English and Spanish, it is divided in six sections (Introduction, Pregnancy, Heath, Ergonomics, Pesticides, and Evaluation), and includes four videos played by community members. Training can be conducted on line or on site without electronics, according to technical capability.

532 A Convergence-Building Model of Superfund Site Communication: Building on Lessons from the Paducah Gaseous Diffusion Plant

A.G. Hoover, Superfund Research Program Research Translation Core; L. Ormsbee, University of Kentucky Superfund Research Program / Research Translation Core, Kentucky Water Resources Research Institute

Best practices approaches have guided governmental risk communication efforts at Superfund and other chronic risk sites for more than two decades, playing an important role in the ways in which those most affected by contamination make sense of risk. Such approaches can affect the information environment in two separate but related ways: 1) directly, through the explicit sharing of information, and 2) indirectly, through ongoing stakeholder interpretations of the processes by which that information is shared. To date, the indirect, process-related effects have not been addressed in assessments of communicative efficacy at Superfund sites. Thus, it increasingly is necessary to evaluate not only whether risk communication approaches have been effective for information sharing but if, in fact, their implementation has had unintended consequences for the ways in which stakeholders perceive and interact with each other. Using sensemaking theory as a diagnostic and evaluative tool, particularly when applied to a complex case, provides a promising avenue for identifying and addressing key challenges for sustained communication in chronic risk communities. The myriad environmental, health, and economic risks associated with western Kentucky's Paducah Gaseous Diffusion Plant (PGDP) National Priorities List Superfund site have affected numerous stakeholders for decades. A crystallized case study of risk communication about and regarding the PGDP points to sensemaking constraints across five themes: 1) The Government; 2) The Public; 3) Delays; 4) Secrecy, Deception, and Manipulation; and 5) Competing Risk Perceptions. These findings suggest that a reconceptualization of risk communication is needed that moves stakeholders to the center of the communication model. By acknowledging the multiplicity of stakes in site-related risks and decisions, the proposed model increases the capacity for shared sensemaking while decreasing the likelihood of adversarialism driving the discourse. Over time, this model should promote increased levels of trust, improving stakeholder expectations and creating a more positive framework within which to make sense of risk-related issues.

533 Integrated Assessment of Small-Scale Artisanal Gold Mining in Ghana: From Engagement to Research to Action

N. Basu, McGill University / Department of Environmental Health Sciences; R. Long, University of Michigan; P. Agyei-Baffour, KNUST; E. Clarke, Ghana Health Service; S. Obiri, University of Ghana; M. Rajaee, T. Robins, University of Michigan; E. Tenkorang, University of Cape Coast; M. Wilson, E. Renne, University of Michigan

The purpose of this presentation is to describe a multi-year Integrated Assessment to assess ecological and public health risks in Ghanaian small-scale gold mining communities. This Integrated Assessment is conducted by an interdisciplinary team of experts and stakeholders from the US (5 members) and Ghana (16 members), including researchers, decision-makers, and community members. In Ghana, the second largest producer of gold in Africa, 10.5% of all gold mined comes from small-scale mines. Exposure to

mercury used in the mining process is a major concern for ecological and human health in mining communities. Yet there is a dearth of knowledge about the interaction among mercury exposure and myriad other public health hazards faced by small-scale gold mining communities, including water contamination, lack of sanitation facilities and infrastructure, inhalation of dust from pulverized ore, and lack of protective equipment. Furthermore, little is known about how these factors are influenced by interactions spanning social, public health, anthropological, political, economic, and ecological domains. This complex problem is being addressed using the Integrated Assessment methodology, which has been applied widely to address sustainability and public health challenges that require integration of complex and diverse data to develop creative and viable solutions. Members have been separated into four technical workgroups: natural sciences, human health, social sciences, and policy and economics. A series of workshops in Ghana are underway. During the first meeting (August 2012), policy-relevant research questions were co-identified across and within each of the four domains. Leading up to the second meeting (April 2013), status and trends related to the problem are being described, and the causes and consequences are being characterized via synthesis of extant data. For the final meeting (summer 2014), desired policy outcomes will be identified based on this information, and policy options developed and evaluated. The benefits of the Integrated Assessment– including the development policy-relevant information, scientific consensus and public support around an issue, and creative responses to difficult problems – will become evident as the solutions to public health hazards in small-scale mining communities in Ghana are developed, implemented, and endured.

Better Benthic Biomonitoring for Risk Assessments, Criteria Development, and Causal Analyses

534 Why I like benthic biomonitoring data and what you can make with them

S.M. Cormier, USEPA

With the maturation of large, geographically explicit, biomonitoring data comes great opportunities to ask and directly answer environmental questions. Under what conditions do species thrive? What is the threshold for extirpation of species? What caused a change in the community? Species sensitivity distributions (SSD), which model a causal relationship between a stressor and an effect, are a convenient way to examine these questions and to graphically depict the answers. For example, extirpation and optimum values can be calculated from these large datasets. These extirpation or optimum values can be used to make a regional SSD. Water quality criteria can be derived from that SSD by estimating the 5th centile of extirpated genera. This is a type of criterion assessment. Using the calculated XC_{95}, one can construct a composite SSD for a watershed and individual SSDs for its contiguous streams to assess condition and site specific causation. Evidence of causation is shown by the relative position of absent genera in the SSD. Condition is shown by the relative diversity of species represented in the SSD. When there is enough information to construct an SSD for many stressors, the condition and cause are revealed by the SSD. An SSD is a strong type of evidence, because it clearly shows a relevant effect. Biomonitoring benthic invertebrates provides information for many genera; thus, enabling us to discover solutions from the biosphere itself. (Views expressed in this abstract are those of the author and do not represent the views or policies of the USEPA.)

535 Biomonitoring and decision making

C. Mebane, US Geological Survey / NOAA Fisheries Liaison

Biomonitoring is widely called for as a means to inform adaptive management in various environmental frameworks in the USA, including effluent permits under the Clean Water Act, evaluating the effectiveness of measures to minimize harm to threatened or endangered species, and to evaluate the effectiveness of Superfund Cleanups. However, the regulatory specifications for biomonitoring may be imprecise, leaving the details to be worked out on a case-by-case basis. This presentation will discuss the pros and cons of several potential decision criteria for assessing biomonitoring data under different interpretive constructs. Examples will include taxonomic completeness and metrics, and "trout chow" objectives that interpret benthic communities from the perspective of providing sufficient forage base to valued fish populations.

536 Toward Better Benthic Community Biomonitoring: Examining How the Taxonomy "Lab Effect" Can Influence Our Understanding of Ecological Impacts

G.T. Lester, EcoAnalysts Inc.

Benthic invertebrate community analyses have been widely used to assess the ecological condition of water quality and habitat quality as part of monitoring programs, ecological risk assessments or natural resource damage assessments. These analyses rely heavily on the technical expertise of taxonomy laboratories to produce scientifically defensible benthic community datasets, yet data users may not be fully aware of the implications that operational decisions made within the taxonomy laboratory will have on the contents of each dataset produced. Some of these decisions will potentially enhance, or confound, the overall conclusions of a benthic community study. This presentation will discuss potential sources of error in benthic sample sorting, taxonomy, data reporting and data analysis procedures, discuss their influence on conclusions, and identify ways to minimize these errors, thereby enhancing the quality of benthic community assessments.

537 West Virginia's Narrative Criteria Implementation-Challenges Associated with Incorporating Benthic Macroinvertebrates without utilizing a Biocriterion

J.L. Yeager, Potesta & Associates, Inc.

In 2010, the West Virginia Department of Environmental Protection (WVDEP), introduced permitting guidance to address a portion of the State's narrative water quality criteria, 47 C.S.R. 2 §§ 3.2.2 and 3.2.i which state, in pertinent part, "No significant adverse impact to the chemical, physical, hydrologic or biological components of the aquatic ecosystem shall be allowed." The goal of the guidance was to provide permit writers a tool to develop site-specific National Pollutant Discharge Elimination System (NPDES) permit conditions for surface mine operations using a holistic watershed approach that would protect the State's narrative water quality standards (WQS). The guidance incorporates the use of whole effluent toxicity testing, the development of Aquatic Ecosystem Protection Plans, Adaptive Management Plans (where applicable), and chemical and biological monitoring which includes the use of benthic macroinvertebrate sampling. The State has a well develop monitoring program, including the development of a statewide multi-metric for benthic macroinvertebrates referred to as the West Virginia Stream Condition Index (WVSCI). The WVSCI was developed by the WVDEP's watershed assessment group as a bioassessment tool to help supplement other data and strengthen the State's biological monitoring program. As stressed in the *permitting guidance*, the WVSCI is simply a tool to be used as a primary indicator of stream health, but not the sole measure. Other watershed data must be used to evaluate the health of the ecosystem, independent of whether the WVSCI score alone indicates good or poor water quality or a potential violations of the narrative WQS. The struggle with implementing the guidance has reflected the difficulties creating permit requirements with a multi-metric index that is not, or cannot be, construed as a site specific biocriteron because the WVSCI has not been adopted into the State's WQS. As a result, when evaluating potential violations of the narrative WQS the WVDEP has to incorporate WVSCI scores, but must consider site specific conditions, which therefore relies more heavily on best professional judgment and a weight of evidence approach to determine applicable violations. Unfortunately applying best professional judgment to determine compliance with a specific permit condition is open to challenge from all sides.

538 Benthic communities in the acid mine drainage-exposed Lac Dasserat system: identifying causes of impairment with multiple lines of evidence

L.C. Grapentine, Environment Canada / Watershed Hydrology and Ecology Resarch Division; W.P. Norwood, Environment Canada / Aquatic Contaminants Research Division

After receiving drainage from an abandoned Cu-Zn mine site for over 70 years, the Lake Dasserat system in western Quebec exhibits a marked spatial gradient of pH and metal concentrations in water and sediment. As part of a multiyear, multidisciplinary study of aquatic impacts related to the mine site drainage, benthic macroinvertebrate communities in sediment from across the gradient were assessed in relation to proximity to the inflow of the mine site drainage, water and sediment quality (including concentrations of 35 metals and metalloids), water and sediment toxicity to the amphipod *Hyalella azteca*, and metal bioaccumulation by *H. azteca*. Total benthos and taxon richness both showed an overall increase with distance from the inflow

of the mine drainage. Community composition (as represented by ordination axis scores) also changed with spatial location. Low dissolved oxygen of overlying water accounted for exceptions to the pattern. Community impoverishment was associated with low pH, high conductivity and high sediment organic content, likely associated with the mine site drainage inflow, and depth, which was inversely related to dissolved oxygen level. Among metals and metalloids, Tl, Pt, Cu, Sr, Pb, Zn, Sb and Fe showed highest association with community impoverishment. Although these contaminants are too correlated to indicate potential causal relationships of individual metals based on spatial pattern alone, maximum Cu and Zn concentrations were markedly elevated above sediment quality guidelines. Of the four measured survival and growth toxicity endpoints, 7-day survival was the most related to community impoverishment. Bioaccumulation of Zn was inversely related to taxon richness. Benthic community structure in the Lake Dasserat system therefore appeared influenced by both habitat factors and mine drainage contaminants. Analyses of ecological traits (mainly metal tolerances) of the resident invertebrate taxa are underway to further link community composition with environmental stressors.

539 An analysis of replicate macroinvertebrate samples to assess uncertainty in measures of presence and absence of taxa in West Virginia streams

S.A. Roark, G.D. De Jong, J.S. Lynch, GEI Consultants, Inc; A.K. Gevertz, ENVIRON International Corp / ESM; R.W. Gensemer, GEI Consultants / Ecological Division; S.P. Canton, GEI Consultants, Inc. / Ecological Division

Recently, field-collected data from paired stream macroinvertebrate and water quality surveys have been used to establish thresholds for water quality parameters such as nutrients and conductivity. This represents a new use for such data; one that differs from that for which they were originally collected. If field-collected data are to be used to establish water quality thresholds, significant consideration must be given to study design, data usability, and quantification of the uncertainty of measures that will be used to determine effects. If the measure of effect is a single parameter (*e.g.*, absence of taxa), and the need to measure that parameter is known *a priori*, the data collection method can be designed to maximize confidence in the parameter of interest. However, if the objective is to develop a water quality threshold from existing data collected to calculate multimetric indices, care must be taken to confirm that the selected biological measures are sufficiently robust to support assessments of association with water quality parameters. For example, the single measure of effect used by USEPA to develop an aquatic life benchmark for conductivity in Appalachian Region streams was the distribution (i.e., presence) of each taxon across the range of conductivity in the data set. However, study design constraints (*i.e.*, fixed-count subsamples) significantly limit the reliability of the inference of absence of taxa from a site, and our analyses suggest that underestimates of taxa presence resulting from study design constraints have led to a downward bias in the conductivity benchmark. To further assess the reliability of absence determined with 200- and 300-count samples, we analyzed macroinvertebrate data from sites on 12 West Virginia streams with five replicate samples per site. These results corroborate our previous analyses, and indicate that absence determination at a site based on a single fixed-count subsample has significant uncertainty. Taxa present at up to 10% relative abundance in some replicates were absent from others. We conclude that unintended bias resulting from a suboptimal sampling design may lead to bias in field-based water quality thresholds, and that a robust data quality objective process should be used when collecting new data or making use of existing data from biomonitoring programs to avoid erroneous conclusions and unintentionally biased water quality thresholds.

540 eDNA (18S rDNA) comparisons of benthic assemblages from five estuaries of varying ecological condition

A. Chariton, CSIRO Land and Water; S. Stephenson; M. Morgan, CSIRO Ecosystem Sciences; C. Hardy, CSIRO Entomology

The health of sedimentary environments is predominately assessed by examining the compositions of macrobenthic assemblages. While there is no doubting the importance of macrobenthic surveys, they are costly; have a long turnaround time to produce data; require local taxonomic expertise; and are often incorrectly used to make assumptions about total ecosystem health, even though they only focus on a minute fraction of the total biodiversity. Recent innovations in DNA sequencing and bioinformatics now make it viable to obtain ecological data which encapsulates a far wider breadth of biota than can be obtained using traditional optical-based techniques. An approach commonly referred to as eDNA surveys or metabarcoding studies. In this study, we sampled five estuaries subjected to varying degrees of urbanization. Ecological data was obtained by high throughput sequencing the 18S rDNA gene, providing profiles of the benthic eukaryl communities. The aims of the study were to: 1) examine whether eDNA biodiversity data could be used to discriminate estuaries; 2) identify whether eDNA derived biotic composition was correlated with nutrients and other physico-chemical variables used to define classify the estuaries; and 3) examine whether the eDNA derived data is ecological informative and suitable for monitoring the study system. Roche 454 FLX sequencing produced more than 1 million reads from approximately 3,000 unique molecular operational taxonomic units (MOTUs). The data encompassed a wide range of taxa including annelids, crustaceans, nematodes, fungi, protists and diatoms. We found that all five estuaries contained different eukaryl compositions, with dissimilarity being greatest between the two most contrasting estuaries. Strong correlations between biotic composition and a number of environmental variables (both natural and anthropogenic in origin) were observed. The study produced some interesting ecological data, including an array of potential indicator taxa for examining the responses of estuarine biota to elevated nutrient concentrations. In addition to the findings of this study, we will discuss some of the broader benefits and current limitations associated with eDNA surveys, as well as our view on the requirements for integrating this approach into routine monitoring programs.

541 Evaluating the use of Next Generation Enviromental Barcoding for improved Environmental Assessment

C.D. Penalva-Arana, USEPA / National Center for Environmental Assessment; J. Martinson, USEPA; C. Ridley, USEPA / National Center for Environmental Assessment; E. Pilgrim, USEPA / Ecological Exposure Research Division

The environmental assessment of aquatic systems relies heavily on water chemistry data, a few habitat quality measurements, and at times the occurrence of a subset of organisms present. Therefore health of ecosystems is determined using very few biological endpoints since current methods are time-consuming, expensive, and prone to high variability and errors. With this in mind, we present the results obtained using a new method we call Next Generation Environmental Barcoding (NGEB) that integrates the use of multiple DNA barcodes and Next Generation Sequencing to increase biomonitoring reliability and efficiency. Our pilot study compares the results obtained by OhEPA scientists using traditional benthic-based indices, with our matching genomic based O/E (observed/expected) indices, acquired concurrently across different sites along the East Fork of the Little Miami watershed. We illustrate how genetic-based data obtained from water, artificial substrates, and periphyton samples, compare with biomonitoring results obtained using traditional field methods. We discuss why we believe an NGEB approach may allow for a more accurate view of overall system health, as it increases our use of more than one functional community.We also investigate if improved measures of biodiversity can help us uncover new bioindicators of system condition, and how these may provide new biological endpoints that can more clearly describe the health of a site or ecosystem. Our results shed light on the vast amount of biomonitoring relevant data that could be exploited but is currently underutilized when assessing the ecological condition of waterbodies.

Assessing Contaminant Effects in Multi-stress Ecosystems: Part A

542 A comparative approach to evaluating the risks due to multiple stressors and regional scales using Bayesian networks

W.G. Landis, K.K. Ayre, Western Washington University / Institute of Environmental Toxicology; E.E. Hines, Western Washington University / Institute of Environmental Toxicology, Huxley College of the Environment,

Ecological structures are exposed to multiple chemical, physical and biological stressors. At regional scales multiple stressors affecting multiple endpoints is the norm. Since 1997 the relative risk model has successfully incorporated the combination of multiple stressors, a heterogeneous landscape and multiple endpoints to a rank based estimate of risk. A recent development has been the incorporation of Bayesian networks to incorporate inherently the combination of causality and uncertainty to estimate risk. In this paper we will use current case studies to demonstrate Bayesian network relative risk model (BN-RRM) and to examine the utility of the approach. Case

studies will included the South River VA contaminated site, the Puyallup WA watershed, and species including non-indigenous species. In our studies the there are two primary impediments. First has been the lack of data that integrates the effects of multiple chemicals, physical parameters and biological interactions across the landscape. Second has been the legacy of site assessment based upon simple comparisons between supposedly uncontaminated sites and contaminated sites without regard for the landscape of other stressors. Advances in performing adequate laboratory experiments to derive exposure-response relationships for multiple stressors and in formulation field assessment can addresses each of these impediments.

543 Mixtures of OCPs in the North Shore Muck Farms of Lake Apopka alter adverse outcome pathways in largemouth bass

N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology; K.J. Kroll, University of Florida / Physiological Sciences; C.J. Martyniuk, University of New Brunswick; A.C. Mehinto, Southern California Coastal Water Research Project / Department of Physiological Sciences and Center for Environmental and Human Toxicology; R.C. Colli-Dula, University of Florida / Physiological Sciences; M.S. Prucha, University of Florida / Dept. of Physiological Sciences; R. Conrow, St. Johns River Water Management District

The North Shore muck farms of Lake Apopka (FL, USA) were used for intense agriculture from the 1940s to the 1980s and today present with elevated levels of organochlorine pesticides (OCPs) in sediments. Experimental mesocosms built in the muck farms were used to evaluate bioavailability of the OCPs to largemouth bass (LMB) (*Micropterus salmoides floridanus*) and to determine whether or not adverse outcome pathways related to reproduction were impacted. LMB collected from a reference site (DeLeon Springs) were stocked during early and late oogenesis into the mesocosms to determine if there were different effects of contaminants on LMB that may be attributed to their reproductive stage. After ~3 months, LMB ranged from 2 to 800 times higher contaminant load for specific organochlorine pesticides (e.g., p,p'-DDE, dieldrin, methoxychlor) compared to control animals. Gonadosomatic index and plasma vitellogenin did not significantly differ between LMB collected at reference sites to those inhabiting the mesocosms. However, circulating 17β-estradiol and testosterone levels were significantly decreased in LMB inhabiting the polluted environment. Gene expression profiles in the LMB ovary were also unique compared to LMB collected from reference sites. Sub-network enrichment analysis for cellular processes showed that reproduction (granulosa function, gonad and oocyte development), endocrine function (steroid metabolism, hormone biosynthesis), immune function (T cell suppression, leukocyte accumulation), and cell signaling were adversely affected in LMB placed into mesocosms. In addition, laboratory-feeding studies with each of the contaminants were also performed and achieved similar body burdens of contaminants. Microarray analyses on livers and gonads of these fish suggest similar effects on adverse outcome pathways as to the fish introduced into the mesocosms. Microarray analysis has been successful in discerning complex gene expression patterns due to multiple stressors.

544 Assessing the Potential for Major Ion Toxicity

P.R. Paquin, Hydroqual, HDR|HydroQual; C.J. Fanelli, HDR|HydroQual

Historically, efforts to develop water quality guidelines (WQG) for trace elements focused on those known to be toxic to aquatic life at relatively low levels (e.g., Ag, Cd, Cu, Ni, Pb and Zn). The integration of well-understood, fundamental concepts involving chemical, physiological and toxicological processes allowed researchers to make significant advances in understanding of how to assess bioavailability and the potential for effects of trace level constituents over a wide range of site-specific water quality characteristics. The derivation of WQGs to protect aquatic life from adverse effects due to relatively high levels of what are commonly called the major ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-} and HCO_3^-) is at a less advanced state of understanding, and is becoming of increasing interest to regulatory agencies, industry and academia alike. Some studies have shown how interactions of one ion with another can serve to mitigate toxicity, and such relationships form the basis of WQG in several jurisdictions. While useful, this approach necessarily overlooks additional complex interactions that might influence the level of protection that is provided. An approach that has the potential to address this limitation is to use multivariate regression modeling to establish exposure-effect relationships for test organisms exposed to well-characterized mixtures of major ions. Other investigators have used more integrative measures of major ion concentrations such as total dissolved solids, salinity or conductivity in an effort to establish concentrations of major ions, collectively, that are protective of aquatic life. Although an expedient way to address the problem, it is relatively insensitive to variations in the underlying composition of the mixture of ions, a characteristic that may influence toxicity levels. Finally, another alternative is to revert to a more fundamental approach that again considers chemistry, physiology and toxicology to predict how major ions affect an aquatic organism (e.g., thru changes in membrane potential). Though not well developed to date, such an approach promotes the realization of an improved understanding of the relatively complex interactions that are likely to influence major ion toxicity. Examples of alternative ways to assess major ion toxicity and important advantages and disadvantages of each will be presented.

545 Separating direct and indirect effects of mine pollution on benthic communities using biomonitoring, microcosm and field experiments

P.J. Cadmus, Colorado State University-Fish, Wildlife & Conserv / Fish, Wildlife & Conservation Biology; W.H. Clements, Colorado State University / Fish, Wildlife and Conservation Biology; H. Guasch, University of Girona (UdG); A. Herdrich, Colorado State University / Fish, Wildlife and Conservation Biology

The North Fork of Clear Creek watershed in central Colorado was placed on the National Priorities List (Superfund) in 1983 as a result of significantly elevated metals concentrations. This stream has healthy and diverse macroinvertebrate and fish populations in upstream reaches, but is heavily contaminated below historical mining sites. An effluent treatment plant to be completed in 2014 is expected to greatly reduce the input of metals to this watershed, providing a unique opportunity to investigate the recovery of a severely degraded ecosystem. We report results of an integrated suite of microcosm, laboratory and field experiments coupled with a before-after-control-impact (BACI) biomonitoring design to make predictions about restoration efficacy and to determine relative importance of indirect stressors associated with mine pollution. Most water quality standards and predictive toxicity models focus on direct effects of aqueous toxicants on pelagic organisms in laboratory conditions. These approaches generally do not consider physical stress, turbidity and habitat loss associated with Fe, Al and Mn deposition. Field manipulation and microcosm experiments were employed to assess indirect effects of Fe pollution alone and in combination with other stressors such as aqueous metals and reduced flow. Naturally colonized communities of macroinvertebrates were reduced by ferric Fe at or well below the national standard of 1.0 mg/l. The sensitive mayfly *Epeorus sp.* was significantly reduced at 0.4 mg/l in a 10 d exposure. Although Ferric Fe is thought to have a protective effect on toxicity of other metals, we observed synergistic effects on function of periphyton when communities were exposed to combinations of ferric Fe (0.6 mg/l) and low levels of Cu and Zn. Increased tourism in the North Fork watershed has led to increased municipal demand for water and reduced flows in North Creek. This suggests that indirect effects of Fe floc may persistent after aqueous metals are removed by water treatment. Field and microcosm experiments found reduced colonization and survival in substrate that was embedded with metal floc. Because management decisions related to development, flow regulation and water diversion in the Clear Creek watershed may compound the ecotoxicological effects of mine pollution, these decisions must be made within the context of multiple environmental stressors.

546 Combined Effects of Selenomethionine and Salinity on the Apoptotic Pathway of Embryo Lethality in Euryhaline, Fish Japanese Medaka (Oryzias latipes)

A. Kupsco, University of California – Riverside; D. Schlenk, University of California-Riverside / Department of Environmental Sciences

As climate change worsens, the salinity of important spawning grounds in certain water-restrained estuaries, such as the San Francisco Bay, is increasing. Hypersalinity may not have direct lethal effects on adult fish, but osmotic stress may alter detoxification strategies of developing organisms. Although Selenium is an essential micronutrient, it has demonstrated embryo toxicity to fish at high concentrations and occurs in areas impacted by hypersaline conditions. Selenomethionine has been previously shown to cause oxidative stress in Japanese medaka embryos and when combined with hypersalinity decreased embryo hatch. The objective of this study was to examine potential mechanisms for embryo lethality caused by combined stressors in developing euryhaline fish. Japanese medaka eggs were treated with 5μM selenomethionine alone and with 16ppth salinity, then sampled

at 2, 4, 8 and 12 hours for measurements of *CASP3A* and *BAX* transcripts. In selenomethionine only treatments expression of apoptotic transcript, *CASP3A,* was up-regulated in a time-dependent manner, after 2 hours of selenomethionine treatment and *BAX* was up-regulated significantly at 12 hours. Additionally, transcripts for genes regulating the unfolded protein response (UPR), *BiP*, *IRE1*, *ATF6* and *Nrf2*, were measured. *IRE1* and *BiP* were up-regulated in a time-dependent manner after 4 and 8 hours and *BiP* continued to increase at 12 hours. Furthermore, *ATF6* was significantly up-regulated after 12 hours after selenomethionine treatment. Salinity stress appears to increase these effects. These results indicate embryo lethality may occur through apoptosis and the unfolded protein response and that hypersalinity may compound these effects.

547 Modulation of Estrogenic Exposure Effects Mediated through Temperature and Dietary Regimens in Male Fathead Minnows

N.W. Shappell, USDA-ARS / Biosciences Research Lab; D. Feifarek, D.C. Rearick, St. Cloud State University; S.E. Bartell, Normandale College Community College; H.I. Schoenfuss, St. Cloud State University

A plethora of studies has examined the biological effects of environmental estrogens on fathead minnows. However, in many cases results from environmental studies do not match the expectations from prior laboratory exposures, which usually are designed to minimize confounding factors such as temperature and food availability. In contrast, the biological effects observed in fathead minnow exposures to environmental estrogens in aquatic ecosystems represent the totality of environmental stressors and seldom match laboratory conditions. To simulate the effects of estrogenic exposure in the wild, we exposed male fathead minnows to low (15 ng/L) and high (100 ng/L) concentrations of estrone for 21 days in a flow-through system. Half of the minnows were maintained at 17 °C the other half at 26 °C, more typical of controlled laboratory conditions. Furthermore, each temperature group was subdivided into a restricted diet group and an *ad libitum* group. At the end of the 21-day exposure, we examined morphometric endpoints (condition factor, GSI, HSI, secondary sex characteristics), physiological data (vitellogenin, blood sugar, cortisol) and histopathology of liver and testis. The entire experiment was replicated once. The GSI was significantly reduced in all *ad libitum* feed treatments with a concurrent increase in body condition factor. As expected, plasma vitellogenin concentrations were elevated ($P < 0.05$) in all high estrone treatments, yet those fish maintained at 26 °C were higher than those at 17°C ($P < 0.05$). Blood sugar concentrations were also significantly increased in high estrone treatments, again with significantly higher concentrations found in fish in the warmer water suggesting greater metabolic stress. This study highlights the impacts of multiple stressors and modulating effects of environmental conditions on the expression of biomarkers associated with exposure to environmental estrogens. In order to realistically expect concordance between laboratory and field investigations of the biological effects of contaminants of emerging concern more attention needs to be focused on establishing environmentally relevant exposure conditions.

548 Use of gene expression responses to evaluate combined effect of low dose gamma radiation and uranium on atlantic salmon (Salmo salar)

Y. Song, Norwegian University of Life Sciences, UMB / Centre for Environmental Radioactivity (CERAD CoE); B. Salbu, Norwegian University of Life Sciences (UMB) / Department of Plant and Environmental Sciences; H. Teien, L.S. Heier, D. Oughton, O. Lind, Norwegian University of Life Sciences (UMB) / Centre for Environmental Radioactivity (CERAD CoE); B.O. Rosseland, Norwegian University of Life Sciences, UMB / Centre for Environmental Radioactivity (CERAD CoE); K. Tollefsen, NIVA / Ecotoxicology and Risk Assessment, Norwegian Institute for Water Research (NIVA); Norwegian University of Life Sciences (UMB); / Ecotoxicology; Centre for Environmental Radioactivity (CERAD CoE)

There is a growing concern that risk could be underestimated for organisms exposed to multiple environmental stressors. In most cases, organisms are exposed to low level of stressors, thus more sensitive endpoints such as gene expression or enzymatic activity are needed to understand combined toxicity and mode of action (MoA). A combination of gamma radiation and uranium (U) is usually observed at U mining sites and other nuclear installations, posing both radiological and chemical risks to contaminated ecosystems. Gamma radiation and U are considered to share similar MoA by inducing free radicals and ROS, leading to oxidative stress and ultimately damage to macromolecules. This study aims to use hepatic biomarker gene and transcriptional responses of Atlantic salmon (*Salmo salar*) to understand the combined effect of low dose gamma radiation and U exposure. Preliminary experiments were conducted to determine the concentration-response relationship of U in fish liver, and to identify responses after exposed to gamma radiation. A 48h exposure study was then carried out based on the preliminary results using 0.25 mg/L U, 70 mGy/5h gamma and their combination. Biomarker gene response was determined using real-time qPCR and global gene expression analysis was performed using a genome-wide salmonid oligo microarray. Results clearly showed that biomarker genes involved in oxidative stress, DNA damage, apoptosis and protein degradation were up-regulated in a concentration-dependent manner by different concentrations of U and by gamma radiation. The microarray analysis showed a more complex pattern of transcriptional responses when exposed to the combination of U and gamma compared to individual stressor. Functional enrichment and Ingenuity Pathway Analysis (IPA) further revealed diverse toxicological pathways induced by single stressors and their combination. This study may enhance the understanding of multiple stressor effect in fish at the transcriptional level.

549 Pesticide Toxicity Index for Freshwater Aquatic Organisms: A Tool for Assessing Complex Mixtures of Pesticides in Streams

L.H. Nowell, US Geological Survey / Placer Hall; J.E. Norman, US Geological Survey / Oregon Water Science Center; J.D. Martin, US Geological Survey / Indiana Water Science Center; N. Nakagaki, US Geological Survey / California Water Science Center; W.W. Stone, US Geological Survey / Indiana Water Science Center

Pesticide mixtures commonly occur in streams with agricultural or urban land in the watershed. The Pesticide Toxicity Index (PTI) is a tool to assess the potential toxicity of complex pesticide mixtures to aquatic life by combining measures of pesticide exposure and acute toxicity in an additive model. Several studies of pesticide mixtures have shown that an additive model predicts toxicity within a factor of 2-3 of the observed toxicity, even for compounds with different modes of action. This study expands the PTI from 127 pesticides to 441 pesticides; modifies the PTI procedure to determine two types of PTI values—the Median-PTI and Sensitive-PTI—for use in different applications, depending on study objectives; and tests how well the PTI model predicts toxicity using data from published studies. The PTI is determined separately for fish, cladocerans, and benthic invertebrates. Toxicity data were obtained from publicly available databases and documents, and consist of 10,483 bioassays representing 547 species. The Median-PTI is calculated from median toxicity concentrations for individual pesticides, so is robust to outliers and is appropriate for comparing relative potential toxicity among samples, sites, or pesticides. The Sensitive-PTI typically is calculated from the 5th percentile of available toxicity concentrations, so is a more sensitive indicator of potential aquatic toxicity of mixtures. In data analyzed from the scientific literature, *Ceriodaphnia* survival was reduced to < 50% in 44% of samples with Median-PTI of 0.1 to 1, and in 97% of samples with Median-PTI values >1. Empirical thresholds that correctly predicted toxicity or nontoxicity in 90% of samples in an aggregated dataset were determined to be 0.3 for the Median-PTI and 1 for the Sensitive-PTI. These thresholds are not necessarily applicable to future studies because they are based on a limited number of pesticides from a limited number of studies. Other example applications to be presented include the use of the PTI to interpret invertebrate community condition in a national study of US streams, and the use of median toxicity concentrations to develop Toxicity-Weighted Use for agricultural pesticides as a tool for study design. The PTI is a relative indicator of potential toxicity that can be used in study planning and to interpret water quality data, relate pesticide exposure to biological condition in multi-stressor systems, and prioritize future assessments.

Contaminants of Emerging Concern for Fish: Assessing Exposure and Effects Across Biological Scales: Part A

550 Effects of bifenthrin and bifenthrin metabolite exposure on the endocrine system of Menidia beryllina

S.M. Brander, Bodega Marine Lab, University of California, Davis / Environmental Toxicology, University of North Carolina, Wilmington / Department of Biology & Marine Biology; K.M. Jeffries, B. DeCourten, University of California, Davis / School of Veterinary Medicine, Dept. of Anatomy, Physiology and Cell Biology; B. DeGroot, University of North Carolina, Wilmington / Dept. of Biology and Marine Biology; N.A. Fangue, University of California, Davis /

Wildlife, Fish, & Conservation Biology; R.E. Connon, University of California, Davis / School of Veterinary Medicine, Aquatic Toxicology Laboratory

Pyrethroid pesticides are acutely toxic to fish via interactions with voltage-dependent ion channels, leading to nervous system disruption. It is now known that some pyrethroids also possess endocrine activity. Bifenthrin, a pyrethroid now frequently utilized nationwide for agricultural applications and increasingly for structural pest control, acts as both an estrogen receptor agonist and antagonist at the pptr concentrations commonly present in aquatic ecosystems. Additional concerns have arisen regarding the endocrine activity of bifenthrin metabolites, which are produced via P450 enzymes in fishes or that result from microbial breakdown. As such, our experiments seek to expand upon what is already known regarding the mechanisms by which bifenthrin interferes with endocrine signaling in fish and to link changes in gene and protein expression with reproductive health status. We conducted 14d exposures to three pptr concentrations of bifenthrin and evaluated the expression of a suite of endocrine system-related genes via qPCR in *Menidia beryllina*, an established estuarine model fish that commonly encounters pyrethroids in situ. We found that estrogen-dependent genes were down-regulated overall in response to bifenthrin exposure, indicating either estrogen receptor antagonism or the triggering of negative feedback mechanisms. We also conducted 7d exposures to pptr concentrations of bifenthrin metabolites and to a mixture of bifenthrin and a commonly used pesticide synergist (piperonyl butoxide) that inhibits P450 enzyme activity, to determine the role of metabolism in bifenthrin's already established interference with estrogen signaling pathways. Results of these experiments indicate that bifenthrin influences the expression of the estrogen-dependent protein choriogenin, and that bifenthrin metabolites appear to have greater estrogenic activity than the parent compound. Ongoing work utilizing a *Menidia beryllina* microarray and RNA seq will further clarify the mechanisms underlying bifenthrin's modulation of the endocrine system. Since changes in molecular endpoints have the potential to scale up to higher levels of organization, these results are being considered in the context of reproductive output via data collected from spawning trials currently being conducted in the laboratory with bifenthrin-exposed *M. beryllina*.

551 Bifenthrin exposure increases plasma 17β-estradiol and alters transcripts in the dopaminergic signaling pathway in juvenile rainbow trout

J. Crago, UC-Riverside / Environmental Sciences; D. Schlenk, University of California-Riverside / Department of Environmental Sciences

Pyrethroid insecticides have consistently been measured in surface waters in the low ng/L range, particularly after storm events. Sources of pyrethroids to surface water include urban runoff from storm events and wastewater discharge through treatment facilities. Previous work in our lab has shown that exposure to the pyrethroid, bifenthrin is associated with increased plasma 17β-Estradiol and an increased volume of ovarian follicles in female steelhead trout. However, plasma vitellogenin was unchanged. The mechanism of action (MOA) by which this estrogenic effect takes place is unclear. Pyrethroids prolong neuronal depolarization binding the voltage-gated sodium channels in neurons through binding the voltage-gated sodium channels. One possible MOA is through modulation of Na+ concentrations or Na+ channels in the dopaminergic/GnRH pathway. The goal of the study is to determine the effect of exposure of bifenthrin on dopaminergic signaling in juvenile rainbow trout. Our initial results indicate that increased expression of transcripts involved in dopaminergic signaling are associated with increased levels of plasma 17β-Estradiol. These results indicate that the estrogenic-effects of bifenthrin may result from changes in signaling within the dopaminergic pathway.

552 Bisphenol A accumulation in rainbow trout oocytes impacts growth and development in multiple generations

O. Birceanu, M. Vijayan, University of Waterloo / Biology

Bisphenol A (BPA), a common chemical used in the plastic industry, is commonly found in the aquatic environment, but its impact on the aquatic biota are far from clear. BPA accumulates in lipid-rich tissues, including fish eggs, and this may be a factor leading to developmental defects in offspring associated with maternal exposure to contaminants. However, little is known about the impact of associated with maternal transfer of BPA in fish, and how it can affect growth in subsequent generations. We tested the hypothesis that BPA accumulation in oocytes, mimicking maternal transfer, leads to the disruption of somatotropic axis function and growth in rainbow trout (*Oncorhynchus mykiss*). We exposed rainbow trout oocytes to vehicle (controls; < 0.01% ethanol), and three BPA concentrations (0.3, 3 and 30 mg/l) for 3 h (oocyte enrichment), after which the oocytes were fertilized and placed in clean water for monitoring growth and development. The accumulated BPA at all concentrations in the eggs were completely eliminated by 42 days post fertilization (dpf). However, the impact on growth persisted long after BPA was no longer detectable in the embryos (>42 dpf). In the F1 generation, growth and development were monitored for 3 years, while the F2 generation was monitored for a year post-fertilization. BPA exposure significantly reduced specific growth rate, and this is in agreement with the suppression of key genes involved in the somatotropic axis function reported by us previously. To determine if the effects of BPA in the F1 generation were also present in the F2 generation, eggs from F1 females, developed from BPA exposed oocytes mentioned above, were fertilized with sperm from clean males. The embryos were maintained in clean water as above and growth and development of the F2 generation were monitored for a year post-fertilization. Our results for the first time demonstrate that the specific growth rate of rainbow trout is affected even in the F2 generation by parental exposure to BPA. This finding suggests that maternal transfer of BPA may affect key developmental growth processes leading to long-term and generational effects on growth in fish. The mechanisms leading to the suppression of growth are currently under investigation, and we hypothesize that key proteins involved in the somatotropic axis function are targets for BPA impact. This work was funded by an NSERC Strategic Grant to MMV and an NSERC CGS-D to OB.

553 Mechanistic effects of exposure to ibuprofen on inland silverside (Menidia beryllina)

K. Jeffries, UC Davis; S. Brander, University of North Carolina, Wilmington; B. DeCourten, N. Fangue, R. Connon, University of California, Davis

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) that is often detected in wastewater effluent and in the receiving waters. Ibuprofen has a relatively short half-life and therefore is not persistent in the environment; however its continual introduction into the aquatic environment via municipal wastewater effluent can lead to some fishes experiencing a chronic exposure in areas affected by wastewater. Ibuprofen is reported to inhibit cyclooxygenases, which are involved in prostaglandin production. Prostaglandins are involved in regulating reproductive hormone production and complex spawning behaviors, among other physiological processes, therefore exposure to ibuprofen may influence reproduction in fishes. We used inland silversides (*Menidia beryllina*; 66 days old), an estuarine fish species that is E.P.A. approved for toxicity testing, to determine acute and chronic effects of exposure to ibuprofen in fish at multiple levels of biological organization. Acute 96-hr exposures were used to identify the effects of ibuprofen on survival, which was 98-100% after 96 hr for concentrations as high as 18.8 mg/L and subsequently decreased at higher concentrations. Juvenile fish were then exposed to one of three ibuprofen exposure treatments (0.025, 0.25 and 2.5 mg/L), along with an experimental control group, for two weeks. We used qPCR analysis to determine the effects of ibuprofen on the expression of hormonally-responsive genes and found that ibuprofen affected the expression of hormone receptors associated with sex-steroid and thyroid hormone binding. These dose-response patterns were non-monotonic, which is characteristic of hormone receptors in organisms exposed to low doses of compounds that can elicit hormonal effects. Follow up work will use a species-specific microarray to further examine the cellular responses to ibuprofen exposure. Our results show that at low concentrations, consistent with those found in wastewater effluent, chronic exposure to ibuprofen can induce cellular responses in the inland silverside. Ongoing work conducting spawning trials with ibuprofen exposed adults will evaluate whether concentrations that elicit molecular-level responses in *M. beryllina* are correlated with changes in reproductive output.

554 Overt Toxicity and Behavioral Effects of Organophosphate Flame Retardant Exposure to Developing Zebrafish

L. Dishaw, H.M. Stapleton, Duke University / Nicholas School of the Environment

Concerns about the potential toxicity of organophosphate flame retardants (OPFRs), the primary replacements for the penta brominated diphenyl ether commercial mixture (PentaBDE), have been raised due to their persistence in the environment and structural similarity to neurotoxic organophosphate pesticides. We evaluated the developmental toxicity of four OPFRs (tris (1,3-dichloro-2-propyl) phosphate (TDCPP); tris (2-chloroethyl) phosphate

(TCEP); tris (1-chloropropyl) phosphate (TCPP); tris (2,3-dibromopropyl) phosphate (TDBPP)) and chlorpyrifos (CPF) using overt toxicity and locomotor activity assessments in zebrafish (*Danio rerio*). Embryos were reared in 96-well plates with exposures occurring from 0-5 days post-fertilization (dpf). For the overt toxicity assessment (i.e., lethality and malformations), embryos were exposed chemicals at concentrations ranging from 0.033 – 100 µM (½ log increments; n=4/dose). On 6 dpf, a detailed visual malformation assessment and a high-content image analysis using a Cellomics® Array Scan® system with the Zebratox V4 bioapplication was performed on each larva. For behavioral testing, chemicals were tested on separate plates (5 doses in the sub-overt toxicity range, ¼ log increments; n=24-36/dose). On 6 dpf, a video tracking system assessed larval swimming activity in both light and dark conditions. No mortalities or malformations were observed in embryos exposed to TCPP or TCEP. Larvae developmentally exposed to ≥10 µM CPF or TDCPP, or >1 µM TDBPP showed ≥75% lethality and/or severe malformations. The LC50 values for CPF, TDCPP, and TDBPP were 19, 11, and 2 µM, respectively. Larvae developmentally exposed to TDCPP, TDBPP, TCEP and CPF at concentrations below the overt toxicity threshold exhibited altered swimming activity during behavioral evaluations. These results indicate that, despite structural similarities, the overt toxicity of these organophosphate chemicals varies greatly in developing zebrafish. Two of the four OPFRs tested exhibited greater overt toxicity than CPF and ongoing experiments will determine whether the LC50s are being driven by differences in chemical uptake and metabolism. Importantly, behavioral changes were observed following developmental exposures to levels below the overt toxicity threshold. These data indicate that early life exposure to some OPFRs may alter neurodevelopmental processes in zebrafish.

555 Mammalian read across comparison of the effect of diphenhydramine and acetylcholinesterase inhibitor mixtures on Danio rerio during development

L.A. Kristofco, Baylor University / Department of Environmental Science, Center for Reservoir and Aquatic Systems Research; B. Du, Baylor University / The Institute of Ecological, Earth, Environmental Sciences / Department of Environmental Science, Center for Reservoir and Aquatic Systems Research; K. Chambliss, Baylor University; J.P. Berninger, USEPA; B.W. Brooks, Baylor University / Department of Environmental Science

In rapidly urbanizing regions of the southwestern and south central US, instream flows are increasingly dominated by discharges from wastewater treatment plants. Future population growth and climate change projections for Texas indicate that base flows of many river systems will depend on these effluents, which contain a complex mixture of contaminants. Interactive effects of pesticides and pharmaceuticals, however, are poorly understood. The objective of this study was to examine the influence of mixtures of the antihistamine, diphenhydramine (DPH), and acetylcholinesterase inhibiting (AChEI) insecticides to zebrafish. Though DPH inhibits the H1 receptor, eliciting antihistamine responses, in mammals DPH also inhibits serotonin uptake at the serotonin reuptake transporter and functions as a competitive antagonist for the acetylcholine receptor (AChR). In fact, due to AChR inhibition DPH has been suggested as an emergency medicine treatment for mammalian AChE poisoning. Based on evolutionary conservation of cholinergic neurotransmissions among animals, we hypothesized that the mammalian pharmacology and toxicology data for AChEI and DPH mixtures will predict the nature of chemical mixture toxicity to *Danio rerio*. We employed the Fish Embryo Toxicity (FET) test method to assess the acute toxicity of zebrafish at various ages to single compounds and AChEI/DPH mixtures to define interactive effects over development. In addition to mortality, other endpoints (e.g., biomarkers, developmental abnormalities and behavior) were also assessed. Developmental timing of exposure was found to influence the magnitude of toxicity observed in zebrafish; in a stage dependent fashion, later developmental windows saw increased sensitivity for both DPH and the AChEI insecticide. However, for all developmental periods evaluated, diphenhydramine was not found to confer a protective effect for zebrafish mortality; rather, additive toxicity was observed.

556 Bridging the Gap Between Screening Assays and Estrogenic Effects In Fish

E.E. Yost, North Carolina State University / Environmental and Molecular Toxicology; C. Lee Pow, North Carolina State University / Department of Environmental and Molecular Toxicology; S.W. Kullman, North Carolina State University

Estrogenic contaminants have gained notoriety as one of the preeminent classes of endocrine disrupting compounds in the aquatic environment. In this study, we aim to delineate the ligand interactions that drive the response in teleost fish that are exposed to estrogen receptor (ER) agonists, as well as bridge the gap between human ER-based *in vitro* estrogen screening assays and actual effects in fish. Adult male Japanese medaka (*Oryzias latipes*) were exposed to solutions of individual steroidal estrogens [17β-estradiol (E2β), estrone (E1), 17α-estradiol (E2α), or estriol (E3)]; or to effluent from a swine operation, which contains a mixture of these compounds. All exposures were conducted at concentrations predetermined to be equipotent in the yeast estrogen screen (YES), a widely used *in vitro* assay that reports activation of the human ERα. Response in the medaka was determined by quantifying hepatic expression of estrogen-responsive genes, including vitellogenin and choriogenin. We found these exposures did not elicit equal responses in the medaka: gene expression changes were significantly lower in E1-, E2α-, and swine effluent-exposed fish, versus fish that were exposed to E2β or E3. To explore the ligand interactions that drive these differential responses, competitive ligand binding assays were then used to determine the relative binding affinities of these steroidal estrogens and swine waste effluent for the three medaka nuclear ER subtypes: ERα, ERβ1, and ERβ2. We found the compounds that elicited the most robust estrogenic responses in the fish (E2β and E3) have preferential binding affinity for the medaka ERβ1 and ERβ2, while the compounds that elicited a weaker response (E1 and E2α, as well as the swine effluent) have greater affinity for the medaka ERα. To determine whether this pattern holds true at the transactivational level, *in vitro* transient transactivation assays were conducted using the three medaka ER subtypes in combination with a medaka vitellogenin-1 gene reporter system. These results support emerging evidence that ERβ1 and ERβ2 are critical in driving the teleost estrogenic response. Accordingly, the incorporation of these teleost ER subtypes into *in vitro* screening assays may increase the ability of these assays to recapitulate estrogenic effects in exposed fish, and in turn help to better inform the ecological risk assessment process.

557 Insights from a Series of Intensive Time-Course Studies in Fish Exposed to Endocrine Active Chemicals

D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory

Over the past several years, our research team has conducted an extensive series of laboratory-based experiments characterizing the time-course of fathead minnow (*Pimephales promelas*) responses to endocrine active chemicals (EACs). Eight EACs with varying modes of action were tested. Samples were collected at multiple time points following one to eight days of exposure as well as additional time-points following cessation of chemical delivery, to evaluate the ability of the fish to recover from the stressors. Additionally, a number of shorter time-course studies examining responses occurring within the first 24 h of exposure were conducted. Endpoints examined included gonadal gene expression as assessed using both targeted (i.e., real-time quantitative PCR) and non-targeted (i.e., microarray), ex vivo steroid production, hepatic metabolomics, and plasma steroid and vitellogenin concentrations. Focusing on the targeted endpoints, we observed rapid responses to most EACs tested, particularly relative to ex vivo steroid production and/or plasma steroid concentrations. There was significant evidence of compensatory responses over time, likely mediated through feedback mechanisms within the hypothalamic-pituitary-gonadal axis, during periods of continuous exposure as well as typically rapid recovery after cessation of chemical delivery. The data reflect a challenge to the use of molecular and biochemical responses in vivo as a reliable methods for detecting exposure to, or predicting effects of EACs. Specifically, the responses vary with time such that an effect identified as significant at one time point, may be identified as non-significant, or even opposite in direction (i.e., a significant increase as opposed to decrease, or vice versa) at another time-point. Relative to effects-based exposure assessment, the data suggest that short-term exposures that capture initial direct effects may have some advantages over longer-term exposures over which compensatory and indirect effects take place, for sensitive detection of EACs. They also suggest the importance of effective modeling of compensatory response if short-term tests are to be used to predict long-term adverse outcomes. Overall, these data underscore the need to understand temporal dynamics when designing and implementing effects-based methods to detect EACs. The contents of this abstract do not necessarily reflect USEPA policy.

Wastewater Effluents: Chemical and Ecotoxicological Characterization

558 A PNEC proposal for a complex mixture: case study for the commercial dye Disperse Red 1

F.I. Vacchi, Faculty of Pharmaceutical Sciences; A.F. Albuquerque, University of Campinas; J.A. Vendemiatti, University of Campinas; G.J. Zocolo, Institute of Chemistry of Araraquara; M.V. Zanoni, Unesp / Analytical Chemistry, Institute of Chemistry of Araraquara / Quimica Analitica; H.S. Freeman, North Carolina State University; G.D. Umbuzeiro, University of Campinas / Faculty of Technology

Disperse dyes are synthetic colorants for hydrophobic substrates and are often used as commercial mixtures in textile coloration. As the main dye class for polyester fibers, they are used in great quantities and the associated dyeing processes can generate high levels of wastewater containing residual dyes and dyeing auxiliaries. The most often used disperse dyes are azo compounds, some of which are known to have toxic and genotoxic properties. This makes the textile activity important from an environmental standpoint. The commercial dye Disperse Red 1 was characterized and its individual colorants were evaluated for ecotoxicity. Acute toxicity tests were performed with *Ceriodapnhia dubia, Ceriodaphnia silvestrii, Daphnia similis, Daphnia magna, Hydra attenuata*, and chronic tests, with *Pseudokirchneriella subcapitata, Ceriodapnhia dubia,* and *Hydra attenuata.* Chemical analysis using TLC and HPLC MS/MS were used to verify the occurrence of Disperse Red 1 in river water under the influence of textile discharges. The commercial dye contained 60% (w/w) of CI Disperse Red 1, other dye components (20%) and a surfactant (20%). A PNEC was derived for the Disperse Red 1 dye, based on the toxicity of the commercial mixture. This was possible because the toxicity of the Disperse Red 1 was accounting for the toxicity of the mixture. Considering 60% of Disperse Red 1 in the commercial product, a PNEC of 120 ng/L for the CI Disperse Red 1 ((*N*-Ethyl-*N*-(2-hydroxyethyl)-4-(4-nitrophenylazo) aniline) was proposed based on the lowest NOAEC (*P. subcapitata* – 0.006 mg/L) divided by 50 of assessing factor. Disperse Red 1 was found in river waters that receive textile effluents in São Paulo state, Brazil, in 4 different samples in concentration up to 109 ng/L. Exposure values of Disperse Red 1 are in the same range of the PNEC, so ecotoxicological risk is expected. More data are being obtained to assess the risk of the presence of this compound in river waters where aquatic life needs to be protected. Acknowledgment: FAPESP, CAPES, USP, UNICAMP.

559 Spatial and Temporal Occurrence of Estrogenic Activity in Urban Effluent-Dominated Systems

D. Martinovic, University of Saint Thomas / Dept. of Biology, USEPA, Mid-Continent Ecology Division / Mid-Continent Ecology Division; T. Minarik, MWRDGC / Associate Aquatic Biologist; M.M. Schultz, College of Wooster / Department of Chemistry; H.I. Schoenfuss, St. Cloud State University

The present study investigated occurrence of environmental estrogens (EEs) in waterways managed by the Metropolitan Water Reclamation District of Greater Chicago ('District') – one of the largest and most complex water districts in the United States. The objectives of the study were: (i) to document spatial and temporal occurrence of EEs in the Chicago Area Waterways (CAWs); (ii) to determine whether water reclamation plant (WRP) effluents contribute to estrogenic pollution of the receiving streams; (iii) to determine whether the mandated water quality monitoring data could be used to predict estrogenic pollution in the receiving streams; and (iv) to determine whether snow melt, storm runoff and combined sewer overflows may be contributors of estrogenic activity to these systems. The estrogenic potency of the waterways was assessed using a cell-based reporter gene assay. The water quality data was readily available as part of the District's regular monitoring program. Our findings indicate that EEs are commonly found in the CAWs, and that WRP effluents are one, but not the only important contributors to estrogenic activity. Mean estrogenic activities in CAWs (11 ng estradiol equivalents (EEQs/L) are well within the values reported for other urban areas and WRP effluents. The estrogenic activity exhibited significant seasonal variation with highest values noted during the spring and summer months. When comparing mean estrogenic activity of general use waters, secondary contact waters and WRP effluents we found that general use waters had significantly lower estrogenic activity (ca 5 ng EEQ/L) than the other two matrices (ca 15 and 17 ng EEQ/L respectively). Our analyses indicate that estrogenic activity of the waterways was not reliably associated with mandated water quality parameters, and that such measurements may not be useful for predicting estrogenic activity, especially so in the complex urban systems. One of the prominent findings of this study is that EEs do not follow predictable spatial patterns – many of the upstream sites in the heavily urbanized areas had levels of estrogenic activity comparable to those found in the effluents and downstream locations. Our data suggest that surface runoff and snow melt are estrogenic (0-9 ng EEQ/L), and given that their estrogenic activities are similar to those of their receiving waterways (0-7 ng EEQ /L), we conclude that these non-WRP sources are important contributors to estrogenic activity of the CAWs.

560 Endocrine Disrupting Compounds in Wyoming Surface Waters: Assessment of Presence, Suspected Sources, and Impacts to Exposed Fishes

E.O. Johnson, Department of Zoology & Physiology; H.L. Bergman, University of Wyoming / Department of Zoology & Physiology

In the last two decades, studies in North America and Europe have demonstrated the widespread occurrence of intersex condition and other reproductive abnormalities in fish related to the presence of endocrine disrupting compounds (EDCs) in surface waters. Many of these studies point to the discharge of steroid hormones and industrial chemicals from wastewater treatment plants (WWTPs) as the principle source of these abnormalities, but the deposition of airborne pollutants and leaching of steroids from livestock waste pose similar threats. Because there is little information about the presence of EDCs in Wyoming, and because livestock outnumber the human population more than 2 to 1, Wyoming provides a unique scenario for studying the impacts of EDCs from both WWTP and agricultural sources. Pursuant to assessing the presence, sources, and impacts of EDCs in Wyoming, we collected white suckers *(Catostomus commersonii)* in the fall of 2011 and the spring of 2012 from three sites on the Laramie River including: (1) a site upstream of potential runoff from Wyoming rangelands, (2) a site downstream of potential runoff from Wyoming rangelands, and (3) a site immediately downstream of the Laramie WWTP discharge. Following collection, histological examination of the gonads revealed a low occurrence of intersex condition and oocyte atresia in downstream fish. In addition, water samples were analyzed from all three sites showing low levels of steroid hormones, alkylphenols and contaminants of emerging concern, with highest concentrations in the Laramie WWTP effluent . In August of 2012, caged fathead minnows *(Pimephales promelas)* were deployed for a seven day exposure at 7 surface water sites across the state of Wyoming, including those where white sucker had previously been collected. Quantification of hepatic vitellogenin expression following the 7 day exposure showed no significant induction at any of the 7 sites across the state.

561 Estrogenic effects on rainbow trout exposed to multiple pulp production effluent and caged along a gradient of pulp mill effluent discharge in Chile

G. Chiang, Canadian Rivers Institute, Biology; P.A. Bahamonde, University of New Brunswick / Biology department; M. Rivas, University of Concepcion / Department of Zoology; M. Diaz-Jaramillo, Environmental Ecotoxicology and Chemistry Laboratory / IIMyC, UNMdP-CONICET; R. Barra, EULA Chile Environmental Science Centre, University of Concepcion, Chile / Aquatic Systems Research Unit; K.R. Munkittrick, Canadian Rivers Institute, Biology

Pulp mill effluents have shown to have different impacts on fish reproduction with a series of reproductive impairments in Northern hemisphere, such as reduced gonads, diminished fecundity with female age, reduction of secondary sexual characteristics in males, and alteration in reproductive hormones. On the other hand, fish reproductive responses to Chilean PPME have been associated with increases in gonad size, induction of maturation and estrogenicity in female juvenile rainbow trout, while in wild native fish populations there is evidence of increased gonad size in males, increased *in vitro* production of 17β-estradiol in females and decreased in vitro production of 11-ketotestosterone in males. We assessed reproductive (plasma Vitellogenin, gonad development) and metabolic responses (EROD activity, relative liver size and condition factor) of juvenile female and male rainbow trout (*Oncorhynchus mykiss*), exposed separately to tertiary treated PME from *E. globulus* and *P. radiata* pulp production, along with an *in situ* bioassay downstream of the discharge of the same pulp mill. EROD activity was induced in all the experiments, at the higher PPME concentration and downstream of the discharge. Despite the average increment in GSI in exposed fish, there was no significant difference between exposed

and unexposed individuals. Despite the lack of significant differences in gonad size, female and male fish showed very high concentrations of plasma Vitellogenin, especially in the fish exposed to Eucalyptus. Despite the low concentration of effluent in the river, similar responses were observed in the caged fish. In fact, male fish showed higher plasma vitellogenin concentrations in all experiments and intersex was observed in a few male exposed in the bioassays and in the cages. Despite previous evidence of estrogenic effects of PPME in Chile, this is the first study confirm estrogenic effects in male fish.

562 The response of wild fish to municipal wastewater effluent exposures at sites in Canada

G. Tetreault, Environment Canada / Aquatic Ecosystem Protection Research Division; C.J. Brown, University of Waterloo / Department of Biology; R. Tanna, University of Waterloo / Biology; P. Marjan, University of Waterloo; M. Fuzzen, University of Waterloo / Biology; K. Hicks, Wilfrid Laurier University; B.M. Smith, University of Waterloo / Department of Biology; K. Shires, Environment Canada; J. Bennett, M.E. McMaster, Environment Canada / National Water Research Institute; M.R. Servos, University of Waterloo / Department of Biology

The extent and implications of municipal wastewater effluent (MWWE) of fish in the Canadian environment at various levels of biological organization is currently poorly understood. The objective of this research was to examine the impact of municipal wastewater effluents discharged into the Canadian environment on the status of sentinel fish species at various levels of biological organization (cellular, tissue, individual, population and community). Field studies conducted in the Grand River watershed, Ontario, Canada, investigated the cumulative impact of two sewage discharges. Responses of wild fish, Rainbow Darter (*Etheostoma caeruleum)* and Greenside Darters (*E. blennioides*), were assessed in terms of energy storage (condition factor), and energy utilization (reproduction: *in vitro* sex steroid production, gonadosomatic indices, and histology [cellular development and intersex]). Both sentinel species collected downstream of both discharges demonstrated significant changes in size, lower gonadosomatic indices, impaired steroid production capacity, and altered sperm cell staging. Exposed female fish also had impair capacity to produce estrogens in vitro, however did not demonstrate differences in oocyte development. Male darters and other fish species collected downstream of both sewage discharges had elevated incidence of intersex. The fish communities downstream of the these outfalls demonstrated differences in abundance, diversity, and species composition (to larger more mobile, tolerant-omnivourus fish species such as suckers and sunfish. The potential exists for a cumulative impact of multiple outfalls of treated wastewater effluents as changes were more pronounced downstream of the second sewage discharge. The next steps of this research include investigation of responses of fish to remedial actions at these plants using wild fish and controlled lab and caging experiments. Ultimately these results will be utilized to develop and evaluate a cumulative effects assessment framework.

563 An ecosystem model to assess the ecotoxicological impacts of endocrine disrupters released by wastewater treatment plants

L. Clouzot, Universite Laval / Département de génie civil et de génie des eaux; M. Paterson, Fisheries & Oceans-Canada / Freshwater Institute; A. Dupuis, P. Blanchfield, Fisheries & Oceans Canada; K.A. Kidd, University of New Brunswick; P.A. Vanrolleghem, ModelEAU – Université Laval / Département de génie civil et de génie des eaux

The ecotoxicological impacts of micropollutants (MPs) released by wastewater treatment plants (WWTPs) on receiving waters are a current research priority. There is limited knowledge of how WWTP design and operation can be modified to reduce the ecological risk imposed by the effluent. Models are used to predict the ecotoxicological impacts of organic matter and nutrients discharged by WWTPs but MPs have not been considered. "Model-based benchmarking" is an approach used by the wastewater industry to compare the efficacy of WWTPs. It is performed by comparing results from realistic simulations with models of different WWTPs anda ggregated criteria such as an operating cost index or the average and peak concentrations of ammonia and BOD_5 in the effluent. A simulation benchmark criterion for ecotoxicological impacts of MPs would be a very useful and relevant addition. This study takes up the challenge to develop such benchmark criterion by assessing the potential ecotoxicity of an effluent through simulation of its impact with a model of a typical aquatic ecosystem. In the case study, the model considers endocrine disturbing effects on individual aquatic organisms, as well as whole ecosystem responses that occur through ecological interactions, i.e feeding and competition. The experimental data used to develop the model come from a multi-year whole-ecosystem study performed at the Experimental Lake Area (ON, Canada). The synthetic hormone 17α-ethinylestradiol (EE2) was added during three years in an experimental lake at environmentally relevant concentrations. Experimental data were collected before, during and after EE2 addition. Endocrine disruption was observed in the fish species with a collapse of *fathead minnow* after the second year of EE2 addition. The developed ecoystem model is based on simplified AQUATOX equations for the species naturally present in the experimental lake (benthic invertebrates, phyto- and zooplankton, fish). The novelty of the study is to add appropriate equations for endocrine disruption. Two fish classes are used in the model: juveniles and adults. The developed model can simulate endocrine disruption based on (i) an increase of gamete mortality, (ii) a decrease of gamete production or (iii) an increase of fish mortality. The ecological benchmark criterion is then calculated as a single index (e.g., Simpson's index of diversity) that is deduced from the simulated abundance of the different populations.

564 Monitored organic sum-parameters in industrial effluents: can we quantify the associated ecotoxicity?

C.E. Raptis, Institute of Environmental Engineering; R. Juraske, Swiss Federal Institute of Technology Zurich, ETH Zürich; S. Hellweg, ETH Zürich / Institute of Environmental Engineering

Commonly measured organic sum-parameters, such as total organic carbon (TOC), are often monitored during wastewater treatment stages for process control, but also at the end of pipe, to gauge the quality of treated industrial effluents. Despite its ubiquity, there are no indications of the ecotoxicological implications associated with this organic sum-parameter, or of how these would vary depending on the industry. Here, we propose a methodology to better understand the nature of TOC in terms of its ecotoxicity, for industries with relatively constant processes and effluent profiles. The methodology is based on results obtained from whole effluent toxicity (WET) bioassays in combination with metal toxicity modelling via biotic ligand models (BLM) or free ion activity models (FIAM), and the overarching assumption is the mixture toxicity principle of concentration addition. Additivity of toxicity is assumed for either fraction of an effluent, broadly split into an organic and an inorganic part. Accordingly, the toxicity thought to be caused by the organic fraction of the effluent, in other words TOC, is isolated by subtracting the modelled toxicity due to the metals from the observed whole effluent toxicity. We present the results of a test-of-concept study with long-term data WET and effluent chemistry data from treated pulp and paper mill effluents. We explore the possible connection between TOC and toxicity, as well as the implications and limitations of the assumptions made, and we discuss the usefulness of deriving conventional ecotoxicity metrics for TOC: median effective concentrations (EC50) for sublethal endpoints for *P. subcapitata* (growth) and *C. dubia* (reproduction) were estimated at 95.3 mg/L TOC (95% CI: 76.0 – 114) and 222 mg/L TOC (95% CI: 164 – 274), respectively; achieving similar ecotoxicity metrics for TOC measured in effluents of other industries could provide some insight into the relative ecotoxic potency of this widespread organic sum-parameter. Such results can be particularly useful in environmental decision support tools, like life cycle assessment (LCA).

565 Advanced treatment technologies as an additional step in wastewater treatment – evidences for beneficial effects on organic matter decomposition

M. Bundschuh, Department of Aquatic Sciences and Assessment; Swedish University of Agricultural Sciences; R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences

Ozone is a potent oxidant due to the formation of free radicals, while activated carbon adsorbes substances from the surrounding medium. Therefore, both advanced treatment technologies have the potential to remove pharmaceuticals and other organic micropollutants from wastewater. The project "Strategy MicroPoll" examined the application of these techniques at municipal wastewater treatment plants in Switzerland. The ecotoxicological effects of the advanced treatment technologies were investigated in several experiments in the laboratory, in situ and field microsoms using *Gammarus fossarum* test organism, which is an important leaf shredding organism in European catchments. A first laboratory experiment investigated the effects of ozonation on the food choice of *G. fossarum* found a significant preference of the test organism for leaves conditioned in wastewater treated with

5 mg ozone/L when offered together with leaves conditioned in non-treated wastewater. In addition, the feeding activity *G. fossarum* was significantly higher when exposed towards ozone treated wastewater compared to non-treated wastewater and was linearly related as *Gammarus* was exposed to different mixtures of ozone treated and non-treated wastewater. In situ experiments were conducted in one receiving stream, i.e. the Futbach, before and during the operation of the ozonation. Those experiments confirmed feeding activity inhibition in *Gammarus* exposed at downstream sites compared to upstream sites and a disappearance of the effect with the beginning of the ozonation. Finally, population sizes of *G. fossarum* in outdoor microcosms were also significantly reduced by approximately 60% in non-ozone treated compared to ozone treated wastewater. Potential mechanisms of ozonation that lead to an increase of food quality for invertebrates are discussed.

Application of Bioavailability in Risk Analysis and Remedial Decision-Making

566 Relative Bioavailability Methods

B.H. Magee, ARCADIS; G.C. Hoeger, ARCADIS / Environmental Science, Assessment and Planning

Data from animal studies used to derive toxicity factors for human health risk assessment are rarely measured as internal doses of chemicals. Toxicity factors are issued and later applied in risk assessments in administered dose units. Toxicity studies typically use highly bioavailable forms of the chemicals, such as dissolved metal ions or crystalline organics dissolved in solvents, to ensure the maximum amount of chemical is delivered to the target tissue to characterize the effects of exposure. Bioavailability studies over the past 20 years have shown that chemicals in complex matrices or chemicals that have been in contact with soils for many decades result in lower absorbed doses compared to doses reported in toxicity studies. To determine the degree by which bioavailability of chemicals in environmental media is reduced, one need not totally characterize the pharmacokinetics and disposition of the chemical in all compartments and all excreta. Pharmacokinetic characterizations often require radiolabeled analytes, which cannot be aged to the degree that chemicals are aged in environmental media. Thus, radiolabelled studies do not provide information about site-aged soils. For risk assessment purposes, one need not know the absolute bioavailability of a chemical. The most relevant information for site-risk assessment is the relative bioavailability of a chemical. Relative bioavailability refers to the degree to which the environmental matrix reduces bioavailability compared to the bioavailability in the controlled laboratory study from which the toxicity factor was derived. This presentation will focus on methods used to evaluate relative bioavailability of chemicals and incorporation of data into human health risk assessments.

567 State of the Science in Arsenic Bioavailability for Use in Human Health Risk Assessment

Y.W. Lowney, Exponent, Inc. / Health Sciences

Arsenic is a chemical that is identified as a potential contaminant of concern at many contaminated sites, and appropriate assessment of potential risks requires meaningful assessment of exposure, including understanding bioavailability. Several studies have been conducted to characterize the bioavailability of arsenic from soils, and there is an emerging body of literature regarding *in vitro* methods to estimate bioavailability for this chemical. This presentation will provide a summary of the currently-available information regarding the bioavailability of arsenic from soil for use in human health risk assessment, including a compilation of data from existing animal studies. The presentation will also address efforts to validate in vitro methods to estimate this exposure parameter: including the strengths and limitations of the in vitro method development work that has been conducted to date (e.g., regarding study design, sample number, source characterization, concentration ranges, etc.), including a discussion of some of the parameters that appear to affect arsenic dissolution from soil in *in vitro* systems. The presentation will also include a discussion of how to proceed in developing a bioavailability adjustment for arsenic in light of uncertainties, developing a "weight-of-evidence" approach for site-specific application.

568 Identification of arsenic form, bioaccessibility and applicability of phosphate soil washing in smelting activity impacted soil

K. Yang, Seoul National University / Department of Civil and Environmental Engineering; J. Im, Seoul National University; E. Jho, Seoul National University / Department of Civil and Environmental Engineering; K. Nam, Seoul National University / Dept. of Civil and Environmental Engineering

The arsenic (As) bioaccessibility in soil depends on which fraction of soils it is associated with and this, in turn, influences the selection of an appropriated remediation technology. In this study, the relationship between the As-bound soil fractions and the bioaccessibility of As in As-contaminated former Janghang smelter site in Korea was assessed to determine the remediation strategy of this site. In addition, the efficiency of a soil washing treatment using phosphate as a washing solution on the removal of bioaccessible As in the site soils is investigated. Total As concentrations in 96 surface soil samples (depth of 0-15 cm) determined by *aqua regia* ranged from 9.8 to 170 mg/kg (average of 43±30 mg/kg) with 81 samples exceeding the Korean soil regulatory level for As (25 and 75 mg/kg for residential and forest areas, respectively). A sequential extraction procedure developed by Wenzel et al. was adopted to analyze As associated with different fractions of soils. The majority of As (59-81%, average of 70±6.4%) could be extracted with oxalate or oxalate with ascorbic acid (i.e., As bound to amorphous or crystalline Fe/Al oxyhydroxides) and the rest of As was associated with the residual fraction (18±6%), the phosphate- (11±5%) and sulfate-extractable (1.0±0.7%) fractions. The bioaccessible As fraction determined with the Solubility/Bioavailability Research Consortium (SBRC) method ranged from 2.2 to 45 mg/kg (average of 11±7.8 mg/kg), which is equivalent to bioaccessibility of 9.0 to 66% (average 29±16%) (i.e., the ratio of the SBRC-extractable concentration to the *aqua regia*-extractable concentration). The bioaccessible As consists of the sulfate- and phosphate-extractable As and 20% of the oxalate- or oxalate with ascorbic acid-extractable As. For this As-contaminated soil, soil washing using ammonium phosphate can be an effective technique for bioaccessible As removal and reuse of the treated soil. The extracted As using 0.5 M ammonium phosphate (16 hr, solid-liquid ratio of 1:25) ranged 30-52% (average of 41±9%). This study shows that soil washing using ammonium phosphate can remove the bioaccessible As and meet the Korean soil regulatory level for the residential area. Subsequent study is being undertaken to determine the optimum washing condition and the changes in ecotoxicity of the soils. These together will contribute towards suggesting the remedial strategy for the former smelter site.

569 Establishing Blood Biological Screening Indices for Cobalt Exposure

A.D. Monnot, Cardno ChemRisk, LLC; A.L. Perez, Cardno ChemRisk; B. Finley, Cardno ChemRisk, LLC / Health Scientist

Excessive cobalt (Co) exposure has been reported to elicit a variety of non-cancer systemic health effects including hematological, respiratory, dermatological, neurological, immunological, reproductive, cardiovascular and endocrine disturbances. In industry, the potential for exposure to Co is particularly important during the production, processing and use of hard metals, as well as, the polishing of diamonds with Co containing disks. Co exposure can also occur via voluntary ingestion of vitamin B_{12} and other Co-containing supplements, ingestion of Co contaminated water and other environmental media, or from the presence of Co-containing prosthetics and other medical devices in the body. This presentation discusses the development of a blood biomonitoring equivalent for Co exposure. In this analysis, animal toxicology and epidemiology literature were evaluated to identify blood Co concentrations at which effects have, and have not been reported. A Co biokinetic model was developed and utilized to convert oral doses to blood Co concentrations in order to determine the blood Co dose-response relationship. In addition, the standard USEPA risk assessment methodology was utilized to establish a chronic oral reference dose (RfD) for Co from human and animal toxicology literature. Following the derivation, the Co biokinetic model was utilized to estimate blood Co concentrations associated with the derived RfD to identify a blood biomonitoring equivalent for Co. A dose of 0.9 mg Co/kg-day was identified as the highest NOEL for endocrine responses from the literature and was chosen as the POD for the RfD derivation. Applying an aggregate uncertainty factor of 30 to the POD yields a chronic oral RfD of 0.03 mg/kg-day. Utilizing a Co biokinetic model, an oral dose of 0.03 mg Co/kg-day corresponds to whole blood Co concentrations of approximately 12–29 µg/L in blood, assuming a 70 kg adult. The results from the dose-response analysis indicated that blood Co concentrations of 300 µg/L and higher were associated with certain

hematological and reversible endocrine responses. These results indicate that the estimated biomonitoring equivalent is well below the blood Co concentration expected to elicit systemic responses. These values could potentially be used as biological screening indices to assess the health risk of individuals exposed to Co.

570 Oral Bioavailability and Dermal Absorption of PAHs in Soil: State of the Science and Applications to Risk Assessment

M. Ruby, Integral Consulting Inc; Y.W. Lowney, Exponent, Inc. / Health Sciences; P. Tomlinson, Integral Consulting Inc.; A. Bunge, Colorado School of Mines; J. Kissel, University of Washington; S. Roberts, University of Florida

This presentation will discuss the state of the science regarding the oral bioavailability and dermal absorption of carcinogenic polycyclic aromatic hydrocarbons (cPAHs) in soil. Direct contact exposures to chemicals in soil can occur by way of the oral, dermal, and inhalation pathways. However, human exposures from direct contact to cPAHs in soil are dominated by the oral and dermal exposure routes because exposure from inhalation of PAH-bearing soil particulates is generally negligible. The discussion will review the *in vivo* studies conducted to date to measure the relative bioavailability of cPAHs from soil, the development and application of *in vitro* tests for evaluation of cPAH bioaccessibility, and the *in vivo* and *in vitro* studies conducted to assess dermal absorption of benzo(a)pyrene (BaP) from soil. Insights from preliminary results from a research program to develop a robust *in vivo* model for measuring the relative oral bioavailability of cPAHs, and an *in vitro* study of dermal BaP absorption, will be presented. The implications of these data for human health risk assessment will be discussed, along with the relative importance of oral and dermal absorption and their potential impacts on soil cleanup goals. Finally, upcoming changes in the regulatory toxicology of cPAHs, such as the pending toxicity reassessment for BaP, EPA's proposed new relative potency factors, and the potential for a dermal cancer slope factor will be discussed, along with the possible implications of these changes on human health risk assessment.

571 Application of Mamalian Bioavailability of Polycyclic Aromatic Hydrocarbons in Site Risk Assessments

S.D. Siciliano, University of Saskatchewan / Toxicology Group and Department of Soil Science; K.J. James, University of Saskatchewan / Toxicology Group and Soil Science; R. Peters, Soil Science Department / Toxicology Group and Department of Soil Science; M.L. Wickstrom, University of Saskatchewan / Toxicology Group

At many contaminated sites, ingestion of contaminated soil particles can be a primary risk driver. At some sites, these soil particles contain polycyclic aromatic hydrocarbons (PAH) which can pose a significant carcinogenic risk to human receptors. The bioavailability, fraction that reaches systematic circulation, of these PAHs is widely known to be less than 100%. However, it is not clear how, or if, these bioavailabilty estimates can be used to estimate risk at a contaminated site for several reasons: (1) it is not known how repeated exposure to PAHs influences PAH bioavailability in animal models used to estimate human bioavailability, (2) it is not known how or if bioavailability is linked to exposure concentrations, (3) it is not known how exposure concentrations interplay with soil properties to influence bioavailability estimates, and (4) it is not clear if bioavailability if a useful concept for PAH cancer risk. Here we assessed the pharmokinetics, bioavailability and effects of exposing juvenile swine to 30 different soils contaminated or spiked with PAHs to provide preliminary answers to some of these questions. Juvenile swine are a strong model for toddler soil exposure because of similarities in gastrointestinal physiology. The effect of repeated exposure did not influence PAH bioavailability with similar PAH bioavailability observed in naive swine and swine exposed for seven days. Soil PAH concentrations influenced bioavailability with higher bioavailability observed at lower doses. As expected, bioavailability differed between soils with soil organic matter contents playing a dominant role in experiment. However, it is not clear how contaminant concentrations and edaphic factors interplay and thus, cross-soil extrapolation is not yet possible. Using biomarkers of PAH effect, we explored how soil factors and PAH concentrations were linked to evaluate if known differences in bioavailability were linked to biological effects of PAHs. This work has begun to fill some of the knowledge gaps required to apply PAH bioavailability estimates for terrestrial site risk assessments.

572 Bioavailability Reduction of TNT in soil via Monopotssium Phosphate and Bentonite Application

J. Jung, Department of Civil and Environmental Engineering; M. Kim, Soil Quality Laboratory; K. Nam, Seoul National University / Dept. of Civil and Environmental Engineering

As an alternative measure for managing TNT-contaminated site such as an active firing range, application of K^+-exchanged bentonite was studied. Monopotassium phosphate (MKP) was used as a K+-providing material. The introduction of MKP and bentonite could reduce the bioavailability of TNT in the firing range soil via sorption enhancement of TNT to K^+-exchanged clay mineral surfaces. In the study site soil, monopotassium phosphate (MKP) application could reduce the hydroxypropyl-β-cyclodextrin (HPCD) extractability of TNT by about 39%. HPCD extraction was used as a chemical surrogate to predict bioavailability. Since TNT sorption enhancement by MKP occurs on mineral surfaces, an expandable clay mineral bentonite was added to further increase TNT sorption and consequently to decrease HPCD extractability. Soil samples amended with MKP of 5% of soil mass were prepared. Then, various amount of bentonite (0, 5, 10, and 15% of soil mass) was applied to the soil and the MKP-bentonite soil samples were incubated for seven days in the dark to prevent possible photolysis of TNT. Water content was kept at the water holding capacity during the incubation. The applied MKP concentration (i.e., 5% of soil mass) was determined because it was the maximum soluble amount at the maximum water holding capacity. When bentonite was not applied (i.e, only MKP was amended), the bioavailable fraction of TNT as determined by HPCD extraction was about 61% of initially applied TNT. However, the bioavailable fractions of TNT were 23, 14, and 16% of initially applied TNT in the presence of 5, 10, and 15% of bentonite (as soil mass), respectively. The dramatic decrease in the extractable fraction of TNT by bentonite amendment indicates that bentonite is a strong sorbent that can retain TNT and thus can play a role as TNT stabilizing agent that can reduce the bioavailability of TNT. However, when applied mass of bentonite exceed 10% of soil mass, no further decrease in extractability was observed and thus it seems that 5% MKP and 10% of bentonite (as soil mass) application is optimal for the reduction of bioavailability.

573 Incorporating Bioavailability Considerations into the Evaluation of Contaminated Sediment Sites

T.L. Sorell, Geosyntec Consultants / Environment and Infrastructure; K. McEvoy, NJDEP; J. Cargill, DNREC; S. Hill, RegTech, Inc.

In 2008, the Interstate Technology & Regulatory Council (ITRC) established a Team focused on Contaminated Sediments. The team developed a Web-based Technical and Regulatory Guidance ("Tech Reg") on the concepts, process, and use of bioavailability in a risk decision-making framework at contaminated sediment sites. This Tech Reg, released in February 2011, provides a common resource for state regulators and practitioners across the public and private sectors to determine the appropriate application of bioavailability concepts in sediment site assessment. The guidance identifies where in the human health and ecological exposure assessment process bioavailability considerations may be pertinent. Available tools are described for the evaluation of bioavailability in the context of evaluating exposures to humans, benthic invertebrates, wildlife, fish and aquatic invertebrates, and plants. Case studies, referenced throughout the document, demonstrate the practical application of bioavailability measures. This guidance assists state regulators and practitioners in understanding and incorporating fundamental concepts of bioavailability in contaminated sediment evaluation including Developing conceptual site models (CSMs) that include bioavailability considerations in prospective exposure pathways; Identifying and describing available tools (biological, chemical, and physical) and models that are used to measure and characterize the fate and transport and potential bioavailability of contaminants; Developing remedial goals based on application of bioavailability concepts in evaluating potential risk. The web-based functionality of the guidance offers streamlined access to items that are the most important to the user. There are options where a pop-up screen will display tables that relate to the narrative within the section. Flow diagrams in each section of the guidance contain interactive links to specific discussion points within the text. ITRC internet-based training sessions on the content and use of the guidance were conducted from 2011 – 2013. A subsequent ITRC team is developing guidance on sediment remediation.

Risk Assessment of Pesticides to Honey Bees: Part A

574 Assessing the Risk of Pesticides to Honey Bees: a Tiered Evaluation

K.V. Garber, USEPA / Environmental Fate and Effects Division; T.M. Steeger, USEPA / Environmental Fate & Effects Division; C. Hart, Health Canada; W. Hou, Pest Management Regulatory Agency, Canada; R. Birely, California Department of Pesticide Regulation

This presentation will provide an overview of the framework for determining the potential risks of pesticides to honey bees (*Apis mellifera*). The framework was developed in collaboration with Health Canada's Pest Management Regulatory Agency and the California Department of Pesticide Regulation. This process is tiered, consisting of an initial screen to identify pesticides that may require more refined assessments. The method identifies the exposure and toxicity data needed to inform the different tiers of a pesticide-specific risk assessment. The initial screen quantifies potential risks to individual bees based on laboratory effect studies and estimated or measured exposure concentrations. For those chemicals that exceed regulatory levels of concern, higher tier studies conducted under semi-field and full field conditions are then used to characterize potential risks under more realistic conditions to the whole colony as well as individual bees, taking advantage of registrant-submitted and open literature studies along with any beekill incident data that may be available. This presentation will also discuss assumptions and uncertainties of the risk assessment framework, as well as data needs that may improve the framework in the future.

575 Dietary Exposure Levels of Honey Bees to a Neonicotinoid Seed Treatment

D. Fischer, D. Dyer, T. Xu, C. Lam, Bayer CropScience, LP; C. Henderson, J. Bromenshenk, University of Montana and Bee Alert Technologies; R. Allen, Valent USA Corporation

More than 100 million acres of crops such as corn, soybeans, and canola are grown each year in North America from seeds coated with systemic insecticides of the neonicotinoid class. The purpose of this study was to quantify concentrations of one such chemical, clothianidin, in pollen and nectar of crop plants grown from treated seeds in order to derive dietary exposure estimates for use in honey bee risk assessments. A second objective was to investigate whether these concentrations are likely to increase as a result of planting treated seeds in multiple years. Average and 95%tile clothianidin concentrations in bee-collected pollen at 53 Midwest cornfield sites were 1.2 ppb and 2.8 ppb. Corn pollen was a small portion of the total pollen collected by most colonies and was much less than available corn resources. At 30 canola sites in Alberta, average and 95%tile concentrations were 1.7 and 3.9 ppb for bee-collected pollen, and 0.8 and 1.4 ppb for bee-collected nectar. In contrast to corn, bees foraged extensively in canola. The portion of canola pollen averaged 72%, and 41% of the samples were 100% canola pollen. Samples from 20 cornfield and 5 canola sites with up to 9 years of planting of clothianidin-treated seeds revealed no accumulation of residues in soil or or increased levels in pollen or nectar. Interpretation of results within the context of a risk assessment for honey bees will be discussed.

576 Predicting honeybee exposure to pesticides from vapour drift using a combined pesticide emission and atmospheric transport model

T.S. Geoghegan, K.J. Hageman, University of Otago / Department of Chemistry; M. Scheringer, ETH Zürich / Institute for Chemical and Bioengineering

A number of approaches have been developed to assess the risk that pesticide exposure poses to honeybees. These assessments have focussed on exposure due to bee foraging in sprayed fields and in the droplet drift zone around sprayed fields. However, exposures due to pesticide vapour drift have not been thoroughly evaluated and the overall aim of this work was to determine if this process presents an additional important exposure route of pesticides to honeybees. The specific objectives of this work were to (1) estimate downwind concentrations of pesticides in air and plants by combining a model for predicting pesticide volatilization from sprayed fields and a chemical transport model, (2) use modelled results to calculate risk quotients for honeybees exposed to volatilised pesticides in non-target areas under varying environmental conditions, and (3) calculate pesticide-specific theoretical "safe distances" from sprayed fields at which hives could be located with no observable effects on foraging honeybees. The pesticide volatilization model incorporated pesticide chemical and physical properties, crop and soil characteristics, and used mass balance and flux equations to calculate pesticide concentrations in air above a sprayed field. Downwind pesticide concentrations in air and plant phases were calculated from characteristic travel distances generated with the Environmental Long-range Transport and Persistence of Organic Substances (ELPOS) model. Honeybee exposure was calculated separately for respiratory, contact and oral routes and these exposures were combined to give total daily exposure. Risk quotients at distances up to 10 km from sprayed fields and theoretical safe distances for hives were calculated for 80 specific pesticides, selected based on high potential risk to honeybees, under a series of different environmental condition combinations. For a number of pesticides, vapour drift was found to contribute enough pesticide to a 10-km distant non-target area to cause an adverse affect on forageing honeybees. Wind speed was found to have an important effect on risk but only for high-volatility pesticides. The relationship between risk and temperature was particularly interesting since increasing temperatures increase both volatilization and degradation rates. Thus, different (non-intuitive) risk-distance patterns were observed for each pesticide, highlighting the usefulness of models such as those developed here.

577 Sub-lethal effects of neonicotinyl insecticides on honey bee (Apis mellifera L.) queens and colony development

J.Y. Wu, M. Spivak, University of Minnesota / Entomology

Bees provide crucial pollination services for natural and managed agriculture systems. In recent years, both wild and managed bee populations have declined. Neonicotinoid insecticides are highly toxic to insects and are systemically translocated to all parts of a treated plant including nectar and pollen. These insecticides are readily used on crops, trees, and ornamental plants visited by bees. Bees become exposed to neonicotinoids while foraging and may return to their hive with contaminated resources. The main objective of this project is to determine if and how neonicotinyl insecticide exposure might contribute to honey bee colony decline. We analyzed sub-lethal effects of neonicotinyl exposure to honey bee colonies on individual bees (queens and workers) and on the colony. We examined queen fecundity and locomotive activity, brood development, and foraging rates in observation hives treated at varying levels of imidacloprid (one active ingredient within this class of insecticides). Preliminary data suggest adverse effects of imidacloprid exposure on queen egg-laying ability, queen locomotive activity, worker foraging activity, and on brood production. This study will improve our understanding of how neonicotinoids may impact core functions, reproduction, growth and development, of honey bee colonies.

578 Honeybee Risk Assessment for Foliar Applied Insecticides: Sulfoxaflor Case Study

V.J. Kramer, Dow AgroSciences LLC / Analytical Chemistry and Environmental Sciences; M. Miles, Bayer CropScience UK / Analytical Chemistry and Environmental Sciences; K.J. Ralston-Hooper, Dow AgroSciences LLC / Analytical Chemistry and Environmental Sciences

Risk assessment procedures have been developed for domesticated honeybees (*Apis mellifera*) potentially exposed to residues of foliar-applied insecticides. Incorporating principles outlined at the January 2011 Pellston Workshop on Pesticide Risk Assessment for Pollinators and in the September 2012 USEPA White Paper on Pollinator Risk Assessment, the proposed risk assessment process includes identification of protection goals for honeybees, quantitative description of exposure pathways for the various castes of bees comprising a hive (based on previously published data), determination of appropriate acute and chronic toxicity metrics and estimation of acute and chronic risk to honeybees covering representative agricultural uses of foliar-applied insecticides. Deterministic risk estimates, in the form of Risk Quotients, are put into the context of the US Environmental Protection Agency's "Level of Concern" paradigm adapted from that used for terrestrial vertebrates. Potential uses of probabilistic risk estimation methods are considered. Areas of uncertainty are discussed including estimation methods for pesticide residues in nectar and pollen (when measured values are unavailable or incomplete), nectar consumption rates and the sugar content of nectar in various blooming crop plants.

579 Assessment of risk for neonicotinoid pesticides to bees

T.D. Anderson, Virginia Tech / Entomology and Fralin Life Science Institute; R. Fell, Virginia Tech / Entomology; J. Purdy, Abacus Consulting Services Ltd.; A. Fairbrother, Exponent, Inc. / EcoSciences

Widespread perception of declines in honey bee colonies has prompted concerns that pesticides are contributing to elevated mortality rates of bees. In particular, neonicotinoid pesticides applied as seed coatings result in

systemic uptake of chemical and partitioning into nectar, pollen, and guttation fluid which can lead to exposure of bees. The European Union recently imposed a ban on the use of these products in crops that are attractive to bees on the basis of a risk assessment conducted by the European Food Safety Agency (EFSA). We reviewed the approach used by EFSA as well as other proposed pollinator risk assessment schemes to investigate their strengths and limitations for supporting regulatory decisions. We highlight problems with standard methodology, in particular the complexity and variability of bee behavior both as individuals and as a colony, inadequacy of laboratory nutrition systems, lack of protocols for investigating pesticide mixtures, and difficulty in determining specificity of responses to pesticide exposures. We explore the difficulties inherent in extrapolating laboratory observations to field conditions for a social species such as honey bees, with an emphasis on understanding how behavior of the bees both within and outside the colony can mitigate exposure. Inaccuracies in exposure predictions based only on field application rates or measurements in nectar or pollen arise as a result of highly conservative and increasingly unlikely assumptions about abiotic factors (e.g., amount of dust drift; juxtaposition of crop fields and foraging plants along field edges) and bee social behavior. While it is standard practice to conduct ecological risk assessments in sequential Tiers, the social nature of bees and their complex behaviors in the environment call into question how predictive the early Tier conclusions can be, and suggests the need for clearly defined decision criteria at each step in the process. We conclude that epidemiological studies and long-term monitoring data are critical components of accurate assessments of the probability of harm to honey bees from neonicotinoid pesticides used under normal field application conditions.

580 A large-scale field study examining potential impacts to honey bees from exposure to clothianidin seed-treated canola

L.W. Brewer, Smithers Viscient, LLC / Department of Wildlife Toxicology; C. Cutler, Dalhousie University / Dept. of Environmental Sciences; C. Scott-Dupree, University of Guelph; M. Sultan, A. McFarlane, University of Guelph / School of Environmental Sciences

Numerous biotic and abiotic stressors have been suggested for the unusually high number of honey bee (*Apis mellifera*) colony losses experienced in many parts of North America and Europe the past decade. The neonicotinoid insecticides are widely used plant-systemic compounds. This class of insecticide contains the active ingredients imidacloprid and clothianidin and has perhaps been subject to more scrutiny and scorn than any other potential cause of honey bee colony declines. Many laboratory studies have shown that neonicotinoids may elicit various acute, chronic, lethal or sublethal effects on honey bees. However, higher-tier studies where dietary exposure to pollen and nectar occurs from soil or seed treatment applications have failed to demonstrate significant colony level effects. Large-scale field studies are usually the most refined and realistic method of characterizing risks of agrochemicals to honey bees, but are rarely undertaken due to their complexity and high cost. In summer 2012 we initiated a large-scale field experiment in southern Ontario to determine whether or not exposure to clothianidin seed-treated canola has any adverse impacts on honey bees. Colonies were placed in the middle of clothianidin seed-treated or control canola fields during bloom, and thereafter moved to an apiary with no surrounding agricultural production. Colony weight gain, honey production, pest incidence, bee mortality, number of adults, and amount of brood were assessed in each colony throughout summer and autumn. Honey, beeswax, pollen, and nectar samples were regularly collected and analyzed for clothianidin residues. Several of these endpoints and overwintering survival were again evaluated in spring 2013. Results will be presented.

581 Cyantraniliprole: A Case study to assess the risk to honey bees of a new systemic ryanodine insecticide in a rapid developing regulatory environment

A. Samel, DuPont Crop Protection / Ecotoxicology; A. Dinter, DuPont / Crop Protection

The toxicity of cyantraniliprole, its formulated products, Cyantraniliprole 100 g/L OD, Cyantraniliprole 100°g/L SE and Cyantraniliprole 200 g/L SC, and several plant metabolites was characterized in acute oral and/or contact honey bee tests. The duration of the toxicity of cyantraniliprole foliage residues was evaluated in a study with Cyantraniliprole 100 g/L OD. It was found that cyantraniliprole foliage residues resulting from sprays up to the maximum intended use rate of 150 g cyantraniliprole/ha and aged for ³ 3 hours pose low risk for honeybees. The potential oral exposure of honeybees to cyantraniliprole and its metabolites was investigated in laboratory translocation studies with different crops and field residue studies comprising non-intended crops (eg *Phacelia*) and intended crops (eg melon, tomato, potato, citrus, and apples). Also residues in bee matrices were determined in honeybee field trials (eg, nectar and pollen). In honeybee tunnel tests with *Phacelia*, apple, nectarine and melon the potential risk for honeybees under worst-case semi-field conditions was assessed. Additionally, a bee brood tunnel test investigated potential effects specifically on bee larval stages. In large scale honeybee field trials in oilseed rape the potential impact on honeybee colonies was investigated. A honeybee melon field trial investigated the potential effects on honeybees for a relevant crop for the intended uses of cyantraniliprole. An overview on the results, risk assessment approaches and data interpretation will be provided. Overall, it was found that the intended uses of cyantraniliprole according to good Agricultural Practice pose no unacceptable acute and chronic risks for honeybees, colony development, colony survival and behavior.

Real-World Applications of Omics Endpoints: Lessons Learned and Challenges

582 Exposure assessment of deployed fathead minnows in an Indiana watershed using microarrays

D.C. Bencic, R. Wang, USEPA / National Exposure Research Laboratory; A.K. Alwan, USEPA / Region 5; A. Biales, US EPA / National Exposure Research Laboratory; J. Dorkin, USEPA / Region 5; R. Flick, USEPA / Office of Research and Development, USEPA / National Exposure Research Laboratory; M. Mitchell, Health Canada / National Exposure Research Laboratory; L. Tedesco, Indiana/Purdue University at Indianapolis / Department of Earth Sciences / Center for Earth and Environmental Sciences; J.M. Lazorchak, USEPA / National Exposure Research Laboratory

The Eagle Creek Watershed (ECW) encompasses over 150 square miles in central Indiana. While the watershed contains three major wastewater treatment plants (WWTPs) and sections continue to undergo urbanization, agriculture remains the dominant land-cover. The watershed supplies the Eagle Creek Reservoir, a public drinking water source for the city of Indianapolis. Water quality monitoring data collected over the last 20 years indicate that both the reservoir and much of the watershed have not met their designated use criteria. While local organizations have made significant advances in identifying and implementing changes to improve the watershed, the problems that have been identified to date do not fully explain the poor water quality and bio-community assessments. The objective of this research was to use biological (molecular) indicators to assess the extent of contaminants in ECW and determine the potential exposure to aquatic life. Adult male fathead minnows (*Pimephales promelas*) were deployed for 7 days in small submerged cages downstream of both a representative agricultural site and a WWTP within the watershed. Week-long deployments occurred at both sites during the high water-flow (May/June) season and again at the same sites during the low water-flow (September) season over multiple years. Livers were collected from fish following deployment, as well as from time zero, non-deployed control fish. Gene expression data were evaluated relative to land use, water chemistry, and flow data. Significant differences among sites, seasons, and years were observed. Moreover, altered gene expression in controls was observed over the duration of the experiment and was unrelated to any exposure, which may have implications in the use of expression-based tools. By helping to identify previously unknown and varying stressors, these molecular data promise to aid in targeting source reduction efforts and improving water quality and bio-community health in the ECW. Although this work was reviewed by the EPA and approved for publication, it may not necessarily reflect official Agency policy.

583 "Noisy" Genes and Pathways: a Transcriptomics Analysis of Untreated Fathead Minnows

R. Wang, USEPA / National Exposure Research Laboratory; N. Garcia-Reyero, Mississippi State University / Institute for Genomics Biocomputing and Biotechnology; D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; A. Biales, D.C. Bencic, USEPA / National Exposure Research Laboratory; E.J. Perkins, Us Army Engineering Research & Development / Environmental Laboratory

The focus of environmental toxicogenomics in recent years has largely been on exposure-related treatment effects including their underlying molecular mechanisms of action and potential biomarkers. While there have been

general assessments of variations in gene expression profiles, those studies were oriented primarily towards identifying responsible technical factors such as microarray platforms and experimental designs in order to minimize such variations and maximize biological signals. The purpose of this study is to characterize transcriptomic variations rooted in fish biology, and identify those genes and pathways most likely to change over time and environmental conditions unrelated to any experimentally introduced treatments. These "noisy" genes and pathways have potential implications in meta-analysis of gene expression profiles composed of samples taken from different sources and time points, as in the case of discovery and application of biomarkers. A preliminary analysis of over 600 microarray samples from untreated fathead minnows assembled from different experiments over several years indicates the existence of substantial variations in their gene expression profiles. A more detailed characterization of the underlying genes and pathways is currently underway and the findings will be presented for discussion.

584 Using microarrays to evaluate gene expression similarities of androgen exposed and paper mill impacted Eastern Mosquitofish (Gambusia holbrooki)

E.K. Brockmeier, University of Florida / Physiological Sciences; W. Pine, University of Florida; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology

Female eastern mosquitofish (*Gambusia holbrooki)* with abnormal male secondary sexual characteristics have resided downstream of a paper mill on the Fenholloway River in Florida for over 30 years. While it is hypothesized that environmental androgens cause this masculinization, neither the causative chemicals nor the ecological impacts are currently known. The objective of this study was to use microarray analysis to determine if an androgenic exposure is occurring via gene expression and biological pathway similarity analysis. A custom microarray was used and coupled with traditional gene expression platforms such as quantitative polymerase chain reaction (qPCR) to evaluate the type of chemical exposure occurring. Tissues of female *G. holbrooki* were collected from the paper mill impacted site (Fenholloway) and a reference site (Econfina) in Summer 2012. qPCR of several endocrine system genes was conducted and a subset of liver samples were analyzed by microarrays. These microarray data were compared to a microarray data set of female mosquitofish exposed to a potent androgen receptor agonist in the lab to determine if gene expression patterns were indicative of androgen exposure. Microarray analysis revealed a set of 62 genes that were similarly expressed between the paper mill exposed and androgen exposed female mosquitofish. Similar gene ontology biological pathways between the two groups include metabolic processes, regulation of transcription, and steroid biosynthesis, demonstrating an overlap in the biological effects of androgen and paper mill exposure. qPCR results demonstrate that expression of the egg yolk proteins vitellogenin and zona pellucida glycoprotein 2 were upregulated in the females at the paper mill site, indicative of a non-androgenic exposure. However, expression of 17β-hydroxysteroid dehydrogenase, previously found to be increased after androgen exposure in females, was also increased in the paper mill impacted female mosquitofish. Overall, our molecular results are supportive of exposure to a mixture of endocrine disrupting chemicals, including unknown estrogenic compounds as well as steroids known to be present at the site including androstenedione and progesterone. Furthermore, this study was a successful implementation of microarray technologies for evaluating the mode of action of the chemical exposure occurring at the paper mill impacted site via comparison of laboratory microarray data to data obtained from field samples.

585 A protein response pattern to test whether fish in the bitumen mining region of the Athabasca River are exposed to industry specific contaminants

D.B. Simmons, Denina Simmons Scientific Consulting / Aquatic Contaminants Research Division; T. Neheli, Environment Canada / Aquatic Contaminants Research Division; F. Gagne, Environment Canada / Emerging Methods; S. Clarence, J. Miller, Environment Canada / Aquatic Contaminants Research Division; J.P. Sherry, Environment Canada / Water Science & Technology Directorate; B. Duncker, University of Waterloo / Department of Biology

Concern about the potential for adverse health effects on aquatic biota downstream of oil-sands operations means there is a pressing need to understand whether fish in the Athabasca River are exposed to industry specific contaminants – as opposed to naturally occurring constituents of bitumen. The health effects of complex mixtures of chemicals, such as the mixtures of PAHs, naphthenic acids, and heavy metals associated with oil sands operations result from exposure and the consequences of exposure. Complex mixtures typically induce a modulated pattern of gene expression in exposed fish. Once characterized and validated, elements of that response pattern can be used to diagnose exposure to the mixture's constituents. We used shotgun proteomics to explore differential protein expression signatures in juvenile rainbow trout (*Oncorhynchus mykiss*) that were exposed to tailings pond (TP) water and Athabasca River water taken from upstream of oil-sands operations. We then exposed rainbow trout in the laboratory for 10-d to water taken daily from sites on the Athabasca River located upstream and downstream of the oil sands operations. The suite of selected proteomic markers were used to test those fish for exposure to tailings pond specific contaminants. *Please note: Denina Simmons is a post-doctoral fellow at both Environment Canada and the University of Waterloo.

586 Amphipod microarray – interlaboratory calibration project

D. Vidal-Dorsch, Southern California Coastal Water Research Project / Toxicology; S.M. Bay, Southern California Coastal Water Research Project / Toxicology Department; B.A. Layton, Southern California Coastal Water Research Project / Microbiology; S.D. Moore, Southern California Coastal Water Research Project; A.C. Mehinto, Southern California Coastal Water Research Project / Department of Physiological Sciences and Center for Environmental and Human Toxicology; M.C. Brown-Augustine, University of California, Berkeley / Department of Nutritional Science and Toxicology; C. Vulpe, University of California, Berkeley / Nutritional Sciences and Toxicology; H.C. Poynton, University of Massachusetts, Boston / Molecular Indicators Research / School for the Environment; L. Escalon, US Army, Engineer Research & Development Center; N. Vinas, Mississippi State University; R.C. Colli-Dula, University of Florida / Physiological Sciences; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology; J. Bruno, Environment Canada; L.L. Brown, Environment Canada / Molecular Biology and Biochemistry / Environmental Toxicogenomics, University of Simon Fraser / Molecular Biology and Biochemistry, Environment Canada / Environmental Toxicogenomics

Marine sediments are monitored in California and other coastal areas using the amphipod *Eohaustorius estuarius* to determine the effects contaminants on survival. In our laboratories, a 15K gene microarray has been developed to improve our knowledge of contaminant effects at the molecular level. This study aimed to evaluate the repeatability and reproducibility of microarray analyses across five laboratories. Amphipods were exposed to cyfluthrin-spiked or negative control sediments for 10 days. Two sample types were generated and sent to the participating laboratories to evaluate the results variability due to sample processing steps. The first sample type consisted of total RNA extracts from exposed and control samples which were generated by one laboratory. The second type consisted of exposed and control whole body amphipods for which RNA was extracted by individual laboratories. Results showed that probe intensity signals were comparable among laboratories. Aliquots of the same RNA extracts analyzed by the different laboratories showed a high correlation, indicating a good interlaboratory agreement. Some clustering of samples by type and laboratory was observed but the differences were not considered statistically significant. These trends could be explained by the differences in RNA quality obtained by each laboratory. Analyses of the RNA quality data indicated that RNA processing, including tissue homogenization, RNA extraction protocol, is a critical component in microarray studies. Overall, this study demonstrates that microarray technology can be transferrable and reproducible among laboratories using standardized protocols. Further analyses on differential gene expression profiles are being conducted, to fully assess the reproducibility of the technique.

587 A transcript response pattern to test whether fish in the bitumen mining region of the Athabasca River are exposed to industry specific contaminants

J.P. Sherry, Environment Canada / Water Science & Technology Directorate; D.B. Simmons, Denina Simmons Scientific Consulting / Aquatic Contaminants Research Division; T. Neheli, Environment Canada / Aquatic Contaminants Research Division; F. Gagne, Environment Canada / Emerging Methods; S. Clarence, J. Miller, Environment Canada / Aquatic Contaminants Research Division

Concern about the potential for adverse health effects on aquatic biota downstream of oil-sands operations means there is a pressing need to understand whether fish in the Athabasca River are exposed to industry

specific contaminants – as opposed to naturally occurring constituents of bitumen. The health effects of complex mixtures of chemicals, such as the mixtures of PAHs, naphthenic acids, and heavy metals associated with oil sands operations result from exposure and the consequences of exposure. Complex mixtures typically induce a modulated pattern of gene expression in exposed fish. Once characterized and validated, elements of that response pattern can be used to diagnose exposure to the mixture's constituents. We used FRAP-PCR to establish a gene expression signature in juvenile rainbow trout (*Oncorhynchus mykiss*) that were exposed to tailings pond (TP) water and Athabasca River water taken from upstream of oil-sands operations. We developed qPCR assays for each of the 20 mRNA transcripts selected for inclusion in the transcript response pattern. We then exposed rainbow trout in the laboratory for 10-d to water taken daily from sites on the Athabasca River located upstream and downstream of the oil sands operations. The suite of qPCRs was used to test those fish for exposure to tailings pond specific contaminants.

588 Improving biorestoration of impacted sites through microbial profiling (metagenomics)

V. Pittet, University of Saskatchewan; J.R. Long, University of Saskatchewan / Computer Science; Q. Yan, University of Saskatchewan; B. Trost, University of Saskatchewan / Computer Science; S. Klatt, C. Majano, Contango Strategies; A. Kusalik, University of Saskatchewan / Computer Science; M. Haakensen, Contango Strategies

Microbial community profiling technologies can be applied to help better understand our environment. While natural microbial communities play a vital role in ecosystem health, fewer than 1% of microbes can be readily grown in a laboratory setting, leaving the vast majority of the microbial world unknown. Metagenomic analyses overcome this limitation by providing an assessment of a microbial community based on DNA that is extracted from a sample such as soil, water, sediment, or waste rock, without laboratory culturing. This technology allows analysis of a variety of phylogenetic branches of microbial life (algae, archaea, bacteria, fungi) by identifying and quantifying their presence based on DNA extracted from a sample. Since its inception, the MAVEN project (Microbial Assessment for Value-added, Environment and Natural resources) has developed laboratory techniques and bioinformatics pipelines that substantially streamline processing and analysis timelines. In contrast to conventional approaches, the tools presented here allow for processing of hundreds of samples at any given time, with turnaround times as short as one week. The resultant metagenomic analyses provide insight into the identity, distribution, and abundance of microbes. Furthermore, the analysis pipeline can define similarities and/or changes in communities over time or between sites, which could be useful to adaptive management strategies. To pilot the MAVEN tools, multiple sediment cores were analyzed from four reference and three exposure lakes downstream from a location releasing effluent. The results indicate both similarities and differences between the diverse microbial compositions in the sediments of the reference and downstream lakes. When paired with concurrent geo- and physicochemical data, insights were also gained into which effluent parameters may be most influential, as well as which effluent parameters have the potential to be managed & immobilized by particular microbial communities. Any given environment includes primary microbial producers, key players in elemental and nutrient cycling, and important food sources in the aquatic ecosystems. As such, this technology is not only useful in guiding the development of potential remedial or mitigative strategies for sites exposed to effluents, but may also prove valuable in monitoring following applied remedial strategies, or in assessing potential cumulative effects and spatial extent of changes within a drainage.

589 Metabolomics from the Lab to the Field: Lessons Learned Along the Way

D.R. Ekman, USEPA / National Exposure Research Laboratory; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; J.E. Cavallin, USEPA, ORISE / National Health and Environmental Effects Research Laboratory; J.M. Davis, USEPA / National Exposure Research Laboratory; E.J. Durhan, US-EPA / Mid-Continent Ecology Division; K.M. Jensen, M. Kahl, USEPA / National Health and Environmental Effects Research Laboratory; E.A. Makynen, USEPA / Mid-Continent Ecology Division; D. Skelton, USEPA; Q. Teng, USEPA / National Exposure Research Lab; D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; T.W. Collette, USEPA / National Exposure Research Laboratory,

Use of metabolomics in laboratory studies for chemical toxicity evaluation is fast becoming an established technique in environmental science, displaying excellent sensitivity, physiological relevance, and providing valuable information regarding toxic modes-of-action. These qualities, in addition to the lack of need for a sequenced genome, make a compelling case for the development and application of metabolomics to important ecological problems. However, a number of considerations must be weighed, both technical and biological in nature, throughout all aspects of a metabolomics study. Moreover, transitioning from lab-based studies to field application involves a myriad of additional complexities that can confound analyses. Our group is currently working to make this transition. This presentation will include a series of considerations and "lessons learned" for both laboratory-based studies and field application of metabolomics to environmentally relevant contaminants. Issues regarding study design, sample collection/handling, data acquisition and analyses will be discussed. In addition, considerations specific to conducting field-based metabolomics studies will be presented, along with examples from recent applications in which metabolomics was used to track impacts of complex environmental mixtures in fish.

Fate and Effects of Metals: Geochemical Perspective

590 Use of passive sampling methods for the assessment of metal-contaminated sediments

W.J. Peijnenburg, RIVM / Laboratory of Ecolog. Risk Assess.; P.R. Teasdale, University of Wollongong / Environmental Futures Centre; D.D. Reible, The University of Texas-Austin, University of Texas / Environmental and Water Resources, The University of Texas at Austin / Dept. of Civil & Environmental Eng.; J.A. Mondon, Deakin University / School of Environmental Science; W.W. Bennett, Griffith University / Environmental Futures Centre; P.G. Campbell, Universite du Quebec, INRS / INRS-ETE

It is generally accepted that freely "dissolved" concentrations of contaminants in sediment pore water (C_{free}) provide a more relevant exposure metric for risk assessment than do total concentrations in sediments. Passive sampling methods (PSMs) for estimating C_{free} offer the potential for cost-efficient and accurate *in situ* characterization of C_{free} for inorganic sediment contaminants. In contrast to the PSMs validated and applied for organic contaminants, the various passive sampling devices developed for metals, metalloids and some non-metals (collectively termed "metals"), have been exploited to a limited extent for the assessment of contaminated sediments. This situation persists despite the recognized advantages of PSMs, which include low detection limits, high spatial resolution and the ability to catch episodic events and cyclic changes that may be missed by occasional grab sampling. In this presentation we summarize the PSM approaches for assessing metal bioavailability to sediment-dwelling biota, including the recognized advantages and limitations of each approach, the need for standardization, and further work needed to facilitate broader acceptance and thus application of PSM-derived information by decision makers. With regard to this latter goal, future acceptance of the results of PSMs for metals by decision makers will in part depend on the ability to reconcile measurement results and the results of calculations with the geochemical speciation models that are currently applied to natural waters. Because regulatory decisions are increasingly based on model calculations, it will be imperative to understand the uncertainties associated with both the PSM measurements and the speciation model calculations.

591 Using the Gellyfish Sampler to Determine Multiple Free Metal Ions in Freshwater Ecosystems

Z. Dong, Harvard School of Public Health / Environmental Health; J.P. Shine, Harvard School of Public Health / Department of Environmental Health

The Gellyfish sampler is a rapid, easy-to-use and inexpensive equilibrium-based sampling device for free metal ions, which is the most bioavailable metal species for aquatic organisms. Previous work has validated that the Gellyfish can accurately determine multiple free metal ions simultaneously in the marine environment using apparent binding stability constants measured for Cu, Zn, Pb, and Ni, while there is a pressing need to extend its use to freshwater ecosystems. Laboratory validation experiments in artificially made freshwater were conducted for different metals to study the performance of the Gellyfish at varying hardness and pHs. Metal concentrations

measured in the Gellyfish following metal spikes were generally found to be higher than expected, and this difference was larger at lower hardness, possibly due to a negative charge on the gel at relatively low ionic strengths caused by reaction products during polymerization. Washing the Gellyfish thoroughly with de-ionized water before deployment to achieve a pH of 6 seemed to reduce the measured-to-modeled ratio of metal concentrations in the Gellyfish, and improve the performance of the Gellyfish in freshwater. Combined with empirical corrections, this method enables the use of Gellyfish in generating reliable free metal ion data in freshwater ecosystems.

592 Role of DGTs in the prediction of mercury bioaccumulation in tubificid oligochaetes (T. tubifex)

D.D. Reible, The University of Texas-Austin, University of Texas / Environmental and Water Resources / Dept. of Civil & Environmental Eng.; A. Offutt, university of Texas at Austin / Environmental and Water Resources Engineering; P. Schierz, University of Texas at Austin / Department of Civil and Environmental Engineering, Duke University / Pratt School of Engineering, The University of Texas at Austin / Center for Research in Water Resources; P. Bireta, The University of Texas at Austin / Environmental and Water Resources Engineering; J. Grundy, The University of Texas at Austin / Civil, Architectural and Environmental Engineering Department-EWRE; R.C. Landis, DuPont

Mercury contaminated sediments of fresh water systems adversly impact benthic organisms and, through bioaccumulation, provide a pathway for chronic mercury exposure in higher trophic levels. Bulk sediment sampling has commonly been used as a line of evidence for this contamination; however, risk is better assessed by considering the bioavailability of mercury species, specifically the rate of methylmercury formation. The formation of methylmercury is governed by mercury availability in porewater, speciation of mercury sulfides, and redox conditions. It is hypothesized that passive sampling of porewater mercury with diffusion gradient in thin film (DGT) technology and an understanding of redox conditions through voltammetry can indicate the rate of formation of methylmercury and its bioaccumulation in benthic organisms. This was tested in laboratory mesocosms which were loaded with sediment from the South River (VA, USA) and populated with a tubificid oligochaete, *Tubifex tubifex*. The mesocosms allowed for realistic redox profiles to be established in order to simulate the mercury speciation and methylation rate. The *T. tubifex* samples, in addition to DGTs and sediment bulk samples, were used to determine the mercury and methylmercury concentrations, and voltammetry was employed to observe the redox profiles. This work was completed in tandem with field work on the South River which includes DGT deployment at the same sampling locations and laboratory studies evaluating the speciation and fraction of mercury that is measureable by DGTs. The results are expected to aid in determining the biological relevance of DGTs as a passive sampling tool for mercury.

593 Effects of freeze-thaw and biochar on sequestration and localization of elements within oxidizing and reducing pilot constructed wetland

M. Haakensen, M.W. Terry, Contango Strategies; V. Pittet, University of Saskatchewan; J.W. CASTLE, Clemson University / Environmental Engineering & Earth Sciences; J.H. Rodgers, Clemson University / School of Agricultural, Foest, and Environmental Sciences

Constructed wetland treatment systems (CWTSs) can be designed and built to decrease concentrations of constituents of concern in a wide variety of contaminated waters, including many produced or impacted by mining or oil and gas operations. Depending on the water characteristics and situational requirements, CWTSs can be designed as stand-alone passive treatment systems, or can be integrated with other treatment technologies to form hybrid systems to reduce operational costs. While CWTSs offer natural environments harboring unique biogeochemical reactions, the operative treatment processes may be influenced by seasonal freeze and thaw cycles or by addition of amendments such as biochar to the hydrosoils. CWTSs have been implemented in cold climates with varying degrees of success. We believe the probability of success, and in turn, the predictability of the long-term functionality of CWTSs can be improved through conducting relevant cold-climate pilot-scale testing, in a rigorous and scientific manner. Here we present data gathered from studies surrounding the freeze-thaw conditions of a pilot-scale CWTS designed to treat waste rock seepage for a mine in the Northwest Territories in Canada. For this pilot-scale CWTS, simulated seepage water was formulated to mimic characteristics of the predicted or anticipated waste rock seepage. In total, four different pilot-scale systems were designed and built to promote specific targeted aerobic or anaerobic processes, each with and without biochar added to the hydrosoils. Each of these four systems were then run in duplicate. The simulated seepage water being treated contained a number of elements in elevated concentrations, including As, Cd, Mo, Pb, Se, U and Zn that are discussed in this presentation. To further scientific understanding of the influence of cold climates on performance of the CWTSs, concentrations of these elements in water, soil, and plants were measured in conjunction with explanatory parameters of redox and pH. Additionally, the sediment microbial communities were profiled over time and through the freeze-thaw stages by growth-based and metagenomic methods.

594 Modeling Metal Cycling and Bioavailability in Lake Coeur d'Alene Using the TICKET Unit World Model

R. Bubnyte, HDR Hydroqual; R.F. Carbonaro, Manhattan College / Civil and Environmental Engineering, Mutch Associates, LLC; K.J. Farley, Manhattan College / Civil and Environmental Engineering

Numerical models can provide insight into the transport, effects and fate of metals and assist in regulatory decision-making. Therefore, a unit world model for metals in lakes, TICKET-UWM, has been in development to assess the potential risks posed by the release of metals to lake environments. The model considers key processes affecting transport, fate, and toxicity including complexation by aqueous inorganic and organic ligands (e.g., DOC), adsorption to particulate organic carbon (POC), binding to biological receptors (biotic ligands), and transport of dissolved metals and solids between the water column and sediment. Recently, the TICKET-UWM code was updated to provide the option for use of either WHAM V, VI or VII for description of metal complexation to DOC and POC. Lake Coeur d'Alene in northwestern Idaho was chosen as a field site to test the capabilities of TICKET-UWM. Historical mining operations along its tributaries has resulted in continued high concentrations of metals to enter the lake. Available literature data for metal loadings was used to parameterize TICKET-UWM, along with lake physical and chemical data. The model was then used to make predictions of metal partitioning, percent removal and water column concentrations of Cd, Pb and Zn. Overall, TICKET-UWM was found to provide a good description for the average-annual cycling of these metals in Lake Coeur d'Alene. Model simulations of WHAM V, VI and VIII separately indicated that WHAM VII provided the most accurate description of metal partitioning for Lake Coeur d'Alene.

595 Modeling Metal Release and Bioavailability during Resuspension of Anoxic Sediments

K.J. Farley, P.T. Heegle, Manhattan College / Civil and Environmental Engineering; K.J. Rader, Mutch Associates, LLC, Manhattan College / Civil and Environmental Engineering; R.F. Carbonaro, Manhattan College / Civil and Environmental Engineering, Mutch Associates, LLC

The presence of sulfides in sediments has been shown to play an important role in sequestering metals (e.g., Cd, Cu, Ni, Pb, Zn) and in limiting metal bioavailability. This effect has been considered in regulatory decision-making through static or "snap-shot" measures of simultaneously extractable metals (SEM) and acid volatile sulfide (AVS) ratios. A potential weakness in this approach is the oxidation of metal sulfides and associated changes in metal bioavailability that are likely to occur during sediment resuspension events (e.g., due to episodic storms, propeller-induced disturbances and dredging activities). A reactive-transport model was therefore developed using the TICKET framework and was applied in evaluating metal release during resuspension in laboratory chamber studies. Overall results show that the TICKET model provides an appropriate framework for evaluating the complexities of metal behavior during resuspension events. Results also show that metal release and bioavailability during resuspension are intricately linked to metal sulfide oxidation rates, pH buffering, and metal binding to natural organic matter and oxide surfaces. Based on these findings, SEM/AVS, BLM and other regulatory assessment tools should be considered in a more dynamic framework to properly address the potential impacts of metals during sediment resuspension events.

596 Fulvic Acid Characterization and Variability in Alpine Watersheds

K.T. Dee, Colorado School of Mines / Geochemistry; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry

Dissolved Organic Carbon (DOC) is a widely known regulator of carbon, source of energy, and binding ligand with metals in freshwater aquatic environments. DOC chemical characteristics may vary among watersheds and can be influenced by vegetation, climate, geology, and hydrology. DOC has two operationally-defined components consisting of hydrophobic acids (HPOA) and hydrophilic acids (HPLA). The HPOA component consists of approximately 40% humic acid and 60% fulvic acid. Acid Mine Drainage (AMD) originating from abandoned hardrock mines characteristically has low pH (< 4.0) and high concentrations of Cu, as well as dissolved and particulate forms of Al and Fe. Interaction of DOC with particulate Fe and Al oxides has been shown to cause chemical fractionation. This study examined the relative importance of source and fractionation on DOC characteristics. Three watersheds (Upper Snake River, Colorado Gulch, and St. Kevin's Gulch) are included in this research where DOC was characterized from AMD impacted and non-AMD impacted tributaries. All three watersheds are located in high alpine ecosystems (2500 – 3000m amsl) with wetlands and boreal forests present. Optical properties including $SUVA_{254}$, excitation-emission matrices (EEMs), and the fluorescence index (FI) of the fulvic acid (hydrophobic) fraction of DOC were examined. Differences that are associated with DOC source, season, and aqueous fractionation mechanisms were assessed. $SUVA_{254}$ analysis of fulvic acid showed differences between the AMD impacted (0.53 – 2.68 L mg^{-1} m^{-1}), non-AMD impacted tributaries (0.77 – 3.49 L mg^{-1} m^{-1}), and below their respective confluences (0.58 – 2.93 L mg^{-1} m^{-1}). Fluorescence index (FI) values showed differences between the three watersheds (1.11 – 1.61). EEM plots showed similar patterns for the fulvic acid fluorophores but the maxima and intensity values were variable. Variability of $SUVA_{254}$ and FI suggest that variability throughout the watersheds included in this study result from a shift in sources due to seasonal changes and fractionation mechanisms when AMD impacted water mixes with non-AMD impacted waters. Understanding the significance of fulvic acid variability throughout any given watershed will provide a better understanding of aquatic metal toxicity including parameterization of modeling programs such as the BLM specific to AMD-impacted watersheds.

597 Aquatic Geochemical Analysis and the Toxicological Implications of Acid Mine Drainage Inputs into the North Fork of Clear Creek

J. Williamson, Colorado School of Mines; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; J.S. Meyer, Arcadis US, Inc., ARCADIS; M. Pastorinho, Universidade de Aveiro; E. Traudt, Colorado School of Mines / Department of Chemistry and Geochemistry

Legacy acid mine drainage (AMD) resulting from turn-of-the-century metals mine waste has adversely affected the aquatic geochemistry and ecology of the North Fork of Clear Creek in and downstream of Blackhawk, Colorado. Below the two major AMD inputs (National Tunnel and Gregory Incline), the creek has high concentrations of metal flocculants (primarily Al- and Fe(III)-oxides) and elevated concentrations of dissolved metals (dominated by Fe(II), Cu, Mn and Zn). Other water chemistry parameters, including pH, hardness and alkalinity, are also affected by the AMD. In order to examine the relationship between AMD and stream ecosystem health, we performed a series of experiments designed to analyze the water chemistry and sediment geochemistry in the creek concurrently with in-stream toxicity/bioaccumulation tests. These tests were performed over a 10-d period in November 2012. Metals and major inorganic cations were analyzed in water samples and acid leachates of streambed rock samples, the later primarily representing surface coatings and sorbed metals. In-stream toxicity/bioaccumulation tests were conducted with two aquatic invertebrate species: *Daphnia magna* (a cladoceran; exposed for 2 d) and *Lumbriculus variegatus* (a worm; exposed for 10 d). After the in-stream exposure, the *D. magna* and *L. variegatus* were acid-digested and analyzed for whole-body concentrations of metals and major cations (Ca, Mg, Na, K). Stream-water chemistry, bioaccumulated metals, and survival were correlated. These results provide a better understanding of this dynamic stream system and thus allow more accurate predictions of how stream geochemistry can affect the fate and transport of metals and the health of aquatic organisms.

Environmental Stereochemistry Then and Now: A Tribute to Wayne Garrison

598 Chiral pesticides: the names have changed but the song remains the same

K.A. Maruya, Southern California Coastal Water Research Project Authority; W. Lao, Southern California Coastal Water Research Project; W. Vetter, University of Hohenheim

Because enantiomers of chiral pesticides may exhibit differential behavior, fate and toxicity, particularly after introduction into aquatic environments, they serve as excellent markers for environmental diagnostic studies. Our work over the past two decades has focused on chiral signatures of legacy organochlorines, and more recently on pyrethroids, a class of currently registered insecticides that are suspected of exerting toxic effects to non-target aquatic invertebrates. One of our first studies showed that elimination of a prominent degradate of technical toxaphene by fish proceeded enantioselectively, suggesting that biotransformation and uptake work in concert to establish steady state chiral signatures. Next, we measured the chiral signatures of *cis*- and *trans*-chlordane in urban stormwater to investigate the possibility of enantioselective weathering in soils mobilized during seasonal rainfall events. With the recent shift in focus on replacements for organophosphate insecticides, we have characterized the isomers and enantiomers of commonly used pyrethroids (e.g., cyfluthrin, cypermethrin) using negative chemical ionization GC-MS. This latest work is critical for accurate identification and quantification of pyrethroid residues in environmental samples.

599 A Reflection on the Fate of Chiral 1,2,4-Triazole Fungicides in Biological Systems

J.F. Kenneke, National Exposure Research Laboratory

In biological systems, stereoisomers of chiral compounds can exhibit significantly different pharmacokinetics (absorption, distribution, metabolism, and elimination) and pharmacodynamics (physiological effects). Pharmacokinetic processes (i.e., what the body does to the chemical) can have a profound impact on the internal dose of a chemical. In the context of risk assessment, internal dose provides a critical linkage between exposure and effects. Approximately 30% of pesticides are chiral and used as mixtures of two or more stereoisomers; however, these mixtures are often treated as a single compound in human health and ecological risk assessment. To that end, we utilized a variety of in vitro approaches to evaluate the stereospecific differences of select pharmacokinetic properties for more than 25, chiral 1,2,4-triazole fungicides and their individual stereoisomers. We studied the metabolic stability (intrinsic clearance) of these fungicides and their individual stereoisomers in hepatic microsomes, hepatocytes, and purified cytochrome P450s (P450). Stereoisomers of the same compound exhibited significantly different clearance rates. From this data we developed quantitative structure activity relationship (QSAR) clearance models. We found that the prochiral carbonyl of triadimefon undergoes stereoselective carbonyl reduction to produce four stereoisomers of triadimenol; the relative formation of triadimenol stereoisomers varied among 16 vertebrate species. We observed that the Individual triadimenol stereoisomers exhibited different toxicities in cell-based assays and different degrees of P450 inhibition. In vitro and in vivo metabolomic studies were conducted with the individual stereoisomers of triadimefon and triadimenol, and differences in metabolomic responses were observed in all cases. Triadimefon was found to undergo abiotic racemization (the irreversible transformation of one enantiomer into the racemic mixture), which could be a significant, but overlooked transformation route for chiral compounds. Collectively, these results illustrate that the stereoisomers of chiral compounds can have significantly different pharmacokinetic and pharmacodynamic properties, which should be considered when conducting risk assessment.

600 Chiral chlordane components in environmental matrices

R.L. Falconer, Colorado School of Mines / Dept of Chemistry and Geochemistry; E.M. Ulrich, USEPA / Methods Development and Applications Branch

Chlordane, a persistent, bioaccumulative and toxic organochlorine pesticide, has been studied for many years. Since the advent of chiral analysis for environmental samples, over 2,400 measurements have been made of various chiral chlordane components. Chlordane enantiomer fractions most often have been reported for air and soil with studies suggesting volatilization from soil is an important source to ambient air, although urban termiticide

usage also can influence chiral chlordane measurements. Sediment core studies suggest the small amount of enantioselective degradation of chlordane likely occurs prior to deposition. In general, enantioselective degradation of chlordanes in biota occurs more frequently resulting in more nonracemic values than in other environmental media. There is also more diversity in range and enantiomer preference in biota. Analysis in plants has shown the ability to enantioselectively uptake and transport chlordane compounds from soil to root, from air to leaf and within the plant itself. Observation and measurement of chlordane enantiomers can provide a better understanding of the fate, exposure, toxicity, and risk of chlordanes and other chiral compounds in the environment.

601 Temporal Trends of Organochlorine Pesticide Enantiomers in Arctic Air Suggest Changing Sources and Pathways

T.F. Bidleman, Umea University / Department of Chemistry; L.M. Jantunen, H. Hung, M. Jianmin, Environment Canada / Science and Technology Branch; G.A. Stern, University of Manitoba / Centre for Earth Observation Science; B. Rosenberg, Department of Fisheries and Oceans / Freshwater Institute

Concentrations of most legacy organochlorine pesticides (OCPs) have declined in arctic air over the last two decades, likely due to regional and international controls on production and usage. Although the downward trend is encouraging, the rates of decline for some compounds have slowed in recent years. This has been linked to rising temperatures and decreasing ice cover in the Arctic, which suggests revolatilization from secondary sources. Nonracemic proportions of chiral OCPs in air infer secondary emissions; e.g., from microbially processed residues in soil and water. Here we report enantiomer fractions, EF = (+)/[(+) + (–)] enantiomers, of alpha-hexachlorocyclohexane (α-HCH), *trans*-chlordane (TC) and *cis*-chlordane (CC) in air samples collected at Alert, Canada (82° 30′ N, 62° 20′ W) between 1994-2000. EFs of α-HCH cycled seasonally from ≥0.500 in winter-spring to ≤0.500 in summer-fall. The changing profiles suggest distant transport of racemic (EF = 0.500) or soil-derived (EF >0.500) α-HCH in the cold season vs. revolatilization from the Arctic Ocean (EF < 0.500) upon loss of ice cover. The concentration fraction TC/(TC+CC) and EFs of TC also showed seasonal cycles which suggest transport of relatively unweathered chlordanes (possibly from termiticide sources) in winter-spring vs. soil emissions in summer-fall. Changes in soil temperature, land use and nutrient loading may alter the capability of soils to enantioselectively transform chemicals, as shown in a classic paper by Lewis, Garrison et al. (Nature 401, 898-901, 1999). Including enantiospecific analysis in air monitoring programs could track the continuing transition from primary to secondary sources, and integrate EF changes caused by shifts in the diagenesis of chiral compounds in soil and water.

602 Enantiomer Selective Estrogen and Androgen Activity of Chiral Pesticides

T.E. Wiese, Xavier University of Louisiana / College of Pharmacy; P. Ma, Xavier University of Louisiana; E. Skripnikova, Xavier University of Louisiana / College of Pharmacy

A number of chiral pesticides are hormone active with endocrine disrupting potential. This report reviews the *in vitro* estrogen and antiandrogen activity of the racemic mixtures and individual enantiomers of a number of pesticides including: o,p'-DDT, o,p'-DDD, o,p'-methoxychlor, a-chlordane, b-chlordane, heptachlor, ruelene and Fipronil. Optically pure samples and racemic mixtures were evaluated for estrogen receptor binding, reporter gene, endogenous gene, and cell proliferation activity as well as androgen receptor binding and reporter gene activity. Many racemic mixtures and pure enantiomers tested were found to have some estrogen agonist activity with a number of specific enantiomer selective induction effects observed. Some racemic mixtures and pure enantiomers also displayed antiandrogen activity with enantiomer selective antiandrogen activity observed. Differences between apical, *in vitro* endocrine determinations and measures of endogenous gene regulation for characterizing the endocrine activity of chiral pesticides are highlighted. Recent developments in the use of virtual screening to identify enantiomer specific regulation of nuclear receptors is also presented. This review highlights the importance of resolving the enantiomers of chiral pesticides for endocrine disruptor characterizations.

603 Use of Chiral Alcohols for Elucidating the Mode and Kinetics of Degradation of Fluorotelomer Compounds

J. Washington, USEPA; J.J. Ellington, USEPA / Ecosystems Research Division; T. Jenkins, SEEP/EPA; J.E. Naile, USEPA / Ecosystems Research Division

Fluorotelomer polymers are the dominant product line of the fluorotelomer industry. Fluorotelomer polymers have been shown to degrade under environmental conditions to form numerous fluorotelomer and perfluorinated monomers that are of environmental and toxicological concern; however, because fluorotelomer polymers are notoriously difficult to experiment with, details of the rates and paths of degradation for these major market-place polymers remain ill-defined. In an effort to help elucidate the degradability of fluorotelomer polymers and their monomers, we studied the degradation of primary fluorotelomer alcohols (*n*-FTOHs), both mass-labeled and unlabeled, in selected soils in which we monitored loss of *n*-FTOH and ingrowth of numerous degradation products using a GC/MS system operated in positive chemical-ionization mode and an LC/MS/MS system operated in negative electrospray-ionization mode. Among these degradation products, we quantitated sec fluorotelomer alcohols (*s*-FTOHs) which have a chiral center, as well as determining their enantiomeric ratio using a chiral capillary column. Informed by these experiments, we embarked on experiments with commercial fluorotelomer polymers in soil. Here we report observations of changes in *s*-FTOH concentration and enantiomeric ratio. Variables in the experiments included incubation time, *n*-FTOH and *s*-FTOH homologue length, incubating soil, and soil-spiking mode. In a soil that was spiked with 8:2*n*-FTOH, ingrowth of one enantiomer of 7:2*s*-FTOH dominated, strongly supporting that the generation/loss of 7:2*s*-FTOH is biologically mediated. In contrast, when the same soil was spiked with 10:2*n*-FTOH, in at least one incubation series, no 9:2*s*-FTOH was observed. When 7:2*s*-FTOH was spiked into soil directly, the alcohol was lost during incubation, but the enantiomeric ratio remained close to racemic suggesting that the loss was not biologically mediated, perhaps a volatilization or irreversible sorption artifact. It is noteworthy that within test soil and incubation time, the enantiomeric ratio generally remains coarsely internally consistent among separate microcosms, so these data support the notion that modes of fluorotelomer biotransformation remain internally consistent between microcosms for the periods of incubation we have completed.

604 PCB 91 vs. PCB 95: Chiral congener behavior and biodegradation conditions

C.M. Lee, Clemson University / Environmental Engineering and Earth Sciences Department; V.D. DANG, University of Florida / Physiological Sciences; T.M. Sattler, Clemson University; D.L. Delach, Clarkson University / Environmental Toxicology

Chiral data collected from a watershed contaminated with polychlorinated biphenyls (PCBs) provide evidence in support of the hypothesis that PCB 91 (2,2',3,4',6-hexachlorobiphenyl) is subject to both aerobic and anaerobic biodegradation processes and that PCB 95 (2,2',3,5',6-hexachlorobiphenyl) is amendable to anaerobic processes but recalcitrant to aerobic biodegradation processes. Results from measurements in the water column of shallow streams show racemic or nearly racemic enantiomeric fraction (EF) values for PCB 95 and distinctly non-racemic (EF) values for PCB 91. The conditions of the sampled shallow streams suggest that aerobic processes are likely. Measurements of EF values for the two congeners in sediments and biota indicate that both are distinctly non-racemic. PCB 91 has both ortho,meta and meta,para vicinal hydrogen atoms, but PCB 95 only has meta,para vicinal hydrogen atoms, which may influence their ability to serve as substrates for enzymes such as P-450 isoforms. Both congeners are at significant weight percent (>0.1wt%) in most of the commercial Aroclor mixtures that were commonly used in North America. They are also common products of reductive dechlorination of higher chlorinated congeners. Therefore, paired enantioselective measurements of PCBs 91 and 95 may be useful markers of aerobic versus anaerobic biological processes.

605 A Fasination with Chirality

A.W. Garrison, Private Citizen. retired from the USEPA / NA

From 1848, when Pasteur separated the different-shaped crystals of tartaric acid and so discovered chirality, consideration of this phenomenon has become essential to the explanation of many natural processes and, more recently, to the development of more effective and safer medicines. Studies of chiral agrochemicals soon followed and, since the early 1990s, the chirality of pesticides and their consequential enantioselectivity has been the

subject of hundreds of investigations by scientists around the world. This is not surprising, being that about one-third of pesticides now on the market are chiral molecules. My involvement in such research began in 1993, when Iwas fortunate enough to be assigned by my EPA management to work with the Institute for Ecological Research in Munich, Germany, on the application of capillary electrophoresis (CE) to the study of environmental pollutants. We observed that when dichlorprop herbicide was sprayed on a field, one enantiomer degraded much faster than the other. My coworker and I were amazed at this discovery – both the enantioselectivity and the good CE separation of the enantiomers – and this began to greatly influence my career. This personal note is to preface the essence of this talk – the chirality of environmental pollutants and the importance of enantioselectivity/stereoselectivity. We will discuss several examples of the stereoselectivity of chiral pesticides and its effects on toxicity and persistence of the enantiomers/stereoisomers. Various approaches to experiments on stereoselectivity will be summarized, as will caveats that must be considered for quality experimentation. For example, one must determine whether enantiomerization occurs under conditions of the experiment and, if so, discuss its impact. We will also show the application of such research to chiral non-pesticide environmental pollutants such as certain PCBs and even chiral PFOA isomers.

A WET Conceptual Approach to Water Management Using Next Generation Bioactivity and MOA Bioassays

606 Bioactivity measure and bioassays for regulatory application in water media

J.M. Lazorchak, USEPA / National Exposure Research Laboratory; E. Doyle, USEPA; V.S. Wilson, USEPA, ORD, NHEERL, TAD / Reproductive Toxicology Branch; A. Biales, USEPA / National Exposure Research Laboratory

There is a recognized need for improved approaches for identifying, monitoring, and criteria setting for chemicals of concern (COCs) or contaminants of emerging concern (CECs) in surface, ground, and finished drinking waters. Current risk assessment and chemical approaches for assessing COCs or CECs are not designed to address cumulative or mixture effects and therefore, may be insufficient to address the Clean Water and Safe Drinking Water Acts. Additionally, current effects-level-criteria based assessments are not adjusted for changes in human health or ecological vulnerabilities (i.e., increasing U.V. and ionizing radiation, chemical sensitivities, chemical induced feminization of human and animal populations, etc). Cairns and Mount addressed the issue of chemical mixtures and why chemical approaches are insufficient by the following quote: "No instrument has yet been devised that can measure toxicity! Chemical concentrations can be measured with an instrument but only living material can be used to measure toxicity." With the advent of bioactivity measures and expanded use of bioassays, the ability to directly measure toxicity can become a reality. Limitations of traditional monitoring schemes include that 1) chemical measures only provide results for the contaminants that you are analyzing for, and 2) even if all the chemical constituents could be measured, our understanding of the interactions of toxicants, additivity, synergism, antagonism, is inadequate to predict biological responses. This presentation will provide an overview of the thinking of a workgroup that was organized to look at bioactivity and bioassay tools and how they could be used to evaluate various water sources (e.g., surface water, ground water and drinking water systems) for cumulative biological activity of contaminants with common adverse effects. The following questions will be discussed: What are bioactivity measures and bioassays? What advantages does the use of bioactivity/bioassays bring to water regulation? What examples are currently available on the use of bioactivity, and how do we know they work? How can bioactivity and bioassay data be used to evaluate risk? How do bioactivity/bioassays fit into regulatory paradigms under the SDWA and CWA? The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the US Environmental Protection Agency.

607 A whole-effluent toxicity method for estrogenic wastewater and inter-laboratory comparisons from mesocosm, laboratory, and field exposures

A. Schwindt, Colorado State University / Fish, Wildlife, and Conservation Biology; A. Jastrow, US EPA / Region 5; D.A. Gordon, US Environmental Protection Agency / National Exposure Research Laboratory; K. Arcaro, Umass Amherst; K. Auger, Umass Amherst / Veterinary and Animal Sciences; K.A. Keteles, USEPA / Region 8; D. Winkelman, Colorado State University; J.M. Lazorchak, USEPA / National Exposure Research Laboratory

There is increasing motivation for monitoring and regulating estrogenic chemicals known to be in wastewater effluent because of the reported effects on fish reproduction. Prior to regulation, and incorporation into discharge permits, there is a need for a Whole Effluent Toxicity (WET) method for testing estrogenicity of effluents. The primary goal for this study was to develop a gene expression based WET method for determining the estrogenicity of effluent. In the development of this method, we 1) calibrated vitellogenic responses in fish to 17α-ethinylestradiol (EE2), 2) estimated a minimum gene induction level, 3) evaluated variability among various laboratories conducting such assays, and 4) determined the real world utility of the method. RNA was used from three experiments conducted prior to and during this project: 1) male fathead minnows (*Pimephales promelas*) exposed for 7d in PVC cages in aquatic mesocosms (n = 5) supplied with lake water and dosed with EE2 at 0 , 2.5, 5, 10, and 20ng/L; 2) 5 adult male fathead minnows exposed to 0, 1, and 2ng/L of EE2 for 48h in moderately hard water in the laboratory; and 3) 15 male fathead minnows caged at reference site and below a waste water treatment plant (WWTP) in Colorado for 5d. Following all experiments, fish were euthanized and livers were collected and snap frozen. Samples were split among labs and vitellogenin (VTG) mRNA expression was measured using quantitative real-time QPCR. In the mesocosm study, one lab detected significantly increased VTG expression relative to control at the 2.5ng/L exposure level. At 5ng/L EE2, all labs detected significantly elevated levels of VTG (p< 0.05) relative to control. Among laboratory variability was significant in fish exposed to 2.5ng/L EE2, but not at EE2 concentrations > 5ng/L. In the laboratory study, three of four labs were able to detect a difference between the control and 1 ng/L (1.33ng/L measured). VTG expression in fish below a WWTP was elevated compared to a reference site and was comparable to that observed in the 3.7ng/L (measured) EE2 mesocosm exposure. These results taken together indicate that we successfully standardized a WET method for assessing the estrogenicity of effluents and receiving waters that can be used in multiple labs using multiple QPCR instruments and reagents. The views expressed in this presentation are those of the authors and do not necessarily represent the views or policies of the US Environmental Protection Agency.

608 Adapting cell-based bioassays for screening of water quality

K.A. Maruya, Southern California Coastal Water Research Project Authority; A.C. Mehinto, Southern California Coastal Water Research Project / Department of Physiological Sciences and Center for Environmental and Human Toxicology; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology; S. Jayasinghe, University of Florida / Department of Environmental Health Sciences; S. Westerheide, J. Mendez, University of South Florida; D. Schlenk, University of California-Riverside / Department of Environmental Sciences; J.P. Crago, Great Lakes WATER Institute, University of Wisconsin-Milwaukee, UC-Riverside / Student; S.A. Snyder, University of Arizona / Chemical and Environmental Engineering; A. Jia, University of Arizona

Current strategies to monitor contaminants in potable and receiving waters target individual or select groups of chemicals that are known to occur and for which robust analytical methods exist. With the advent of state, national and global initiatives to identify, prioritize and mitigate the impact of chemicals that pose unacceptable risks to ecological and human health, it has become apparent that a new, more efficient strategy is needed to screen for all chemicals – known and unknown – that occur in our water resources, now and into the future. In response, an effort to adapt high throughput in vitro cell based assays for water quality screening has been initiated by the State of California. This study identifies endpoints of concern for humans exposed to chemical residuals in recycled/drinking water, and evaluates the utility of commercially available cell-based test kits for screening of samples representing a range of water quality. The majority of endpoints being evaluated (e.g., estrogen, androgen and progesterone receptor based kits) are hypothesized to respond to a wide variety of endocrine disrupting compounds at toxicologically relevant (i.e., ng/L range) concentrations. Successful elements of this effort (e.g., the estrogen receptor bioassay) will be next evaluated for monitoring of receiving waters, i.e.,– those receiving discharges of treated wastewater effluent and stormwater. In parallel, these evaluations will be coupled with investigations of the linkages among bioassay responses and higher order impacts to ecological receptors in aquatic systems.

609 Coupling in vitro and in vivo neurochemical-based assessments of wastewater effluents from the Maumee River Area of Concern (AOC)

A. Arini, School of Public Health; J. Cavallin, J. Berninger / Mid-Continent Ecology Division; R. Marfil-Vega, American Water, Innovation and Environmental Stewardship / National Risk Management Research Laboratory; M. Mills, USEPA / National Risk Management Research Laboratory; D. Villeneuve, USEPA / Mid-Continent Ecology Division; N. Basu, University of Michigan / Department of Environmental Health Sciences

Here we utilize *in vivo* and *in vitro* approaches to study whether real world effluents released in the Maumee River (Toledo, OH) Area of Concern (AOC) contain neuroactive substances that may impair fish reproduction and behavior. Our approaches help extend the concept of endocrine disruption beyond routine bioassays (ER, AR, TH) under the premise that toxicants may also interact with and disrupt the function of neurotransmitter receptors and enzymes that play critical roles in vertebrate reproduction and behavior. Cell-free methods were used to study such interactions, and to also compare the *in vivo* and *in vitro* responses. First, 288 fish (fathead minnow) were exposed in cages to river water at 8 different sites along the Maumee River, including several in close proximity to wastewater treatment plant (WWTP) discharges. After 4 days of *in situ* exposure, brains were sampled and analyzed for GABA, dopamine-2 and N-methyl-D-aspartate (NMDA) receptor binding, and monoamine oxidase (MAO) and glutamine synthetase enzyme activity. The preliminary work shows that fish caged downstream of a major WWTP had increased MAO activity (66% and 35% in males and females respectively), and that NMDA receptor binding was also significantly changed (30% decrease in females). Second, *in vitro* studies were performed on river water extracts (final concentration 5x) to see if they interfere with the aforementioned neurochemicals studied *in vivo*. This was performed in 5 model species of aquatic relevance (fathead minnow, rainbow trout, bald eagle, river otter, human). The initial *in vitro* results suggest that extracts optimized for recovery of alkylphenols significantly impacted the NMDA receptor binding (15% decrease), whereas those optimized for recovery of steroid hormones induced binding (up to 34%) in fish. In vitro responses for other organisms and endpoints will be presented. In summary our work thus far suggests that wastewater effluents discharged to the Maumee River AOC contain chemicals that may directly interact (and possibly interfere) with neurochemicals that are important in fish reproduction and behavior. In addition, the work here (via a USEPA STAR grant) is taking next steps to identify key neurochemical indicators, resolve *in vitro* and *in vivo* responses, and compare responses across taxa.

610 The development of expression based biomarkers for pyrethroid pesticides in Pimephales promelas

A. Biales, D.C. Bencic, USEPA / National Exposure Research Laboratory; R. Flick, USEPA / Office of Research and Development / National Exposure Research Laboratory; M. Kostich, USEPA / EERD; A.L. Batt, USEPA Office of Research and Development / National Exposure Research Laboratory; J.M. Lazorchak, USEPA / National Exposure Research Laboratory; M. Mitchell, Health Canada / National Exposure Research Laboratory; D.A. Gordon, US Environmental Protection Agency / National Exposure Research Laboratory

Aquatic ecosystems are exposed to diverse groups of environmental pollutants from a number of sources including waste water treatment plant effluent and agricultural run-off. Due to the complexity of these exposures, identifying causative agents for ecological impairment has become increasingly difficult. Chemical methods are typically employed to identify potential drivers of impairment, however, these analyses are limited to only those chemicals with developed methods and are often difficult to link to observed effect due to unanticipated interactions of mixture components. Expression based biomarkers of exposure may prove useful in strengthening the linkage between chemical occurrence data and higher order effects. These biomarkers provide a needed biological context to occurrence data and can account for mixture effects and other complicating factors, such as bioavailability. Ideally, sources of chemical contaminants and surface waters can be evaluated for biologically active levels of contaminants using expression based biomarkers in surrogate model organisms. These endpoints can then be extrapolated to endemic species to help determine cause of ecological impairment. Here we describe the development of an expression based biomarker for pyrethroid pesticides using an aquatic vertebrate toxicity model organism, *Pimephales promelas* (FHM). Larval FHM were singly exposed to concentrations of four pyrethroid pesticides; esfenvalerate, cypermethrin, bifenthrin and permethrin for 48 hr in a flow-through system. Transcriptomes of exposed individuals were interrogated for genes differentially expressed relative to controls. Random forests were used to generate classifiers for individual chemicals and classifiers were evaluated using leave one out cross validation. Classifiers were subsequently evaluated by their ability to accurately classify individuals from independent experiments. The results of this work demonstrate the development of an expression based classifier for pyrethroid pesticides that has the potential to be extended to incorporate additional chemicals that act along similar modes of action.

611 Toxicogenomic Approach for Investigating the Combined Effects of Mixture

N. Gou, Northeastern University / Civil and Environmental Engineering; A. Gu, Northeastern University / Department of Civil & Env Engineering, Biotechnology Initiative Program

The combined effects provoked in environmental species and human through the exposure upon numerous chemicals in water body have been a major concern in water toxicity assessment. Recent years, toxicogenomic approaches provide tools to improve our understanding and predictability of the combined effects. However, most of the published mixture assessments from toxicogenomic studies is as yet mainly observational and needs further improvement. In this study, we demonstrated the application of a quantitative toxicogenomic-based approach for investigating the combined effects of various binary mixtures with a range from environmental relevant concentration to the benchmark level. Regression-based quantitative analyses were also performed. Six binary mixtures were investigated at various concentrations with a fixed ratio. Cellular level responses (indicated by Transcriptional Effect Level Index (TELI)) evoked by most of the binary mixtures exposure exhibited dose-response patterns. One of the TELI based endpoints TELI-max has a clear linear relationship (r^2=0.6108) with phenotypic endpoint EC50 (luminescence inhibition test using *V.fisheri*), which can further link the molecular level response to phenotypic outcomes. All three combined effects, synergism, additive, antagonism, are all observed, while most of the mixtures exhibited additive effects. Only partial exposures of the two binary mixtures with most distinct MOA, hydrogen peroxide-sucralose and mitomycin-sucralose, exhibited synergism/antagonism effects. For example, mixture of hydrogen peroxide- sucralose exhibited significantly synergism effects at the concentration lower than 1 mg/L (w/w=1:75), and additive with the concentration higher than 10 mg/L. These non-immutable combined effects show that few benchmark concentrations with little chemical combinations are not enough for assessing the mixture toxicity in reality. Our results demonstrated that quantitative toxicogenomic method can detect effects at environmental relevant concentrations and evaluate a large number of samples in a more efficient manner. Our results not only illustrated a rapid, sensitive, and informative approach for mixture toxicity studies, but also for the first time demonstrated the possibility to explicitly predict the combined effects at the molecular level, and this quantitative cellular response can be further interpreted in context with consented phenotypic outcomes.

612 Reproductive and Developmental Signaling Pathway in Crustaceans

E. Medlock, North Carolina State University / Environmental and Molecular Toxicology; G.A. LeBlanc, North Carolina State University / Department of Toxicology

Alterations in offspring development can be initiated through maternal exposure to environmental stimuli—both chemical and physical. These environmental stimuli induce phenotypic changes through activation of relevant signaling pathways. One example is the methyl farnesoate hormone/receptor pathway and its association with environmental sex determination in Branchiopod crustaceans. The juvenoid hormone, methyl farnesoate, can orchestrate daphnia environmental sex determination, in the early embryo, to the male phenotype. We recently described methyl farnesoate-activated transcription factor (methyl farnesoate receptor, MfR), a complex of two bHLH-PAS proteins, dappu*Met* and SRC that activates a reporter gene in response to the endogenous hormone. Moreover, environmental juvenoids not only stimulate male sex determination *in vivo* and activate reporter gene expression *in vitro*, but maternal pyriproxyfen exposure elicited transgenerational effects by significantly altering sex ratios of second generation organisms, and reducing the number of second and third generation organisms produced. Whole animal expression levels of MfR genes, post juvenoid exposure, did not parallel each other. Met expression was severely suppressed (approximately 10-15-fold decrease relative to controls) at higher exposure concentrations, while SRC expression was more variable. Presently, a novel

multifaceted Bioluminescence Resonance Energy Transfer (BRET) reporter assay is being used to elucidate MfR dimerization, DNA-binding and transcription activation intricacies. This assay will serve as a high-throughput chemical screening tool to determine potential reproductive and developmental signaling disruptors for crustaceans.

613 Coupling online effects-based monitoring with physicochemical, optical, and spectroscopy methods to assess quality at a surface water intake

J. Allen, M. Elovitz, USEPA / Office of Research and Development; C.T. Nietch, USEPA / Water Supply Water Resources Division; J.M. Lazorchak, USEPA / National Exposure Research Laboratory

Effects-based monitoring of water quality is a proven approach to monitoring the status of a water source. Only biological material can integrate factors which dictate toxicity. Online Toxicity Monitors (OTMs) provide a means to digitize sentinel organism responses to dynamic water quality with subsequent input into a water quality management decision structure. These systems assess the toxicity of water samples by monitoring responses of living organisms in a continuous, time-relevant manner. As part of a larger watershed research effort within the East Fork of the Little Miami River Watershed, an effort is underway to deploy a suite of water quality monitors at the drinking water intake structure located on Lake Harsha, a reservoir located in Clermont County, OH during the 2013 season. Historically, harmful algal blooms (HABs) have been observed in Lake Harsha and, along with contaminants associated with mixed agriculture and suburban land uses, represent a significant challenge to the cost-effective production of safe drinking water. The goal of this monitoring effort is to provide time-relevant feedback to the drinking water producer and watershed water quality managers regarding potential HABs and episodic contamination events. The approach described here couples OTMs with basic physical/chemical, optical, and spectroscopy methods to provide a relatively comprehensive water quality data set applicable to both ecological and source water process managment. The selected suite includes three OTMs (*Daphnia magna* behavior, *Aliivibrio fischeri* luminescence, and algal PAM fluorescence), an optical particle analysis system, a multiparameter sonde, a UV-vis absorbance spectrophotometer, and total organic carbon analyzer. All data streams will be telemetered to a central database. Real-time analysis of the streaming data will be developed. Details of this approach and experience gained through the process will be presented.

Communities, Ecology, and Health: Making the Connection – Part B

614 The Need to Cross-Inform between Human Health and Wildlife Data for Environmental Health Assessments

D. Henshel, Indiana University

Wildlife are exposed to the same environmental contaminants as humans. There is currently a wealth of under-utilized wildlife toxicological data that could be used to inform human health studies and evaluations of public health impact of environmental contaminants; however these studies tend to be ignored by laboratory scientists, public health professionals and regulatory agencies. This paper presents parallels between wildlife biology and health impacts of environmental contaminants and the biology and toxicological evidence for the same groups of chemicals in humans and acknowledged laboratory models for human health. Finally, this paper will conclude with an initial assessment of some observed wildlife health impacts of environmental contaminants that have not yet been evaluated in humans according to the published literature. Public health impacts of environmental contaminants are typically evaluated by examining public health data bases (data mining), epidemiological studies, case controlled studies, or studies that assess individual exposures, and compare them to individual health records, measures, or health diaries. Of these, data mining is the tool that is least expensive and most able to evaluate potential public health impacts at a landscape or regional scale. However, any study must first identify the potential health endpoints to be evaluated, as well as potential confounding factors. These evaluation endpoints are usually determined by either noting clustered health impacts usually derived from community reports, or by following up on observations made in laboratory models for human health effects. Wildlife have similar biological mechanisms, disposition and toxicological effects as humans and laboratory animal models. Unlike humans, wildlife are consistently exposed to environmental contaminants in their home range and exposures are therefore limited to the contaminants in their food (and environmental media) found in that home range, rather than in food or other ingesta making it easier to draw a clear link between environmental contaminants and health impacts. In order to fully understand the impacts environmental contaminants are having on public health, the full range of available knowledge needs to be used in the assessments; to do so, wildlife contaminant-related health data must be incorporated into the growing database of potential health impacts determined from human and laboratory studies.

615 Research Outcomes: How can we make a difference in regulatory science

R.S. Wentsel, Exponent / Ecological Sciences

This paper will discuss how research can be more directly applied for utilization in regulatory decision making and in the determination of the long term outcome of remediation efforts. Methods to assess research outcomes will be discussed and they will provide a framework on what is and is not a research outcome. A review of successful approaches that utilize a partnership between researchers and regulatory scientists will provide information on various routes where science has proactively informed regulatory decisions. The science policy issues and barriers to the application of new tools will also be presented. This paper was inspired by a recent SETAC EU workshop sponsored by the SETAC Ecological Risk Assessment Advisory Group on the gap between academic research and regulatory risk assessment of chemicals.

616 Nanomaterials and public health: What we have learned from nano-TiO2/ZnO sunscreens and selected nanosilver-based products?

H. Ma, Mid-Continent Ecology Division, USEPA; S.A. Diamond, NanoSafe Inc. / MED

As nanotechnology brings enormous benefits to human society in every aspect from manufacturing to medicine, human exposure to engineered nanomaterials becomes inevitable as these materials are incorporated into a large variety of commercial and consumer products. Potential health effects from exposure to nanomaterials is a major concern among many entities including scientific communities, general public, and regulators. As researchers have made significant progress in understanding biological responses to nanomaterials, science-based risk assessment guidelines to support regulatory decision making has not been available due to insufficient understanding of potential exposure and other related challenges. Before such official guidelines are available, the public acceptance or antagonism of nanotechnology and nano-based products is largely dependent on the ability of the scientific community to communicate effectively with the public, regulators, and other parties. The current work presents a discussion on how the current scientific knowledge on health effects of nanomaterials can be used to guide the decision-making by regulators to protect human and environmental health, with a focus on partnership for communication among different entities and community engagement. Two examples, sunscreens incorporated with nano-ZnO or nano-TiO_2 and selected nanosilver based products (i.e., washing machine, fabrics, toys) are explored to answer the following questions: 1) What we have known about the potential adverse health effects of the nanomaterials? 2) How is this knowledge communicated among scientist, regulators, and general public (communities)? 3) How does community engagement impact the decision making by regulators regarding to acceptance or withdrawal of a product? 4) What are effective ways to promote community engagement? Our findings from these case studies suggest that although the current level of knowledge about potential health impact of nanomaterials remains insufficient for adequate risk assessments, there is much we can do to improve this situation. Community engagement during communicating the linkage between the nanotechnology/nanomaterials and human health proves to be an effective way to improve the translation of scientific knowledge to policy and practice to protect public health.

617 Successful nutritional modulation of polychlorinated biphenyl toxicity

M.C. Petriello, University of Kentucky / Graduate center for toxicology; B.J. Newsome, University of Kentucky / Department of Chemistry; B. Hennig, University of Kentucky

An emerging body of evidence correlates persistent environmental toxicants, such as PCBs, with human diseases such as diabetes, obesity and atherosclerosis. Although complete remediation of persistent environmental pollutants may be preferred to eliminate their contribution to disease pathologies,

this process is extremely time-consuming and cost-prohibitive. Therefore, it is necessary to identify sensible means to provide physiological "buffers" against toxicant-induced diseases. We have shown through *in vitro* and *in vivo* studies that the use of healthful nutrition effectively downregulates the pro-inflammatory events related to PCB-induced vascular toxicity and protects against toxicant-induced chronic disease. Diets centered on fruits, vegetables and healthier fats contain bioactive food components such as polyphenols and omega-3 polyunsaturated fatty acids and have been shown to decrease inflammation and chronic diseases, but their use as a modulator of toxicant-induced diseases is limited. We have shown that mice supplemented with the tea catechin EGCG, a polyphenol, are protected against PCB-induced inflammation and oxidative stress by exhibiting a more efficient antioxidant response. Similar results have been seen in mice supplemented with DHA, a long-chain omega-3 PUFA, and subsequently exposed to coplanar PCBs. Understanding the mechanisms behind nutritional modulation of persistent organic pollutant-induced toxicity will better allow for effective human dietary interventions in at-risk populations. As part of the University of Kentucky's Superfund Research Center, active community engagement aims to educate and inform inhabitants of communities directly impacted by daily exposures to persistent environmental pollutants. Through nutritional education, counseling, and demonstrations we empower individuals to make smarter eating decisions which can help to buffer against chronic exposures to environmental contaminants. Funding source: P42 ES007380

618 Can Pharmaceutical Take Back Programs (TBPs) Improve Public and Environmental Health? Results from an Interdisciplinary Investigation of a TBP

K.I. Stoddard, University of North Texas / Department of Biological Sciences; G. Maxey, C. Tiwari, University of North Texas; V. Hodge, City of Denton; D.B. Huggett, University of North Texas / Department of Biological Sciences

Research continues to show that pharmaceutical environmental contamination causes adverse effects, with one of the most studied effects being feminization of fish exposed to endocrine disruptors. Additionally, there are also public health risks associated with pharmaceuticals because in-home reserves of medications provide opportunities for accidental poisoning and intentional medication abuse. Pharmaceutical take back programs (TBPs) have been seen as a potential remedy for these issues; however a thorough review of peer-reviewed literature and other information on these programs indicates limited research has been conducted to substantiate these programs as a purported solution. Furthermore, there are significant data gaps on key factors relating to take back programs. To address these gaps in knowledge and ultimately determine if take back programs could improve public and environmental health, social and scientific research was conducted in conjunction with the TBP Denton Drug Disposal Days (D4) held in Denton, Texas. Socioeconomic, demographic, and geographic characteristics of D4 participants were investigated using surveys and geographic analysis. Potential impacts of the D4 program on public health were determined by comparing medications collected at D4 events with medications reported to the North Texas Poison Center as causing adverse drug exposures in Denton County. Potential improvements to environmental health were determined by monitoring hydrocodone concentrations in wastewater effluent released from Denton's wastewater treatment plant before and after D4 events. Data collected and analyzed from the D4 events and the wastewater monitoring program suggests D4 events were successful in contributing to improvements in public and environmental health; however there was insufficient evidence to prove that D4 events were exclusively responsible for these improvements. An additional interesting finding was that there was a definitive travel threshold which influenced participation in D4 events. This geographic information, combined with other findings from this research, have the potential to help improve the effectiveness of future take back events. Such improvements could allow TBPs to more effectively meet their common goals of improving both public and environmental health, which this study has shown is achievable to some degree.

619 Puerto Rico Testsite for Exploring Contamination Threats (PROTECT): Recruitment Profile and its Impact on Community

L. Anzalota, University of Puerto Rico Medical Sciences Campus / Department of Human Development; J. Meeker, University of Michigan, School of Public Health / Department of Environmental Health Sciences; D.R. Kaeli, A.N. Alshawabkeh, Northeastern University, College of Engineering / Department of Civil and Environmental Engineering; P. Brown, Northeastern University / Departments of Sociology & Anthropology, and Health Sciences; C. Velez Vega, University of Puerto Rico, School of Public Health / Department of Social Sciences; D. Cantonwine, University of Michigan, School of Public Health; L.O. Rivera Gonzalez, University of Michigan, School of Public Health / Department of Environmental Health Sciences; B. Jimenez Velez, University of Puerto Rico, School of Public Health and School of Medicine / Department of Biochemistry; J.F. Cordero, University of Puerto Rico, School of Public Health / Dean

Puerto Rico has the highest rate of preterm births (PTBs) among all United States jurisdictions and the highest density per square mile of Superfund Sites containing toxicants such as phthalates, chlorinated solvents, and pesticides in its groundwater. PROTECT (Northeastern University's Superfund Research Program) is a transdisciplinary program that seeks to identify environmental risk factors associated with PTBs. A key component is the recruitment of 800 pregnant women before 16 weeks gestation and their follow-up to pregnancy completion in the northern region of Puerto Rico. Biological and environmental samples are collected from each subject and from their immediate environment at three times (16-20, 20-24, and 24-28 weeks gestation) during pregnancy and at birth. In addition, medical and pregnancy history, demographic variables, dietary patterns, psychosocial factors, and self-reported environmental exposures are also collected. We have recruited 532 pregnant women through May 17, 2013. The median age of participants was 27 years old. 57% of participants were married. 14.8% reported being enrolled students at high school or university level. 30.5% reported having completed a college degree and 21.5% reported having an associate degree. 58.1% of participants were employed, but family annual income for 42.1% percent of the participants was less than $19,000. The demographic characteristics of our study subjects vary by recruitment site, which is evident when comparing private health care institutions with the local community clinics. Understanding the socioeconomic profile has provided insights on success of recruitment efforts and in the future may lead to reduce exposures that promote better health of our community and future generations. Our deep connections with participants through both the interactions with PROTECT recruitment team and March of Dimes has led to a highly engaged cohort that seeks additional involvement. Such level of engagement has helped us to formulate a plan to personally report back individual health data to participants, in order to democratically provide public health knowledge, to help in exposure reduction, and in empowering individuals and communities to expand their environmental health awareness and practice.

620 Well testing and treatment behaviors in Central Maine: Engaging the community for arsenic mitigation

S.V. Flanagan, Columbia University, Lamont-Doherty Earth Observatory; R.G. Marvinney, Maine Geological Survey; Y. Zheng, LDEO, Columbia University, Queens College, CUNY

Naturally occurring arsenic (As) in the groundwater of New England has led to often unsafe levels in private wells, which are the household's responsibility to test and treat. The Maine CDC, using the Behavioral Risk Factor Surveillance System (BRFSS), estimated that 45.6% of homes with private wells in Kennebec County, Central Maine have tested for As as of 2003. Between 2006 and 2011 1,428 out of about 17,600 well households in 17 towns of Kennebec County volunteered to have their well tested through Columbia University. All households received full reports on water quality results and a Maine CDC brochure on arsenic. In January 2013, we followed up with the 466 households (31%) with high (>10μg/L) As well water through a mail survey that achieved a 60% return rate despite 17% of surveys being undeliverable. 41.5% of respondents reported installing a treatment system for arsenic, 29.8% switched to drinking bottled water or from another source, and another 28.2% took no action. Of those households that took no action, the majority had lower levels of exposure (10-50 μg/L) and the most common reasons for lack of action were "not concerned" about the As level and treatment options are too expensive. Use of a treatment system for As was also significantly positively associated with education and income. We also implemented a mail survey to understand factors influencing well water testing and treatment in 13 of 17 towns in January 2013, achieving a response rate of 58% out of 900 randomly selected households. About 58.7% of those respondents report that their well has been tested for As. Both surveys were designed using a model of integrated health and social psychology theories, measuring the RANAS (Risk, Attitude, Norms, Ability, Self-regulation) factors that influence testing and treatment behavior. The follow-up survey of high As households demonstrates that risk information alone (i.e., providing well test results) does not always guarantee

health behavior change (i.e., arsenic mitigation). Recently, a Maine Arsenic Reduction for Community Health (MARCH) citizen advisory committee has been established to guide Columbia University's Community Engagement activities to increase well testing and treatment. Knowledge gained through the two surveys is being used to develop new tools and strategies for working with communities and communicating messages that go beyond risk information for more effective behavior change.

Environmental Implications of Biochar

621 Characteristics and environmental implications of biochars from sugarcane and rice residues

J.J. Wang, Lousiana State University / School of Plant, Environmental and Soil Sciences; C. Jeong, S.K. Dodla, Louisiana State University Agricultural Center / School of Plant, Environmental and Soil Sciences

Converting sugarcane and rice harvest residues to biochar being used as soil amendment could potentially improve soil conservation. In this study, fundamental properties of biochar produced from sugarcane and rice residues and their environmental functions were evaluated. Biochars were produced at different pyrolysis temperatures were characterized for elemental, molecular and surface structural properties. In general, sugarcane and rice residue biochars exhibited different elemental and molecular compositions. Charring at high temperature decreased N and S contents but increased P, K, Ca, and Mg. Increasing temperature also caused the loss of the surface structures of biochar. Soils treated with either sugarcane harvest trash or bagasse char enhanced water holding capacity as well as adsorption capacity of atrazine, a common herbicide used in sugarcane production.

622 Characterization and In Situ Application of Biochar and Activated Carbon to Minimize PCB Bioavailability

M.J. Denyes, Chemistry and Chemical Engineering, ; B.A. Zeeb, The Royal Military College of Canada / Chemistry & Chemical Engineering Dept.; A. Rutter, Queen's University / School of Environmental Studies

The use of biochar to immobilize organic contaminants *in situ* and thereby reduce risk to environmental and human health is gaining in popularity. Biochar, due to its production, is a greener more cost-effective material than traditional carbon sorbents such as activated carbon (AC). In the first *in situ* experiment conducted at a Canadian PCB-contaminated Brownfield site, AC and two types of biochar (produced from different feedstocks and under different pyrolysis conditions) performed equally well at reducing PCB uptake into plants. Our supporting greenhouse work has shown that plants grown in PCB-contaminated soil amended with 2.8% biochar grew significantly larger than the plants grown in both the AC amended and the control soil (74% and 99%, respectively). In an effort to explain the similarities and differences between biochar and AC behaviour, extensive laboratory characterization has been conducted including BET surface area, cation exchange capacity, particle size distribution, moisture and organic matter content, pH, and ICP analysis for metals and nutrients (N, P). Results indicate that even biochars with a low specific surface area (SSA) (54.7 m^2/g) can perform as well as AC at reducing PCB uptake, even though AC has a much higher SSA (808 m^2/g). The SSA of most biochars studied were in the range of 320-420 m^2/g. This phenomena may be explained by the particle size distribution in that smaller grain sizes increase the sorbent:soil volume ratio, resulting in low PCB uptake by plants and earthworms. Our work shows that biochars have potential to serve as sorbents for the *in situ* stabilization of organic contaminants, while simutaneously improving the soil quality at degraded Brownfield sites.

623 Environmental implications of water-soluble components from biochars and wildfire residues

C.E. Rostad, US Geological Survey-Water / National Research Program; D.W. Rutherford, C.N. Kelly, R.L. Wershaw, US Geological Survey-WRD

Applying biochar to soil has been proposed both as a means for long-term sequestration of atmospheric carbon in terrestrial ecosystems and for improving soil fertility. Wildfires combust organic matter and leave behind a cover of char on the land surface. The effect of water-soluble char components from amended agricultural soil or wildfire areas on surface-water chemistry is not well understood. Water extractable organic carbon from laboratory chars, wildfire chars, and runoff from wildfire-impacted areas provide a unique opportunity to characterize water-soluble components to assess their environmental implications. Laboratory chars from different vegetation feedstocks formed under a variety of conditions and pine, bark, and grass char from wildfires were extracted with water to determine the effects of these variables on the identities and relative concentrations of water-soluble components. Extracts were analyzed with gas chromatography/mass spectrometry and flow injection/electrospray ionization/mass spectrometry (for organic constituents) and conductance measurements and alkalinity titrations (for inorganic constituents). Dissolved organic carbon was highest in extracts from chars formed at low temperatures; inorganic components were highest in extracts from chars formed at high temperatures. Some typical organic compounds found in the water extracts of low temperature pine chars were guaiacol (a component of pine tar), syringol (found in wood smoke), vanillin, ethyl vanillin, isovanillic acid, and levoglucosan. None of these compounds were found in water extracts of high temperature laboratory chars or from local commercial chars. Several of these pine-related compounds were found downstream of burned areas, including guaiacol, vanillin, ethyl vanillin, and homovanillyl alcohol. Many of these compounds are antioxidants, which may complicate oxidative disinfection treatment at drinking water utilities that process water from burned areas.

624 How toxic is biochar?

S.E. Hale, Norwegian Geotechnical Institute; G. Cornelissen, NGI, Norwegian Geotechnical Inst; J. Lehmann, Department of Crop and Soil Sciences, Cornell University; A. Zimmerman, Department of Geological Sciences, University of Florida; D. Rutherford, USGS, MS 408 Denver Federal Center; R. Bachmann, Malaysian Institute of Chemical and Bioengineering Technology (MICET), Universiti Kuala Lumpur; V. Shitumbanuma, University of Zambia, Department of Soil Science; A. O Toole, Bioforsk, Soil and Environment Department, Frederik A Dahls vei 20; K. Sundqvist, Department of Chemistry, Umeå University; H.H. Arp, Environmental Engineering, Norwegian Geotechnical Institute (NGI)

Placing biochar into soil simultaneously mitigates climate change and improves the quality of marginalized agricultural land in impoverished regions. The positives of adding biochar to soil are well known (e.g., increased CEC, pH and water holding capacity), however one concern is that during biochar preparation toxic polycyclic aromatic hydrocarbons (PAHs) and dioxins are likely formed. These contaminants could leach out of the biochar into the surrounding soil and be assimilated by native organisms. Both from a regulatory and environmental perspective, using a soil amendment that contains PAHs and dioxins that pose a threat to the soil is unacceptable. Here we quantified total and bioavailable PAHs and dioxins in over 50 biochars produced via slow pyrolysis between 250 and 900 °C, using biomass from tropical, boreal and temperate areas. These slow-pyrolysis biochars were compared to some produced using the industrial methods of fast pyrolysis and gasification. Total concentrations were measured with a soxhlet extraction and bioavailable concentrations were measured with polyoxymethylene passive samplers. Total PAH concentrations were low and ranged from 0.07 $\mu g\ g^{-1}$ to 3.27 $\mu g\ g^{-1}$ for the slow pyrolysis biochars and depended on biomass source, pyrolysis temperature and time. Increasing pyrolysis time and temperature caused PAH concentrations to decrease. These total concentrations are below existing environmental quality standards for tolerable concentrations of PAHs in soils. Total PAH concentrations in the fast pyrolysis and gasification biochar were 0.3 $\mu g\ g^{-1}$ and 45 $\mu g\ g^{-1}$, with 45 $\mu g\ g^{-1}$ exceeding some quality standards. Concentration of bioavailable PAHs in slow pyrolysis biochars were low due to the strong binding of these compounds to black carbon matrices, and ranged from 0.17 $ng\ L^{-1}$ to 10.0 $ng\ L^{-1}$. These values can be compared with the concentration of bioavailable PAHs in clean urban sediments which vary from 0.08 to 342 $\mu g\ g^{-1}$. The gasification produced biochar sample had the highest bioavailable concentration (162 ± 71 $ng\ L^{-1}$). Total dioxin concentrations were low (up to 92 $pg\ g^{-1}$) and bioavailable concentrations were below the analytical limit of detection.

625 Role of naturally-occurring chelating agents on the interaction of biochar with Cd(II) and Sb(V)

S.M. Uchimiya, L. Wartelle, USDA-ARS Southern Regional Research Center

Microorganisms and plants are known to engineer specific chemicals, (phyto)siderophores, and excrete within rhizosphere for nutrient acquisition and other survival strategies. These chelating agents, e.g., mugineic acid, are designed to have selectivity not only for Fe^{3+} over Al^{3+}, but +III over +II metals, and will change the local redox equilibrium. Research is necessary

to understand how the structure of these selective chelating agents will influence the sorption of metal ions on biochar. Of inorganic contaminants, Cd(II) and Ni(II) are less sensitive to surface complexation and precipitation than Pb(II) or Cu(II), and are intrinsically more soluble in soil matrices. Compared to cations, sorption mechanisms of Sb, As oxoanions on biochar are less well understood. Batch sorption-desorption experiments were combined with equilibrium speciation calculations to quantitatively predict Cd(II) and Sb(V) speciation-sorption relationships on pecan shell biochars produced at 300-700 ºC. Metal sorption behaviors responded to the changes in solution chemistry: pH; concentration of oxalate, citrate, EDTA and model NOM; and ionic strength. Uptake and release behaviors of protons and alkali/alkaline earth metal ions were used to understand how Cd(II) and Sb(V) (1) respond to biochar-induced changes in solution chemistry, and (2) become coordinated by chelating agent in solution and on the surface of biochar.

627 Mechanistic evaluation of sorption of apolar and polar aromatics to wood char produced at varying heat treatment temperatures

C. Lattao, The Connecticut Agricultural Experiment Station; J.J. Pignatello, Connecticut Agricultural Experiment Sta. / Soil and Water; X. Cao, J. Mao, Old Dominion University; K. Schmidt-Rohr, Iowa State University

The sorption mechanism of hydrophobic organic compounds (HOCs) in black carbon (BC) is not well understood. Char properties vary as a function of heat treatment temperature (HTT) and starting material and would therefore translate to differences in its capacity to sorb HOCs. We aimed to investigate the role of char properties, sorbate structure and steric effects on sorption capacity and non-linearity. Maple wood chars produced at 6 different environmentally relevant temperatures from 300

628 Biochar in Zambia, Indonesia, Malaysia and Nepal: Technologies, Field Trials, and Socio-Economy

G. Cornelissen, NGI, Norwegian Geotechnical Inst; S.E. Hale, Norwegian Geotechnical Institute; M. Sparrevik, NGI; J. Mulder, V. Martinsen, UMB; C.E. Rostad, US Geological Survey-Water / National Research Program; D.W. Rutherford, US Geological Survey-WRD

The Norwegian Geotechnical Institute (NGI) and the Norwegian University of Life Sciences (UMB) have set up four biochar field research initiatives in Zambia, Indonesia, Malaysia and Nepal. Total funding of the multidisciplinary research projects is around 5 million US$. The agronomical soil work is combined with research on biochar generation technologies and socio-economic aspects of biochar implementation, through life-cycle and cost-benefit analyses. Laboratory work is carried out on nutrient availability, plant-available water, GHG emissions and PAHs/dioxins in biochar. The main overall goal is to come to an evaluation of the overall potential of biochar in Asia and Africa. A complete life-cycle analysis of biochar implementation in tropical countries showed that traditional earth-mound kiln technology is not to be recommended because of PM10, methane and CO emissions. Thus small-scale stoves and retort kilns have been introduced. The results of these are being integrated in life-cycle analyses and implemented via business concepts and cost-benefit analyses. Agronomic field trials in all 4 countries have shown that biochar addition leads to up to 400% increase in yield in some places at dosages as low as 4 tons/ha, whereas it is not effective on others. The reason that the low application rate was so effective is the combination of poor, sandy soils with conservation farming, where planting is done in basins that only cover 10% of the soil surface. We hypothesize that the biochar is so effective because of a doubling of plant-available water, as well as a decrease in available Al. In a side study, the sorption of N_2O to four biochars was measured at a range of 20 pressures in an anhydrous system with pure N_2O. The biochar data were compared to those for two activated carbons, uncharred pine wood, peat, and five inorganic metal oxides. Biochar could bind N_2O around one order of magnitude more strongly than both mineral and organic soil materials. Q_{max} of N_2O on biochar exceeded the N_2O emission suppressions observed in the literature by several orders of magnitude. Thus the hypothesis could not be falsified that N_2O sorption to biochar causes N_2O emission suppression.

Assessing Contaminant Effects in Multi-stress Ecosystems: Part B

629 Silent stressors: Contaminant-mediated energy balance, temperature tolerance, and body condition on a warming planet

M.L. Brooks, Southern Illinois University / Dept of Zoology; T.A. Hallman, Southern Illinois University / Zoology

Ecologists are challenged by predicting how aquatic organisms will respond to climate change as compounded by other anthropogenic stressors. Using geospatial models, we identified intersections between current mean summer temperatures and waters of legally impaired water quality (303(d) listing) across the central United States. Regions of concern have high summer temperatures, low water quality, and maximal increased temperature projections (IPCC 2007). As a case study, I present results from outdoor mesocosm experiments during which we evaluated the temperature tolerance and growth of amphibians. Cope's gray tree frogs (*Hyla chrysoscelis*) were subjected to marginal-to-poor water quality under ambient, +1.5, and +2.5 ºC temperatures. Experiments were conducted in filtered lake water and mixtures of copper, cadmium, and lead. We characterized metals concentrations as Bioavailable Chronic Concentrations Units (BCCUs), the sum of the bioavailable fractions of the three metals relative to the criteria protective of freshwater organisms for each. Interestingly, in metals levels < 10 x BCCU warming had no effects. However, in mixtures >10 x BCCU, warming significantly altered growth metrics and growth rates, indicating lower pejus temperature in amphibians. In a separate series of acute toxicity tests in filtered lake water, we show dramatic interspecific differences in how nocturnal relief from thermal stress mitigated acute toxicity of the same metals mixtures for the spotted salamander (*Ambystoma maculatum*), leopard frog (*Rana sphenocephala*) and the gray tree frog. I argue that oxygen capacity limited temperature tolerance when the metabolic demands of sequestering and detoxifying metals drives oxygen demand higher than oxygen availability in warmer waters. Thus, chronic stress from sublethal pollution complicates predictions of how aquatic ectotherms will cope with the energetic costs of global warming.

630 Water quality and biota patterns in multi-stressor systems: patterns from a multi-faceted, large-scale, long-term dataset

C. Flinders, R.L. Ragsdale, W. Arthurs, J. Ikoma, NCASI; D.L. Cook, NCASI / Department of Chemistry; D. Campbell, R. Messmer, J. Napack, NCASI

Lotic systems are naturally spatially and temporally variable, and elucidating the relative response of water quality and biological endpoints to multiple stressors benefits from long-term data and multiple sampling locations within a reach. Our study used a multi-year (n=15), seasonally-sampled dataset of water quality (temperature, pH, color, conductivity, turbidity, hardness, COD, nutrients, and metals) and biota (fish, macroinvertebrates, periphyton chlorophyll *a* [chl *a*]) from multiple sites (n=5-7) in four streams (Codorus Creek, PA; Leaf River, MS; McKenzie and Willamette Rivers, OR) to examine patterns relative to non-point source run-off, and point-source inputs from tributaries and pulp and paper mill effluent discharge. Most measured water quality parameters were variable across seasons and years, although annual patterns were generally consistent across sites. Statistically significant increases in a number of parameters (COD, color, conductivity, hardness, and some metals (chromium, iron, nickel, and magnesium)) were seen at sites downstream of the effluent discharges relative to upstream sites. However, several water quality parameters measured in the tributary streams at all four study streams were similar or substantially greater than those at main channel sites regardless of effluent exposure. Calculated loadings based on measured flows from effluent and tributary streams showed that tributaries contributed nutrients and metals at similar or substantially greater levels than that of the mill effluents. Analysis of biological data in the context of water quality endpoints and known stressors is underway, and will be discussed. However, preliminary findings from this ongoing study highlight that non-point source discharges can significantly affect lotic water quality, and that incorporating tributary streams in studies assessing water quality and biota may provide important information in explaining upstream-downstream patterns.

631 USGS-USEPA Midwest Stream Quality Assessment: A regional-scale study to assess multi-stressor effects on stream ecology

P.C. Van Metre, J.W. Frey, US Geological Survey; L.H. Nowell, US Geological Survey / Placer Hall; P.W. Moran, US Geological Survey / Washington Water Science Ctr.; B.J. Mahler, US Geological Survey / Water Resources Discipline; D. Carlisle, US Geological Survey

In 2013, the US Geological Survey began a study to assess stream quality in the Midwestern United States in cooperation with the US Environmental Protection Agency (USEPA). The study is in support of the USEPA's National Rivers and Streams Assessment (NRSA) program. The goals are to characterize water-quality stressors—contaminants, nutrients, stream flow, temperature, and sediment—and ecological conditions in streams across the Midwest during spring-summer 2013 and to determine the relative effects of these stressors on aquatic organisms in the streams. Of 100 stream sites sampled, 50 sites were selected using a NRSA probabilistic design and 50 sites were selected using a targeted design. Inclusion of targeted sites helps ensure coverage of a wide range of land-use settings and other factors (such as base-flow index) that affect ecological condition and water quality. The study includes ecological assessment of fish, benthic invertebrates, and algal communities (in July and August 2013); toxicity tests with water and sediment; analysis of pesticides, nutrients, and suspended sediment in weekly water samples for 3 months prior to the ecological assessment; analysis of bed sediments for metals, pesticides and selected other constituents; and use of passive samplers to collect dissolved pesticides for 6-8 weeks prior to ecological sampling. Special studies at subsets of sites include assessments of mercury in water and fish tissue; halogenated organics and PAHs in bottom sediment and passive samplers; daily pesticide sampling using a newly-developed, small-scale auto sampler; and effects on reproduction and health of caged fish and frogs in streams from agricultural areas. This presentation describes study design and preliminary results from the chemistry and toxicity sampling. Ultimately, study findings will contribute useful information for communities and policymakers by identifying which human and environmental factors are the most critical in controlling stream quality.

632 Benthic conceptual site model for the Lower Passaic River Study Area (LPRSA): important considerations for exposure and remedy effectiveness

M. Johns, L. Saban, J. Toll, Windward Environmental LLC; B.G. Church, Windward Environmental LLC / Department of Environmental Sciences; R. Law, De Maximis, INC

Exposure to the benthic community depends on many factors in an eccosystem, including factors such as grain size, total organic carbon, and depth of the redox (rpd) layer. In most sediment investigations the biologically active zone is operationally defined as a depth of 15 cm for sediment investigations and data to characterize sediment exposure is based on the chemical concentrations from samples homogenized over the 15 cm depth. However, benthic communities may only occupy a small segment of the operationally defined biologically active zone, resulting in exposure that is far different than the chemical concentrations found in the homogenized 15 cm sample. Often times benthic communities that occupy the upper portion of the surface sediment may be more influenced by the chemical concentrations in the unconsolidated sediment layer (the "floc" layer). The importance of the "floc" layer to benthic exposure and ultimately to fish tissue concentrations is important in understanding both the benthic conceptual site model, but also ultimately in management decision for the site. Urban systems such as the LPRSA have the added complexity of general urban inputs that aggregate in this upper unconsolidated layer. Ingestion of this layer provides a pathway for chemical transfer to the biota that can be disconnected from the chemical concentrations in the sediment. This is particularly true if chemical concentrations in the deeper sediments are not reflective of floc layer concentrations. This presentation will discuss the LPRSA benthic conceptual site model initial findings, including implications for chemical transfer to upper level trophic organisms. Management and remedial considerations will be discussed.

633 Assessing effects of organic micropollutant mixtures in coastal stormwater retention ponds impacted by runoff from a wastewater-irrigated golf course

L. Ferguson, Pratt School of Engineering / Department of Civil and Environmental Engineering, Duke University; T. Sabo-Attwood, University of Florida / Department of Environmental & Global Health, Center for Environmental and Human Toxicology; A.S. Kolok, University of Nebraska – Omaha / Nebraska Watershed Network; K.J. Ralston-Hooper, Dow AgroSciences LLC / Analytical Chemistry and Environmental Sciences; G.J. Getzinger, Duke University / Nicholas School of the Environment; A.C. Mehinto, Southern California Coastal Water Research Project / Department of Physiological Sciences and Center for Environmental and Human Toxicology; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology; G.A. Dominguez, School of Freshwater Sciences – University of Wisconsin Milwaukee; K.A. Stencel, Duke University / Nicholas School of the Environment; S.P. McGee, Bayer CropScience LP / Environmental Health Sciences; A.J. Bone, Nicholas School of the Environment

We have utilized a combined analytical and ecotoxicological approach to examine the *in vivo* and *in vitro* effects of wastewater-derived and turf grass management organic contaminants in stormwater retention ponds impacted by sewage-derived irrigation water within a coastal golf course community at Kiawah Island, SC. Targeted quantitative analysis of wastewater-derived and agricultural micropollutants revealed widespread occurrence of both turf management chemicals (e.g., fipronil and propiconazole) as well as xenoestrogens (e.g., nonylphenol and bisphenol A) at low (ng/L) concentrations in stormwater retention ponds adjacent to wastewater-irrigated fairway turf. *In vivo* activity of stormwater and wastewater was tested by exposing male fathead minnows (*Pimephales promelus*, FHM) for 14 days to treated wastewater in an effluent holding pond as well as 3 stormwater retention ponds receiving irrigation runoff from land-application of wastewater effluent. The fish were exposed to pond water and wastewater using a novel, field-deployable, flow-through chamber system. Following the exposure, vitellogenin mRNA was quantified by qRT-PCR in livers. Additionally, to determine other biomarkers of exposure to complex mixtures containing xenoestrogens, we performed microarray and proteomic analyses on liver, gonad and brain tissues from the fish. Results indicate that the effluent holding pond and one of the stormwater retention ponds induced significant biomarkers of estrogenicity in FHM (by qRT-PCR and microarray response). Biological pathway analysis of both global gene expression (microarray) and protein abundance (proteomic data) revealed complex and exposure-specific patterns in biological response of fish, illustrating the complexity inherent in assessing effects of contaminant mixtures (such as stormwater) on aquatic organisms. Significant regulated pathways included cholesterol metabolism, immune response, and glucose metabolism. Our results will be discussed in context of the challenge and opportunities associated with diagnosing stress response in aquatic organisms after exposure to contaminant mixtures in the environment.

634 Pharmaceuticals and Personal Care Products in the Environment: from exposure to population and community response

A.J. Jones, Clemson University / Institute of Environmental Toxicology; C. Marion, M. Scott, South Carolina Department of Natural Resources; E.R. Carraway, Clemson University / Environmental Engineering & Earth Sciences, Clemson Institute of Environmental Toxicology

At present, little is known about population and community level responses to low levels of pharmaceuticals and personal care products (PCPPs) in the environment. While effects at a community level are the ultimate goal of many researchers, often we extrapolate potential community effects based on exposures to small populations that may or may not be endemic to the system of interest. Research in small, rural watersheds may have the benefit of lower background contamination and lower thresholds of effect than larger urbanized watersheds. However, the myriad biological, physical, and environmental stressors often compound determination of community level effects of individual compounds, and in many cases mixtures of compounds are present. The research presented here employs multiple multi-variate statistical techniques to elucidate potential effects of PPCPs to the community level. Biological, ecological, hydrological, physical, and chemical characteristics of over 200 small streams are incorporated into a model of overall stream health. Results indicate that major drivers of overall community decline are physical, chemical, and hydrological regimes in small streams, however the presence of PCPPs does have a small, but not insignificant, effect on community health. These results indicate that while PCPPs are of concern in the environment, many other environmental factors must be considered when extrapolating data to the community level.

635 Interactive effects of CO2 and trace metals on the proteasomal activity, metabolism and stress response on two estuarine bivalves

O. Matoo, University of North Carolina at Charlotte; S. Gotze, R. Saborowski, Alfred Wegener Institute for Polar and Marine Research; I. Sokolova, University of North Carolina at Charlotte / Department of Biology

Increased anthropogenic emission of CO_2 changes the carbonate chemistry and decreases the pH of the ocean which can affect the speciation and bioavailability of metals in polluted habitats such as estuaries. However, the effects of acidification on metal accumulation and stress response in estuarine organisms including bivalves are poorly understood. We studied the interactive effects of CO_2 and two common pollutants copper (Cu) and cadmium (Cd) on metal accumulation, ATP/ubiquitin-dependent intracellular protein degradation, stress response and energy metabolism in two common estuarine bivalves – *Crassostrea virginica* (eastern oyster) and *Mercenaria mercenaria* (hard shell clam). Bivalves were exposed for 30 days to clean seawater (control) and to either 50 µg L^{-1} Cu or 50 µg L^{-1} Cd at one of the three partial pressures of CO_2 (P_{CO2}): ~400, 800 and 1500 µatm representative of the present-day conditions and projections of the Intergovernmental Panel for Climate Change (IPCC) for the years 2100 and 2250, respectively. Clams accumulated lower metal burdens than oysters. Elevated P_{CO2} enhanced Cd and Cu accumulation in mantle tissues of clams and oysters, and higher Cd and Cu burdens were associated with elevated mRNA expression of metal binding proteins (metallothionein and ferritin). Activities of three main catalytic centers of the proteasome (responsible for chymotrypsin-, trypsin- and caspase-like activity) were differently affected by Cu and Cd exposure depending on P_{CO2} indicating enhanced protein degradation and possibly a shift in the predominant protein substrates of the proteasome. Levels of mRNA transcripts for ubiquitin and a tumor suppressor p53 were suppressed by metal exposures in normocapnia but this effect was alleviated or reversed at elevated P_{CO2}. Exposure to 800 µatm P_{CO2} and Cu significantly reduced muscle glycogen, cellular ATP and ADP levels in clams, whereas Cd-exposed oysters had significantly elevated cellular ATP levels under all CO_2 levels. Other combinations of metals and CO_2 levels had no effect on cellular energy status in clams or oysters. The data suggests that environmental CO_2 levels can modulate metal accumulation and toxicity in the studied bivalves in a species-specific manner which can affect their fitness and survival during the global change in estuaries. Supported by NSF award IOS-0951079 and UNC Charlotte's Faculty Research Grant.

636 What's Causing Toxicity in Sediments? Results of Twenty Years of Toxicity Identification and Evaluations (TIEs)

K.T. Ho, USEPA / Atlantic Ecology Division; R.M. Burgess, USEPA / Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory / ORD/NHEERL

Sediment Toxicity Identification and Evaluation (TIEs) methods have been used for twenty years to identify the causes of toxicity in sediments around the world. We summarized and categorized results of more than 80 peer-reviewed TIE studies into non-ionic organic, cationic, ammonia and "other" toxicant groups. We further categorized results by whether the study was performed in fresh- or marine water sediments, and whether the study was performed using whole sediment or interstitial water TIE methods. When all studies were grouped, non-ionic organic toxicants, either singly or in combination with another toxicants were implicated in 75% of all studies. When studies were divided into interstitial water TIE methodology compared to whole sediment TIE methodology, results indicated that studies performed using interstitial water TIE methods had cationic metals and nonionic-organic toxicity in approximately equal roles in sediment toxicity. In contrast, studies using whole sediment TIE methods report nonionic organic chemical toxicity, either singly or in combination with another toxicant, in 95% of all sediments tested. Cationic metals play a much smaller role in whole sediment TIE studies– only about 22% of all sediments had a metals signal. The discrepancy between the two methods can be attributed to exposure differences. Contrary to earlier studies, ammonia plays only a minor role in sediment toxicity.

Contaminants of Emerging Concern for Fish: Assessing Exposure and Effects Across Biological Scales: Part B

637 Nitroarenes and induction of micronuclei in tilapia (Oreochromis niloticus) exposed to waters of Butuanon River (Cebu, Philippines)

E.T. Bacolod, The United Graduate School of Agricultural Sciences; J. Koyama, Kagoshima University / Faculty of Fisheries; S. Uno, Education and Research Center for Marine Resources and Environment, Faculty of Fisheries

Fish has been a model for accumulation and genotoxicity studies of pollutants, and rivers are the ultimate sink for residential and industrial wastes including traffic discharges. This is a first report regarding accumulation of nitroarenes or nitropolycyclic aromatic hydrocarbons (NPAHs) in freshwater fish exposed to river water. In addition, micronucleus test (MNT) was also conducted to determine genotoxic effects of river water to fish. In this experiment, fish were exposed to river water collected at two different sampling sites for 14 days, followed by 7 days depuration period. NPAHs in samples were quantified using LC-MS/MS. Results showed that NPAHs were detected in river water and were accumulated in fish. Total NPAHs concentration in river water were 311 and 1050 pg/L in upper and lower stream, respectively, while total NPAHs concentration in fish body at last day of exposure were 6150 and 15000 pg/g dry in upper and lower stream, respectively. In addition, carcinogenic 1-nitropyrene (1-NP) was also detected. However, most NPAHs were detected in the site affected by traffic, residential and industries. Genotoxicity study using MNT showed induction of micronuclei (MN) and other nuclear abnormalities (NA) in fish erythrocytes. Induction of MN and NA were higher in fish erythrocytes exposed to river water affected by traffic, residential and industrial establishments. This study suggests that NPAHs is one of the agents that induced genotoxic effects in fish erythrocytes.

638 Impact of Animal Feedlot Operations on Receiving Streams: Larval Fish Survival, Growth and Behavior under Laboratory Rearing Conditions

H.I. Schoenfuss, St. Cloud State University; W.T. Foreman, US Geological Survey / National Water Quality Laboratory; J.L. Gray, US Geological Survey / National Water Quality Laboratory; M.T. Meyer, US Geological Survey / Organic Geochemistry Research Laboratory; J.M. Lazorchak, USEPA / National Exposure Research Laboratory; S.K. Haack, D.W. Kolpin, USGS

Non-point sources of contaminants of emerging concern likely contribute substantial pollutant burdens to many aquatic ecosystems, especially in rural and agricultural watersheds. Among non-point sources runoff from agricultural practices, including feedlot operations and field-applied manure, has been identified as a substantial source of environmental estrogens (EEs) to receiving waterways. Yet little is known about the biological effects of agricultural runoff from diverse sources on receiving aquatic ecosystems. Larval fathead minnows were exposed for 21 day in a 50% daily static renewal system to water samples collected on 16 occasions from 11 animal feedlot operations impacted streams during baseflow and after manure applications. Environmental estrogen (EE) concentrations were highest in water samples collected after dairy manure application and subsequent precipitation. However, EEs were also found in stream located in watershed with intensive poultry or swine production and in one instance in an assumed reference stream. Significantly reduced survival or reduced growth of larvae was observed in 49% of exposures to water samples collected in animal feedlot impacted stream but in none of the three exposures to waters from reference streams. These adverse effects occurred during all flow conditions and seldom co-occurred. Predator avoidance performance was also altered in exposed larval fish in three of the 16 exposures but did not trend consistently with other exposure effects or total EE load. The detection of EEs in 38% of water samples and frequency of adverse exposure outcomes in exposed larvae warrant further study of agricultural sources of EEs. However, the occurrence of EEs under varying flow conditions and the variability of biological effects also hint at the complex relationship between EEs and adverse biological outcomes.

639 Multi-level biological effects in yellow perch (Perca flavescens) environmentally exposed to a municipal wastewater effluent

M. Houde, Environment Canada / Centre Saint-Laurent; M. Douville, Environment Canada; B. Bougas, Institut National de la Recherche Scientifique / INRS-ETE; M. Giraudo, S. Despatie, Environment Canada; P. Couture, INRS / Centre Eau Terre Environnement; M. Pilote, Environment Canada; J.

Verreault, Université du Québec à Montréal / Department of Biological Sciences; A.O. De Silva, Environment Canada; S. Lair, Universite de Montreal; L. Bernatchez, Universite Laval / Department of Biology; C. Gagnon, Environment Canada

Municipal waste water effluents represent important and continuous sources of aquatic contamination. Numerous wastewater treatment plants (WWTP) discharge their effluents in the St. Lawrence River, an important North American waterway that connects the Great Lakes and tributaries with the Atlantic Ocean. A study was conducted in order to investigate impacts of a major municipal effluent on environmentally exposed fish. The project specifically aimed 1) to evaluate biological impacts of the municipal effluent on fish using a multi-level biological approach, i.e., gene transcription (microarray and quantitative PCR), enzyme activities, and histology and 2) to investigate the bioaccumulation of existing and novel flame retardants, perfluoroalkyl substances, and metals in fish tissue. Yellow perch (*Perca flavescens*) were selected based on their wide distribution, their industrial and recreational importance, and the availability of a customized microarray for this species. St. Lawrence yellow perch were sampled in 2012, upstream of an important WWTP, and 4 km and 8 km downstream the point of discharge. Genomic results indicated that the transcription of 144 genes, out of the 1000 analyzed, was significantly different between fish captured upstream the WWTP compared to fish from sites down in the effluent dispersion plume. Among these genes, 38 were found to be differentially transcribed at both downstream sites. Impacted genes were associated with biological processes and molecular functions such as cellular respiration, ion transport, pyruvate metabolic process, ribosome biogenesis, and DNA. Oxidative and reproductive enzyme activities were quantified in liver and histological analyses conducted in liver and gonads. Bioaccumulation of flame retardants, perfluoroalkyl substances, and metals were found in fish at concentrations comparable to other industrialized regions of the world. The detection of novel organic compounds in fish and relationships between fish physiological parameters (i.e., length, weight, girth, condition index, and age), gene transcription, enzyme activities, histology, and chemical tissue concentrations will be presented and compared among sites.

640 Biomarkers of Exposure of Fish and Mussels to Contaminants of Emerging Concern Downstream of Wastewater Treatment Plants

C.D. Metcalfe, Trent University / Environmental & Resource Studies; P. Gillis, Environment Canada; F. Gagne, Environment Canada / Emerging Methods; G.J. Van Der Kraak, University of Guelph / Department of Integrative Biology; E. Jasinska, University of Alberta; G.G. Goss, University of Alberta / Biological Sciences; T. Moon, University of Ottawa; M.R. Servos, University of Waterloo / Department of Biology

There is evidence from many laboratory experiments and a few field studies that contaminants of emerging concern (CECs) in municipal wastewater can induce adverse effects in aquatic organisms, including reduced reproductive capacity. However, there are few methods available for monitoring aquatic organisms that are exposed in situ to municipal wastewater to evaluate whether these contaminants are causing adverse effects. We addressed this challenge by identifying rapid and sensitive endpoints in aquatic organisms to indicate exposure to CECs discharged into receiving waters. Biomarker studies were conducted to assess effects on fathead minnows and freshwater mussels caged downstream of municipal wastewater discharges in the St.Lawrence River, Grand River and North Saskatchewan River in Canada. Data from passive samplers deployed at the study sites showed that concentrations of pharmaceuticals, personal care products, estrogens and androgens were elevsated at sites downstream of the WWTP discharges. Several of the biomarkers tested in this study could be used to monitor for effects in aquatic organsms exposed to municipal wastewater. Biomarkers that are specific for exposure to CECs in wastewater include CYP3 induction in fish and mussels, induction of vitellogenin in fish and vitellogenin-like protein in mussels, and molecular markers of modulation of steroidogenesis in fish. Biomarkers of oxidative stress and immuno-modulation may be general indicators of exposure to wastewater, and may not be specific to contaminant exposure. The induction of microsomal enzyme activity and vitellogenin are relatively simple and rapid techniques that could be used for monitoring aquatic organisms, The molecular techniques are more sophisticated methods, but they are sensitive approaches that could be applied for targeted monitoring studies. Overall, these studies show that CECs in wastewaster are inducing biological responses in effluent plumes at least 1 km downstream, but responses further downstream are not obvious relative to upstream reference organisms.

641 Does intersex matter? A case study of the rainbow darter in the Grand River watershed

M. Fuzzen, University of Waterloo / Biology; A. Crichton, University of Waterloo; G. Tetreault, Environment Canada / Aquatic Ecosystem Protection Research Division; M.E. McMaster, Environment Canada / National Water Research Institute; M.R. Servos, University of Waterloo / Department of Biology

The presence of oocytes in male gonad tissue in a gonochoristic fish species, a condition known as intersex, has been reported in contaminant exposed fish worldwide. While this condition is known to be linked with the exposure of fish to estrogenic and anti-androgenic compounds, it is unclear whether the intersex condition is directly associated with decreased reproductive fitness. The Grand River in the area of Kitchener-Waterloo, Ontario, Canada has been shown to have a high incidence of intersex in male fish downstream of wastewater treatment plants (WWTP). While this high rate of intersex is associated with decreases in androgen production and delayed gonad development, it is unclear whether there is a direct impact on reproductive capability. The purpose of this study was to determine if intersex severity is associated with reduced reproductive success in rainbow darter (*Etheostoma caeruleum*). Fish were collected directly up and downstream of the Waterloo and Kitchener WWTPs, and at a reference site upstream of the city limits which has little to no exposure to WWTP effluent. Milt volume, sperm density, fertilization success, and embryo survival were analyzed in fish collected from the field to evaluate reproductive success. Additionally reproductive behaviour was assessed in rainbow darter from these sites which were acclimated in the laboratory. While sperm density did not differ between sampling sites in the field or laboratory, fertilization success and embryo survival were lower in fish collected from the WWTP exposure sites. The relationships between intersex severity and fertilization success, embryo survival, and reproductive behaviour are being developed.

642 A survey of wastewater-derived compounds and their effects on field-exposed salmonids in the Stillaguamish watershed, Washington

P.W. Moran, US Geological Survey / Washington Water Science Ctr.; J. Pope, Stillaguamish Tribe of Inidans / Natural Resources Dept.; R. Wagner, US Geological Survey / Washington Water Science Cntr.; J. Barbash, US Geological Survey / Washinton Water Science Cntr.; J. Sevigny, Stillaguamish Tribe of Indians / Natural Resources Dept.

Wastewater compounds (WWC) are often studied for their potential to cause endocrine disruption. This study investigated the presence of WWCs and other contaminants in the surface waters of the Stillaguamish River watershed, Washington, USA, as well as the physiological responses of fish exposed to these waters. The objectives of the study were to evaluate; 1) the presence and concentration of WWCs in surface waters of the Stillaguamish watershed, 2) the percent removal of WWCs in three wastewater treatment plants (WWTPs) in the watershed, and 3) the physiological consequences of field-exposed juvenile salmonids to these compounds. WWC concentrations were measured in; surface water 'grab' samples, Polar Organic Chemical Integrative Sampler (POCIS) devices, and fish tissues. The water samples included paired influent and effluent samples of WWTPs in the watershed as well as samples from small streams receiving inputs from non-point sources. Results from the chemical analyses suggests large but variable percent removal in the WWTPs (18 – >99% among different compounds and facilities), low detection frequencies in small streams, and low concentrations in fish tissue. Assessing the physiological responses of juvenile salmonids to Stillaguamish waters involved a 28-day caged Chinook salmon (*Oncorhynchus tshawytscha*) study in 2011 and collection and sampling of resident cutthroat trout (*Oncorhynchus clarkii*) in 2012. Liver, gallbladder, and blood plasma samples were collected from these fish and used for chemical and physiological measurements. Metabolites from polynuclear aromatic hydrocarbons (PAHs) were evaluated in composite gallbladder tissues from the caged Chinook salmon; however, most analytes were below the recovery-corrected reporting limits of 6-22 ng/mL bile. Vitellogenin protein was assessed in blood plasma from juvenile, male cutthroat trout, but infrequently detected. A targeted cDNA microarray designed for rainbow trout (Wiseman and others 2007) was hybridized with RNA extracted from the liver tissues with both the 2011 caged Chinook and 2012 field collected trout. Analysis of the gene responses is ongoing, but preliminary results suggest weak overall response, relative to controls, and similar patterns of response between caged Chinook salmon and resident cutthroat trout.

643 Bioaccumulation and biological effects of contaminants of emerging and legacy concern in Columbia River foodwebs

E.B. Nilsen, US Geological Survey / OWSC; J. Morace, W.B. Temple, US Geological Survey / Oregon Water Science Center; J. Davis, US Fish & Wildlife Service

Several interdisciplinary studies have been carried out in recent years to assess impacts of different classes of contaminants of emerging concern (CECs) in several levels of the foodweb in the Columbia River Basin. This work has been motivated by previous work showing that CECs are present in effluent reaching the Columbia River and in bed sediments of the river. The ultimate goal of these studies is to determine whether exposure to and uptake of these contaminants is linked to detrimental biological endpoints in fish and wildlife. Organisms studied have included species of biological and cultural importance including largescale sucker (*Catostomus macrocheilus*), Pacific lamprey (*Entosphenus tridentatus*), white sturgeon (*Acipenser transmontanus*), and osprey [*Pandion haliaetus*]. Different foodweb levels have been investigated including primary producers (phytoplankton), primary consumers (macroinvertebrates), secondary consumers (largescale suckers, Pacific lamprey) and tertiary consumers (white sturgeon and osprey). Transport pathways and effects of polybrominated diphenyl ethers (PBDEs) and other CECs were investigated in a limited foodweb including water, surface bed sediments, resident largescale suckers, and osprey eggs. Chemical concentrations increased in all media along the exposure gradient towards the urbanized downstream sites. Biomagnification was shown to occur and environmental quality benchmarks were exceeded in some cases. Biomarker results showed that bioaccumulation caused negative effects in this foodweb. In another study, PBDEs and other CECs were investigated in white sturgeon. Concentrations in this long-lived tertiary consumer were nearly an order of magnitude larger than those measured in largescale sucker. Concentrations exceeded cancer-screening levels in several fillet samples for several legacy contaminants. In a third study, Pacific lamprey were studied. Concentration patterns of flame retardants, pesticides, industrial, and personal care compounds were somewhat different in tissues as compared to sediments. Several contaminants, including pesticides and flame retardants, were identified as posing the greatest risk to Pacific lamprey based on prevalent concentrations, aquatic life ratios, bioaccumulative potential, and endocrine disruption potential. These projects have provided important information about distributions, levels, bioaccumulation, transfer, persistence and effects of CECs on key species and foodwebs.

644 A Systems-based Approach to Predict Biological Responses of Aquatic Organisms to Complex Environmental Mixtures

A. Schroeder, G. Ankley, J. Berninger, E. Durhan, USEPA / Mid-Continent Ecology Division; N. Garcia-Reyero, Mississippi State University / Institute for Genomics Biocomputing and Biotechnology; K. Lee, US Geological Survey / Minnesota Water Science Center; Z. Jorgenson, US Fish and Wildlife Service / Environmental Contaminants; E. Makynen, USEPA / Mid-Continent Ecology Division; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory; K. Stevens, D. Villeneuve, USEPA / Mid-Continent Ecology Division

Contaminants of emerging concern (CECs) such as new-generation pesticides, pharmaceuticals, household and personal care products, steroid hormones, and flame retardants enter the aquatic environment through multiple sources such as wastewater treatment plants and agricultural operations. CECs may have adverse effects on aquatic organisms, including fish. However, attempting to predict biological responses to environmental mixtures containing tens to hundreds of measured CECs can be an arduous task. In this presentation we describe a systems-based approach to develop chemical-gene interaction networks to help predict biological responses of fathead minnows to environmental mixtures of CECs. Caged fathead minnows were deployed for four days at multiple sites within five areas of concern (AOCs) in the Great Lakes. Ambient water samples also were collected from these sites by either grab or composite sampling. A suite of CECs including wastewater indicators (e.g., caffeine, cholesterol), human pharmaceuticals, and steroid hormones were analyzed. Of the 137 analytes measured, the number detected at any particular AOC ranged from 62 to 79. The Comparative Toxicogenomics Database and STITCH (http://ctdbase.org and http://stitch.embl.de, respectively) were used to identify known interactions between chemicals detected at our sites and the top 20 genes that the chemicals interact with. The criteria for selecting the top genes were based on the highest number of known interactions of a chemical with a particular gene from the published literature. Cytoscape was used to visualize chemical-gene interaction networks for each site. Analysis of the networks was then used to develop testable hypotheses concerning potential biological responses to the complex mixtures detected at the sites and prioritize the caged fish samples for subsequent transcriptomic analyses using microarrays, with the intent of comparing predicted responses with those observed in vivo. This approach illustrates a method for generating hypotheses for aquatic organism responses to complex environmental mixtures. The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.

Ecotoxicology and Risk Assessment of Aquatic Primary Producers

645 Cerium oxide nanoparticles induce biochemical but not phenotypical modifications in rice

C. Rico, Chemistry; J. Hong, Environmental Science and Engineering Program; A.C. Barrios, The University of Texas at El Paso / Chemistry; M.I. Morales, University of Texas at El Paso; R. McCreary, The University of Texas at El Paso / Department of Chemistry; W. Lee, J.R. Peralta-Videa, The University of Texas at El Paso; J. Gardea-Torresdey, The University of Texas-El Paso

Cerium oxide nanoparticles (nCeO$_2$) have significant interactions in plants; however, their impactsin rice (*Oryza sativa*) are not yet well understood. Rice is an important crop that support the economic activity, and nutritional and health status of more than half of the world's population. This study was performed to determine the influence of nCeO$_2$ on rice at seedling stage. Rice seeds were grown for 10 days in nCeO$_2$ suspensions (0, 62.5, 125, 250 and 500 mg l^{-1}) and the oxidative stress, membrane damage, antioxidant enzymes' activities and macromolecular changes were measured by employing biochemical assays, in vivo imaging, and ICP, FTIR and synchrotron spectroscopy techniques. Results showed that Ce in root increased as the external nCeO$_2$ increased without visible signs of toxicity. The H_2O_2 generation decreased at 62.5 mg nCeO$_2$ l^{-1} and increased at 500 mg nCeO$_2$ l^{-1}, while the membrane damage was enhanced at 125 mg nCeO$_2$ l^{-1}. Fatty acid and lignin contents were markedly reduced at 500 mg nCeO$_2$ l^{-1}. The FTIR spectromicroscopy showed modifications in the biomolecular components of root xylem while synchrotron m-XRF analysis revealed the presence of Ce in the vascular tissues of the roots. Although no phenotypical changes were observed, the results illustrate that nCeO$_2$ cause toxicity to rice. This study sheds light on the physiological impacts of nCeO$_2$ on rice.

646 Wetland macrophyte community responses to direct application of glyphosate-based herbicides

J.F. Mudge, J. Houlahan, Canadian Rivers Institute, University of New Brunswick / Biology

Widespread use of glyphosate-based herbicides has led to concerns that it may have indirect effects on wetland ecosystems through disturbance to the plant community. Impacts of glyphosate-based herbicides on wetland macrophytes can vary in magnitude depending on the species examined. Wetland macrophyte community level resistance and resilience to disturbance in the form of direct application of glyphosate herbicides remains poorly understood. From 2009 to 2013, I have been monitoring wetland macrophyte community level responses to glyphosate-based herbicides at the Long-term Experimental Wetlands Area in New Brunswick, Canada as part of a broader project aimed at evaluating the long-term, ecosystem-level impacts of glyphosate herbicides in wetland systems. This research involves 34 wetlands, each bisected by an impermeable high-density polyethylene barrier and treated on one side with either an environmentally observed concentration or a predicted maximum environmental concentration of a glyphosate-based herbicide under an array of agricultural and silvicultural scenarios. Temporal trends in wetland macrophyte abundance, richness, evenness, and community similarity between treated and control sides of wetlands indicate that while the plant community on the treated sides has rebounded from glyphosate treatments occurring in 2009 and 2010, the community composition of plant regeneration on the treated sides remains different from that on control sides of wetlands. Lasting changes in wetland macrophyte community composition in the years following direct application of glyphosate-based herbicides may have important indirect impacts on wetland algal, invertebrate and/or amphibian communities through changes to habitat structure and/or function. Investigation of the ecosystem-level effects of glyphosate-based herbicides in wetland systems at the Long-term Experimental Wetlands Area is currently underway.

647 Oxidative stress and photosynthesis in phytoplankton species exposed to glyphosate-based herbicide

T. Chesney, P. Juneau, Université du Québec à Montréal

Increasing agricultural activity has triggered the development of many pesticides in order to protect crops against various threats. The effects of glyphosate, a non-selective herbicide and one of the most frequently used herbicides, on non-target organisms, such as phytoplankton, are not fully understood. Glyphosate is a molecule that interferes on the activity of the enzyme 5-Enolpyruvyl-shikimate 3-phosphate synthase (EPSPS), essential for amino-acid production such as tyrosine and phenylalanine, two amino-acids involved in photosynthesis. Tyrosine is an important component of the photosynthetic electron transport chain, while phenylalanine is found in D1 a photosystem structuring protein. Degradation of photosynthetic apparatus reduces the efficiency to assimilate light energy, which may generate production of reactive oxygen species that cause damage to various cell components. This study aimed to evaluate the impact of exposure to glyphosate on green algae (*Scenedesmus obliquus*) and cyanobacteria (*Microcystis aeruginosa*) in order to understand the effect of this herbicide on photosynthesis and induction of antioxidant enzymes. We also evaluated the effect of pre-exposure to high light stress in order to generate oxidative stress and induce antioxidant enzyme activity, and ultimately to increase phytoplankton ability to cope with the glyphosate action. We have shown that the efficiency of photosynthesis is reduced in presence of glyphosate, while the activity of the antioxydant enzymes is increased. It confirms then, at least partly that glyphosate affects photosynthesis. We also showed that, D1 protein was degraded, probably due to oxidative damages, which can also be responsible for the observed decrease in the photosynthetic efficiency. It seems that previous exposure to high light improves the ability to resist against the effect of glyphosate by previous stimulation of antioxidant enzymes. Our study demonstrates that commercial formulation of glyphosate may affect non-target organisms like phytoplankton.

648 Sensitivity of an alga to atrazine is not enhanced by previous acute exposure

L.R. Baxter, University of Guelph / School for Environmental Science; R. Brain, Syngenta Crop Protection, Inc. / Department of Environmental Risk Characterization; R.S. Prosser, School of Environmental Sciences; K.R. Solomon, University of Guelph / School of Environmental Sciences; M.L. Hanson, University of Manitoba / Department of Environment and Geography

Exposure to herbicides in small lotic systems can be episodic, with short-term pulses followed by diminishing concentrations. Consequently, the sensitivity of a green alga (*Pseudokirchneriella subcaptitata*)to atrazine following an initial pulsed exposure was assessed. The exposure profile was designed to simulate an exaggerated worst-case chemograph associated with a potential runoff event in a highly vulnerable watershed. Concentrations were based on amplifications of those seen in streams from regions of high intensity use, such as the Midwestern US Initial pulsed atrazine exposure of 0, 150 or 300 µg/L for 24-h was followed by continual 72-h exposure to 0, 5, 10, 25, or 50 µg/L. Measured responses were cell density, growth rate, chlorophyll-a, and quantum yield of photosystem II. Initial pulsed exposure concentration did not significantly increase sensitivity during the subsequent continual exposure, and recovery of cells was rapid (within 24 h). These data do not indicate altered sensitivity from previous exposure to atrazine and illustrate recovery even under continual exposure following an initial pulse, supporting the incorporation of such information into risk assessment approaches.

649 Biomonitoring of Algal Communities Relative to Atrazine and a Variety of Environmental Variables in Select Midwestern Streams

R. Brain, Syngenta Crop Protection, Inc. / Department of Environmental Risk Characterization; M. Andrus, Waterborne Environmental; A.J. Hosmer, Syngenta Crop Protection, Inc.

Atrazine is a triazine herbicide registered primarily for the control of broad-leaf weeds in corn and sorghum. Atrazine has been detected in surface water from watersheds with highly vulnerable landscape characteristics as indicated by the Atrazine Ecological Monitoring Project (AEMP). In order to assess whether atrazine residues measured in surface waters potentially impart biological changes to aquatic primary producer communities, a biomonitoring assessment was conducted in six streams throughout the Midwestern US (Iowa, Missouri, Illinois and Ohio). Based on weekly sampling, the community dynamics (structure, diversity, richness, and abundance) of native phytoplankton and periphyton were assessed over a four-month period encompassing the spring planting window and early growing season for corn (April to August). Several environmental variables, including atrazine, were concurrently monitored and multivariate statistical approaches were used to evaluate the extent of association between community metrics and variation in environmental characteristics as well as the extent of association between community metrics and historic or unique site effects not measured. Assemblage patterns of periphyton and phytoplankton were found to structure according to the predominant environmental gradients of season, year, and site. Approximately 25-50% explained taxa inertia (variation) was related to gradients of site, season and year; substantial inertia was also related to environmental variables exhibiting stochastic variation. Optimized linear and canonical correspondence analysis models for community metrics based on environment and habitat measurements included: habitat score, DO, NO_3^{2-} + NO_2^-, TSS, flow, water temperature, wind speed and atrazine concentration as variables. Habitat quality accounted for the greatest variation in site models followed by normalized flow. No concentration dependence was demonstrated for atrazine as sites with the highest historical average concentrations did not include this parameter as a significant variable in the model, whereas site-models with the lowest historical average concentrations did. An overview of the two-year biomonitoring initiative will be presented.

650 Effects of sediment-exposure on a rooted, submerged macrophyte: a case with linuron

H. Buresova, Institute of Chemical Technology, Department of Environmental Chemistry; S. Crum, J. Belgers, P. Adriaanse, Alterra, Wageningen University and Research Centre; G. Arts, Alterra Wageningen University and Research Centre / Centre for Water and Climate, Environmental Risk Assessment

Sediment-rooted, submerged macrophytes may be exposed to toxic compounds via water or via sediment. For submerged macrophytes, both exposure routes are potentially relevant, as their growth form allows them to adsorb toxic compounds from both compartments. If partitioning to sediment is a concern, leading to root uptake and exposure of rooted macrophytes, a sediment-toxicity test with a rooted macrophyte is an appropriate test design. Although this test design has been proposed as part of a standardized protocol for testing of *Myriophyllum* sp., effects from sediment exposure to aquatic macrophytes have hardly been studied up to now. Most attention was given to exposure via the water compartment in risk assessments. Therefore, a sediment-toxicity test was undertaken including exposure via spiked sediments in a static test-design. For this test design, the draft standard OECD guideline for *Myriophyllum* sp. was followed with slight modifications. The herbicide Linuron was chosen as a model test-compound as it is rather stable ($DT_{50,\text{water-sediment}}$= 46 d) and moderately adsorptive ($K_{oc,soil}$ = 406 L/kg) and therefore highly suitable for studying macrophyte uptake, elimination and effects. Sediment, pore water, overlying water and macrophyte shoots were sampled for chemical analyses weekly. After dosing of the sediment in the test systems, sediment particles and pore water concentrations were in equilibrium rather quickly (after 48 h). Following this equilibrium phase, Linuron concentrations were stable in the sediments. Overlying water concentrations increased over time, but did not reach equilibrium with pore water concentrations and were 100 times lower. Mass balances showed a rapid uptake of Linuron by macrophyte roots. Known pathways and the compound's properties support the conclusion that *Myriophyllum* takes up Linuron from pore water directly through the roots and transports the compound to shoots and leaves. Hence, for the risk assessment, effects on macrophytes in this type of sediment toxicity tests should be expressed in terms of pore water concentrations. The toxicity values generated during the study and based on pore water concentrations, were higher than those based on similar endpoints for water concentrations in water-exposed test designs reported in the literature. In order to simplify the interpretation, we recommend a pre-treatment phase that allows for a full equilibrium between pore water and the overlying water layer. For Linuron, this period exceeds 21 days.

651 Evaluation of Short Term Exposures for Aquatic Plant Risk Assessments of Sulfonylurea Herbicides

M. McCoole, DuPont Crop Protection, Environmental Safety Assessment / Department of Toxicology; H. Ochoa-Acuna, DuPont Crop Protection / Veterinary Pathobiology; T. Scown, DuPont Crop Protection

Evaluation of the effects of variable, short-term exposures in surface water in the context of EU registration of herbicides has previously been addressed by using time-weighted average (TWA) predicted environmental concentrations (PEC_{sw}). Justification of the use of TWA PEC_{sw} values for aquatic macrophyte risk assessments, in support of registration of sulfonylurea (SU) herbicides, is based on providing a bridge between the exposure pattern of laboratory tests (constant concentration exposures over the 7-d test) and the time-variable exposures modeled to occur in the environment. Knowledge of the exposure concentration profile may allow determining the effect of these short-term exposures, provided corresponding effects data are available. We conducted dose-response studies for 6 SUs based on the standard protocol for the sensitive aquatic macrophyte *Lemna gibba*, but with different periods of exposure (0.5 to 4 days) with the remaining of the 7-d test duration in clean media. The data show that *Lemna gibba*'s sensitivity to SUs decreases significantly as the exposure period is reduced, with a strong relationship between exposure duration and growth endpoints for the 6 SUs tested. This study demonstrates the feasibility of using time-variable exposure data to better predict actual potential environmental effects of SUs on aquatic plants.

652 Aquatic Plant Water Quality Criteria

G.B. Thursby, USEPA / ORD / NHEERL / Atlantic Ecology Division; M.A. Lewis, USEPA / ORD / NHEERL / Gulf Ecology Division

The USEPA, as stated in the Clean Water Act, is tasked with establishing criteria values for various pollutants found in the waters of the United States. These criteria serve as guidance for States and Tribes to use in developing their water quality standards. The *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* ("the 1985 Guidelines") describes the current Office of Water methodology for deriving aquatic criteria. These OW guidelines focus primarily on deriving criteria based on animal toxicity data. An acute criterion uses a minimum of eight acute toxicity values for animals, but a chronic criterion uses the most sensitive of the final chronic value (FCV) for animals or the final plant value (FPV). In practices, though, it is essentially always the FCV. Data for plants recently have been considered more important to address, especially for herbicides. There are some limitations; however, associated with the use of the FPV because of an insufficient description of minimum data requirements for plants within the 1985 Guidelines. The availability of a more complete plant toxicity database is desirable. This would allow the USEPA to provide a more definitive recommendation for the optimal minimum dataset needed to assess the risk of chemicals to aquatic plants. This presentation will provide the status of the current effort for creating a methodology for the derivation of aquatic plant water quality criteria. The majority of the data for the analyses comes from EPA's publically accessible ECOTOX database.

New and Emerging Methods in Aquatic Toxicology

653 Validation of the mysid two-generation and copepod lifecycle assays for the regulatory testing of endocrine active compounds

T.A. Verslycke, D.B. Mayfield, Gradient; V.J. Brown, D.P. Houchens, Battelle; P. Browne, USEPA / Office of Science Coordination and Policy; L.W. Touart, USEPA / Office of Science Coordination and Policy

USEPA developed the Endocrine Disruptor Screening Program (EDSP) in response to a US Congressional mandate "to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or such other effects as [USEPA] may designate" [21 USC. 346a(p)]. As part of the EDSP, USEPA is validating assays to identify and characterize the endocrine activity of pesticides, commercial chemicals, and environmental contaminants, specifically in relation to estrogen, androgen, and thyroid hormones. USEPA is also working with the OECD's Endocrine Testing and Assessment Advisory Group to validate and harmonize endocrine screening tests of international interest. Invertebrates (especially arthropods such as insects and crustaceans) constitute the vast majority of animal species on earth. Many invertebrate toxicity test protocols are routinely used in regulatory testing; however, few have been designed with endocrine-specific endpoints in mind. As part of the EDSP, a two-generation mysid toxicity test is proposed to provide a comprehensive Tier 2 evaluation of substances identified as possible endocrine disruptors through Tier 1 screening. Although the focus of the EDSP Tier 1 screening is on vertebrate relevant endocrine processes (estrogen, androgen, and thyroid), the mysid Tier 2 test is deemed necessary to assess potential adverse consequences of Tier 1 possible endocrine disruptors on invertebrate endocrine regulated processes. This talk will present a brief historical summary of the development and validation of a standardized mysid two-generation toxicity test proposed as an *in vivo* Tier 2 EDSP test and the harpacticoid copepod reproduction and development test as a potential alternative or option. We will present the reasoning and judgments leading to the development, demonstration, and validation studies for both invertebrate assays. For example, the proposed mysid two-generation toxicity test was used in an inter-laboratory validation study consisting of six studies carried out at four laboratories with three representative endocrine-active chemicals (*i.e.*, lindane, vinclozolin, and 4-tert-octylphenol). Conclusions regarding advantages and limitations of both the mysid and copepod assays in their current form will be presented.

654 Mission Impossible: can we standardize a Hyalella azteca reproduction test conducted entirely in sediment?

L.N. Taylor, Environment Canada; P.M. Jackman, Environment Canada / Toxicology Laboratory; G. Schroeder, C. Buday, A.J. Bartlett, Environment Canada; T. Watson-Leung, Ontario Ministry of the Environment / Aquatic Toxicology Unit; R.P. Scroggins, Environment Canada / Ecotoxicology and Wildlife Health Division

Environment Canada (EC) has been leading an ad hoc method development group with the goal of creating a *Hyalella azteca* reproduction test run entirely in sediment for 42 days. This is in contrast to existing standardized methods (e.g., USEPA, ASTM) that transfer adults from sediment to a water-only exposure before release of their first brood. One hypothesis in this effort has centered around the recent international advancements in diet quality and quantity of food, which have sped up the time to first brood so that young are larger at test end, thereby easier to recover from sediment. Experiments were successfully conducted first in clean field-collected sediments with a high proportion of sand. In follow up research, a more complex sediment with high organic matter was also tested with this new method. Other areas of method development have focussed on: different test vessels (i.e., imhoff cones, beakers, and mason jars), increased water-to-sediment ratios yielding stable and improved overlying water quality (i.e., 1:10 and 1:50), and changing the starting age of test organisms (i.e., 2-9 vs. 7 -14 day old). All method improvements are evaluated on their ability to 1) lower the coefficient of variation for the young per female endpoint, and 2) meet minimum performance criteria derived from other standardized methods (i.e., minimum survival, starting weight, change in weight, and young per female). So far, four years of research in four different labs have us convinced our mission is lofty but achievable.

655 Validation of EPA's EDSP Tier 2 Ecotoxicity Test Methods

L.W. Touart, P. Browne, S. Lynn, US-EPA / Office of Science Coordination and Policy; M. Manibusan, US-EPA

EPA established the Endocrine Disruptor Screening Program (EDSP) in response to a US Congressional mandate "to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or such other effects as [EPA] may designate" [21 USC. 346a(p)]. As part of the EDSP, EPA is validating assays to identify and characterize the endocrine activity of pesticides, commercial chemicals, and environmental contaminants, specifically in relation to estrogen, androgen, and thyroid hormones. This talk will present a brief historical summary of the development and validation of the candidate test methods including a Japanese quail two-generation test, the Larval Amphibian Growth and Development Assay (LAGDA), a medaka multi-generation test, and an invertebrate test. Although a medaka multigeneration test was the principal fish method considered, an abbreviated medaka reproduction test was also proposed. Additionally, a mysid two-generation toxicity test is recommended as the preferred invertebrate *in vivo* Tier 2 EDSP test, but a harpacticoid copepod reproduction and development test was also considered as a potential alternative or option. We will present the reasoning and judgments leading to the various studies that were conducted as part of the development, demonstration, and validation of the various test methods. In

addition, the outcome and recommendations of a FIFRA Scientific Advisory Panel review (June 2013) of the proposed methods will be summarized and discussed. Conclusions regarding advantages and limitations of the proposed tests will be presented. Disclaimer: This abstract does not represent USEPA policy.

656 Review and Evaluation of the Test of Significant Toxicity Approach

S.P. Canton, GEI Consultants, Inc. / Ecological Division; S. Skigen, GEI Consultants; N. Love, GEI Consultants, Inc.; T. Moore, Risk Sciences; V.K. Yoon, S.C. Paulsen, Flow Science Incorporated; R. Claff, American Petroleum Institute

In June 2010, the EPA issued the *National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document*. The TST approach was proposed by the EPA as an alternative method for evaluating whole effluent toxicity (WET) test results. We have reviewed the TST approach, comparing the TST to current methods, focusing on implementation and regulatory concerns, as well as potential costs associated with the TST. Our evaluation of the approach suggests chronic tests will be the most heavily impacted through TST implementation, therefore the primary issues identified centered on the potential of the TST to adversely affect the outcome of the chronic test method. The Implementation Document was evaluated to determine the potential implications for discharger compliance. After conducting our review, several key issues were identified, including the increased potential for false-positives. Using the original data collected during EPA's Interlaboratory Study (2001), each of the valid method blank samples was reanalyzed using the TST technique. Based on these data, the TST procedure will classify non-toxic samples to be toxic at a rate 2-3 times the current NOEC or IC_{25} methods. These increased false-positives would lead to additional follow-up confirmatory testing and toxicity identification evaluations resulting in an increase in costs to the discharger. Our evaluation has also revealed that the modified test design of reduced concentrations essentially eliminates the use of the dose-response curve, greatly restricting the ability of laboratories to detect when issues, such as pathogen interference, etc., play a role in test outcome.

657 Building models of retrospective Se exposure in rainbow trout, brook trout, and cutthroat trout using otolith microchemistry

V.P. Palace, Department of Fisheries & Oceans Canada, Stantec Consultants Ltd.; L. Friedrich, Stantec Consultants Ltd.; N. Halden, Univeristy of Manitoba; N. Pilgrim, J. Rasmussen, University of Lethbridge; L. Ripley, Allison Creek Brood Trout Station; A. Hontela, University of Lethbridge / Dept. of Biological Sciences

Visualized annual growth zones (AGZ) in sectioned and polished otoliths (inner ear bones), provide the best means for aging fish. Microchemical analysis of AGZ can also be useful for determining relative exposure to trace elements, but there is an urgent need to calibrate concentrations of trace elements measured in otoliths with those in other tissues. Using selenium (Se) as an example, establishing the relationship between concentrations in the most recent otolith AGZ with those in the ovary, would allow concentrations of Se in ovaries in past years to be derived from Se concentrations measured in each AGZ of the otolith. Models based on this premise were investigated in otoliths obtained from adult female rainbow (*Oncorhynchus mykiss*), brook (*Salvelinus fontinalis*) and cutthroat (*Oncorhynchus clarkii lewisi*) trout exposed to Se methionine (SeMet) in the diet at 1.5 (reference), 13 or 35 µg/g for 5 months prior to spawning. Concentrations of Se in eggs, muscle and liver increased dose dependently in all three species and there were quantifiable concentrations of Se in all otoliths. The concentrations of Se attained in otoliths varied among the three fish species, but significant regression models between concentrations in otoliths and ovaries were established for each species. These models provide evidence that retrospective reconstructions of Se in ovaries can be accomplished. Additional field studies are required to validate the models in these, and other, species. Funding for these studies was provided by an internal R&D grant from Stantec.

658 Development and completion of algal inter-species correlation estimation (ICE) models for use in hazard assessment

J.L. Brill, The Procter & Gamble Co. / Environmental Stewardship and Sustainability; S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization; S.D. Dyer, The Procter & Gamble Company / Central Product Safety; D. Versteeg, The Procter & Gamble Company / Environmental Science Department; J. Chaney, The Procter & Gamble Company; S. Raimondo, USEPA / Gulf Ecology Division, National Health and Environmental Effects Laboratory; M.G. Barron, USEPA / Gulf Ecology Division

Web-ICE (Web-based Inter-species Correlation Estimation) is an internet-based modeling platform developed by USEPA to relate acute toxicity of a chemical for one species to that of another. ICE allows the user to input an acute toxicity value for a surrogate animal species and predict the effect value for many other species, thus potentially filling in data gaps for a variety of environmental assessment purposes. The absence of a toxicity estimation capability for algal species in Web-ICE program was addressed by Procter & Gamble. A compilation of public (ECOTOX and scientific literature) and P&G-held algal toxicity data were synthesized into an ACCESS database, data quality was assessed and inter-species correlations were constructed from the most commonly tested algal genera for a broad spectrum of chemicals. Algae-only SSDs were then assessed and an extensive QA of the literature was completed according to USEPA Science Advisory Board. The final approved database consists of 1946 unique studies with approximately 300 unique chemicals and encompasses 22 genera and 38 species of Green Algae, Blue-Green Algae and Diatoms. The last steps included developing ICE regressions for this final database. 100 ICE models were developed at the Genus level with 22 meeting our criteria and 160 ICEs were developed at the Species level with 29 meeting our criteria. Initial assessments of interspecies correlation estimations (ICE) to predict the 5th percentile hazard concentration (HC5; protective of 95% of species) have shown great promise for screening effects assessments without the use of prohibitive assessment factors. Also to be addressed will be additional applications for the use of ICE-based SSDs in regulatory and scientific contexts to derive protective criteria. ICE greatly reduces our overall dependence on animal tests as well as presents another mechanism to reduce uncertainty factors applied to minimal data sets.

660 New characterization factors for PCDD/Fs including oil influence on their fate in a life cycle assessment context

E. Taing, CIRAIG / Polytechnique; C. Bulle, CIRAIG – Polytechnique Montreal / Chemical engineering department; L. Deschenes, École Polytechnique de Montréal / Genie Chimique

Are (eco)toxic impacts of a single contaminant the same as when it interacts with other contaminants in a mixture? Life Cycle Impact Assessment (LCIA) methods currently consider that this is the case whereas some studies have showen that mixture interactions can have a significant influence on contaminant fate. This project aims to adapt the USEtox model and to determine to what extent (eco)toxic potential impacts change (regarding model uncertainty) when including interactions between contaminants. In LCIA, Characterization Factors (CF) calculate the amount of potential impacts by amount of emitted pollutant, and (eco)toxic CFs include the contaminant fate in the environment (Fate Factor FF), exposure (Exposure Factor XF, set to 1 for ecotoxic impacts) and effects (Effect Factor EF). This study only considers influence of contaminant interactions on fate and is based on the specific case study of dioxins and furans (PCDD/Fs) contained in pentachlorophenol (PCP) of PCP pole-treating oil. As the latter is a significant source of PCDD/F emission into soil in the Canadian inventory of release (47% of total emissions in 2003), it may be of interest to see if interactions significantly influence the overall potential impact of Canadian PCDD/F emissions. Due to a lack of precise physico-chemical parameters for PCDD/F congeners present in PCP, the most documented dioxin, 2,3,7,8-TCDD, has been chosen to "represent" the PCDD/F mixture. Oil organic components are gathered into fractions based on similar behaviors in the environment, and their fates are assessed via the USEtox model. Thus, as it is assumed that oil "carries" PCDD/Fs entirely, which represents an "extreme case", oil-receiving compartments where oil degrades become PCDD/F emission compartments, so that 2,3,7,8-TCDD FFs can be calculated. Finally using these new FFs, and XFs and EFs from the USEtox model, new CFs for 2,3,7,8-TCDD are determined. The proposed approach leads to between 8 and 39 times higher toxicity CFs and between 5 and 21 times higher aquatic ecotoxicity CFs than the original USEtox CFs for an emission into soil. For an emission into air and water, modifications are negligible. While applying these new CFs in the Canadian PCDD/Fs release inventory of 1999, both category impacts increase at most by 2%. This is due to a small quantity of PCDD/F emitted into soil but a huge quantity emitted into air. Sensitivity has not yet been estimated.

Risk Assessment of Pesticides to Honey Bees: Part B

661 An Evaluation of the Potential Risk to Honey Bees Exposed to Thiamethoxam Residues in Canola Pollen and Nectar

P.F. Hoekstra, Syngenta Canada / Regulatory and Biological Assessment; C. Harrington, K. Sagan, Syngenta Canada Inc. / Regulatory and Biological Assessment; A. Shulkin, Syngenta Canada / Regulatory and Biological Assessment; J. Overmyer, Syngenta Crop Protection, LLC. / Ecological Sciences, Americas

Canola is one of Canada's most valuable crops, grown on over 8 million hectares and contributing more than $15 billion CDN to the economy. This crop is mainly produced in western Canada and is susceptible to damage caused by insect pests such as flea beetles. Historically, seed-applied insecticides such as lindane were applied to canola seed to protect early stages of plant development that are most sensitive to insect damage. In the early 2000s, neonicotinoids such as thiamethoxam were introduced as an alternative seed treatment option for canola and provide protection against early-season foliar pests. In this field residue study, whole flowers, pollen and nectar were collected during peak bloom from a canola crop that was grown from seed treated at with thiamethoxam. Concentrations of thiamethoxam and the insecticidally-active degradate CGA322704 (i.e., clothianidin) were determined in each matrix. Residue levels of thiamethoxam and CGA322704 were quantified in pollen and nectar and compared against Tier I laboratory toxicity information for adult and larval honey bees as per the guidance of SETAC's Pellston Workshop and the White Paper on pollinator protection jointly developed by the US Environmental Protection Agency, Health Canada's Pest Management Regulatory Agency and the California Department of Pesticide Regulation. Residue levels in pollen and nectar indicate that the potential risk to pollinators is well below the level of concern proposed in the White Paper. These results, in combination with the finding from experimental field studies and real-world examples, demonstrate the coexistence of bee populations with canola production.

662 Predicting honey bee susceptibility based on the conservation of the pesticide molecular initiating event

C.A. LaLone, USEPA; D.L. Villeneuve, C. Russom, USEPA / Mid-Continent Ecology Division; J.P. Berninger, USEPA; J.E. Tietge, USEPA / Office of Research and Development, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division; H. Helgen, D. Lane, S. Walata, Computer Sciences Corporation; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory

Concern surrounding the potential adverse impacts of pesticides to honey bee colonies has led to the need for rapid/cost efficient methods for aiding decision making relative to the protection of this important pollinator species. Neonicotinoids represent a class of pesticides that are widely used to exterminate pest insects through their action on the nicotinic acetylcholine receptor (nAChR) and have been under scientific investigation for their lethal/sublethal effects on various non-target insects, including the honey bee. The goal of our research was to demonstrate how knowledge of nAChR conservation across insect species can aid in predictions of honey bee susceptibility to neonicotinoids. Our strategy makes use of available protein sequence and conserved functional domain information, as well as queries of individual amino acid residues linked to species sensitivity, to compare molecular target similarity across insect species and predict relative intrinsic susceptibility. These analyses could aid in defining the taxonomic domain/s of applicability for the action of these insecticides on the nAChR. Our results indicate that the nAChR is highly conserved across many insect species, including pollinators. Therefore, assuming similar absorption, distribution, metabolism, elimination and dosage implications across species, we would predict that honey bees are likely to be susceptible to neonicotinoids that target the nAChR. In conjunction with defined adverse outcome pathway constructs pertaining to insecticide molecular initiating events, this strategy could be useful for routine evaluation of potential vulnerability of honey bees to insecticides. The contents of this abstract neither constitute nor reflect official USEPA policy.

663 A causal analysis of observed declines in managed honey bees (Apis mellifera)

J.P. Staveley, Exponent, Exponent, Inc.; S. Law, Exponent, Inc. / EcoSciences / Environmental Group; C.A. Menzie, Exponent; A. Fairbrother, Exponent, Inc. / EcoSciences

The European honey bee (*Apis mellifera*) is a highly valuable, semi-free-ranging managed agricultural species. While the number of managed hives has been increasing, declines in over-winter survival, and the onset of colony collapse disorder in 2006, precipitated a large amount of research on bee health in an effort to isolate the causative factors. A workshop was convened to explore this research with respect to the various candidate causes that have been identified by researchers, beekeepers, and regulatory bodies. Bee experts were introduced to a formal causal analysis process that used a weight-of-evidence approach to compare 39 candidate causes against specified causal criteria, to evaluate their relationship to the reduced overwinter survivability observed since 2006 of bees used in the California almond industry. The causal criteria used to evaluate the strength of evidence for each candidate stressor were: evidence of preceding causation, spatial and temporal co-occurrence, stressor-response relationship, interaction, and alteration. Relevant evidence was scored based on the degree of confidence in the supporting weight of evidence. Evidence scores ranged from "convincingly weakens" to "convincingly supports". Candidate causes were categorized as being probable, possible, unlikely or indeterminate. Three of the 39 candidate causes will be discussed further in this presentation. One example for each of "probable," "possible" and "unlikely" candidate causes will be described in detail. *Varroa* mites plus viruses was judged to be a "probable cause," and nutrient deficiency was judged to be a "possible cause" of the reduced survival of commercial honey bee colonies. Neonicotinoid pesticides were judged to be "unlikely" as the sole or a primary cause of this reduced survival probability, although they could possibly be a contributing factor.

664 A Colony Population Dynamics Model to Determine the Effect of Varroa Mites and Pesticide Exposure to Honey Bees

G. DeGrandi-Hoffman, United States Department of Agriculture / Agricultural Research Service; K.V. Garber, USEPA / Environmental Fate and Effects Division

Concern has been expressed about the declining health of honey bees (*Apis mellifera*) in the United States. The current understanding is that this decline can be attributed to multiple stressors, including pests (especially the Varroa mite, *Varroa destructor*), diseases (e.g., *Nosema* spp.), pesticides, poor nutrition and genetics. A colony simulation model was developed to investigate two of these stressors: Varroa pressure and pesticide effects. The population dynamics of honey bee colonies were mathematically modeled without pest pressure or pesticide exposure to generate predictions on population growth and colony survival. Predictions of colony populations generated from simulations indicated that if food is not limited, colony growth and survival are most dependent on the queen's egg laying rate and the longevity of worker bees. Both of these factors are influenced by weather conditions because they affect egg laying and brood rearing rates and foraging that ultimately influence worker mortality. The model was then modified to simulate Varroa mite population dynamics within the honey bee colony. When mite populations reached exponential growth in colonies, the reduction in worker longevity due to parasitism affected the age structure of the honey bee population so that colonies died in the winter or early spring. The original model was also modified to simulate the impacts of pesticide exposures that cause lethal and sub-lethal effects to individual adult honey bees and brood. Of particular interest is how timing of exposure may coincide with periods when colonies are the most or least resilient to population losses. Model simulations such as these will allow for identification of periods with higher or lower risk for colony loss from lethal or sub-lethal exposure to pesticides. Although this model allows for separate examination of effects of Varroa or pesticides on honey bee colonies, it also allows for consideration of the combination of these stressors and may provide more realistic estimates of effects given that these stressors typically occur in combination.

Point and Non-Point Sources of Organic Pollutants and Plastic Debris in the Marine Environment

665 The distribution of floating microplastic debris in the eastern North and South Pacific Oceans

K. Lavender Law, S. Moret-Ferguson, D. Goodwin, E.R. Zettler, Sea Education Association; E. DeForce, Woods Hole Oceanographic Institution; G.K. Proskurowski, University of Washington

Floating plastic marine debris has been documented in all five of the ocean's subtropical gyres, most commonly occurring as microplastic (particles < 5 mm in size). The particles themselves retain little evidence of their origin, and few reliable estimates of plastic input to the ocean currently exist. Nor is the spatio-temporal extent of plastic marine pollution well-known, despite public and media attention to regions such as the eastern North Pacific Ocean dubbed "garbage patches". The distribution of floating plastic debris in the eastern North and South Pacific Oceans (57.5°N to 17.5°S) was analyzed from measurements of plastic concentration from more than 2500 surface plankton net tows conducted by Sea Education Association between 2001 and 2012. From these data an accumulation zone in the North Pacific subtropical gyre was defined (25° to 41°N and 130° to 180°W) that closely corresponds to centers of accumulation predicted by several numerical models, and that results from the convergence of ocean surface currents due to Ekman and geostrophic flow. Maximum plastic concentrations measured from individual surface net tows exceeded 10^6 pieces km^{-2}, with concentrations decreasing with distance from the predicted center of the accumulation zone. Outside of the North Pacific subtropical gyre the median plastic concentration was 0 pieces km^{-2}. Spatio-temporal variability in sampling must be considered when calculating summary statistics such as mean or median concentration in the accumulation zone. No clear trend in plastic concentration was observed within the subtropical accumulation zone over the last decade, despite a 27% increase in global plastic production and a 42% increase in discarded buoyant plastic waste in the United States during the same time period.

666 Plastic contamination in marine habitats may be a source of PBDEs in fish tissue

C.M. Rochman, Marine Ecology/Aquatic Health; R. Lewison, San Diego State University; S. Teh, University of California, Davis; M. Eriksen, 5Gyres

The accumulation of plastic debris in the subtropical gyres, far from land, is a global phenomenon predicted to increase. Plastic debris travels long distances, drifting with ocean currents, and harbors a cocktail of organic pollutants that are ingredients of plastic and/or sorb from ambient seawater. This raises concern that plastic is another source of organic pollutants to remote open ocean habitats and to wildlife that ingest plastic debris and are at risk of bioaccumulating hazardous pollutants. To begin to understand the fate of organic contaminants from plastic in remote open oceans, we quantified the density of plastic debris and examined the relationship between the bioaccumulation of several organic chemicals in mesopelagic fish across the South Atlantic Subtropical Gyre. Mesopelagic lanternfishes from the family Myctophidae were sampled and analyzed for bisphenol A (BPA), alkylphenols, alkylphenol ethoxylates, polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). Plastic contamination was detected at all stations where fish were sampled and we observed a large variability of plastic densities and concentrations of organic pollutants in fish among stations. Patterns of organic pollutant contamination in fish across the South Atlantic will be discussed in terms of their potential point sources (e.g., maritime, urban runoff). Although it is difficult to tease apart the bioaccumulation of organic pollutants from plastic debris or from the food chain, we observed a relationship between plastic density and the bioaccumulation of PBDEs. Myctophid sampled at stations with greater plastic densities had greater concentrations of BDE-183 and BDE-209 in their tissues ($P < 0.05$, R^2=0.24), consistent with patterns observed in seabirds from the North Pacific. This suggests that plastic ingestion may be a source of higher brominated congeners of PBDEs, added to plastics as flame-retardants, to marine life. In the remote open ocean of the subtropical gyres, chemical contamination is relatively low, and the concentration of chemicals sorbed to plastics in these regions is much smaller than in coastal regions. Still, we conclude that even in remote open ocean regions, plastic debris poses a hazard of bioaccumulating chemical contaminants in marine animals.

667 Methylmercury production and distribution in the global oceans

E.M. Sunderland, Harvard University; D. Krabbenhoft, USGS; W. Landing, Florida State University

For most individuals in Europe and North America, the majority of mercury exposure is from marine fish consumption. Methylmercury is the only species of mercury to biomagnify in aquatic food webs. Thus, understanding biogeochemical controls on methylmercury production in the ocean is critical for understanding present and future health risks to humans and wildlife. Here we use new data on methylmercury levels in seawater from the Indian and Southern Oceans, in combination with previously published information from the North Pacific, to develop a model for spatial and temporal distributions of methylmercury in the world's oceans. The model is based on the relationship between organic carbon remineralization and methylmercury concentrations observed across these three major ocean basins. We extend this relationship globally using gridded oceanographic data on apparent oxygen utilization and apparent ages of water masses from the World Ocean Atlas. We evaluate the model using previously published data from various ocean basins and present a new budget for the spatial and seasonal production and distribution of oceanic methylmercury.

668 Vertical eddy diffusion as a key mechanism for removing perfluorooctanoic acid (PFPA) from the global surface oceans

R. Lohmann, University of Rhode Island / Graduate School of Oceanography; E. Jurado, IDAEA-CSIC / Environmental Chemistry; H. Dijkstra, Utrecht University / Institute for Marine and Atmospheric Research (I.M.A.U.), Department of Physics and Astronomy; J. Dachs, Institute of Environmental Assessment and Water Research -CSIC / Environmental Chemistry

Here we estimate the importance of vertical eddy diffusion in removing perfluorooctanoic acid (PFOA) from the surface Ocean and assess its importance as a global sink. Measured water column profiles of PFOA were reproduced by assuming that vertical eddy diffusion in a 3-layer ocean model is the sole cause for the transport of PFOA to depth waters. The global oceanic sink due to eddy diffusion for PFOA is high, with accumulated removal fluxes over the last 40 years of 664 t, with the Atlantic Ocean accounting for 70% of the global oceanic sink. The global oceans have removed 13% of all PFOA produced to a depth greater than 100 m via vertical eddy diffusion; an additional 4 % has been removed via deep water formation. The top 100 m of the surface oceans store another 21% of all PFOA produced (~1100 t).

669 Per- and Polyfluoroalkyl substances (PFASs) in the Coastal and Marine Environment

L. Ahrens, Swedish University of Agricultural Sciences (SLU) / Dept. of Aquatic Sciences and Assessment

The occurrence and fate of per- and polyfluoroalkyl substances (PFASs) in the aquatic environment has been recognized as one of the emerging issues in environmental chemistry. Several PFASs from different classes have been detected ubiquitously in the aqueous environment while the concentrations usually range between pg and ng per liter for individual compounds. Sources of PFASs into the aqueous environment are both point sources (e.g., wastewater treatment plant effluents) and nonpoint sources (e.g., surface runoff). The detected congener composition in environmental samples depends on their physicochemical characteristics and may provide information to their sources and transport pathways. However, the dominant transport pathways of individual PFASs to remote regions have not been conclusively characterized to date. Key loss processes and deposition, the relationship between sources and aqueous environment concentrations, partitioning behavior and transport mechanism are discussed to lead to a better understanding of the global geochemical cycle and fate processes of PFASs in the aqueous environment.

670 Seasonal Variation of Organotin Compounds in Seawater from Cape Town Harbour

H.K. Okoro, University Of Ilorin / Department Of Chemistry, Faculty of Science; O. Fatoki, Cape Peninsula University of Technology; F. Adekola, University Of Ilorin / Chemistry; B. Ximba, Cape Peninsula University of Technology, / Chemistry; R.G. Snyman, Cape Peninsula University of Technology / Biodiversity and Conservation

The annual distribution of OTCs in the seawater of Cape Town harbour was investigated. The concentration of OTCs varies for locations in Cape Town harbour. The concentration ranges from 0.067 ± 0.01 to 111.290 ± 32.20 x

10^{-3} µg/l for TBT while that of TPT ranges between ND to 23008.0±0.03 x 10^{-3} µg/l respectively between locations. The seasonal variation of TBT was also investigated and significant variation of $P \leq 0.05$ was found after the statistical analysis. Seasonal variation in TBT and TPT concentrations with a higher level in summer than in winter and spring was observed. Apparently, the observed high or low values recorded for BT in Cape Town harbour could be the result of an increase or decrease in the traffic of ships and boats. In addition, dilution effect due to increase in water volume could also account for the decrease in concentration of TBT. High values observed for TBT in summer could be associated to steady slower ocean current, while in spring, the ocean tide effect may suggest the reason why we have more TBT in spring or low but this also depends on the direction of the tide.

671 Atmospheric deposition and cycling in the water column of legacy persistent organic pollutants in the Arctic and Southern Ocean

J. Dachs, Institute of Environmental Assessment and Water Research -CSIC / Environmental Chemistry; C. Galban-Malagon, A. Cabrerizo Pastor, E. Jurado, IDAEA-CSIC / Environmental Chemistry

Persistent organic pollutants can reach Polar regions such as the Arctic Ocean and Antarctica through atmospheric transport and deposition. The southern ocean and the North Atlantic ocean adjacent to the ice-sheet are ecosystems characterized by high primary and bacterial productivity. The biological and degradative pumps are processes which can deplete the water column concentrations due to settling of organic matter-bound POPs or bacterial degradation. In this work we present the results from several sampling cruises in the Arctic and Southern Ocean in which the atmosphere, seawater and phytoplankton were sampled simultaneously. In addition, a campaign at Livingston Island was performed to elucidate the potential secondary sources from Antarctic soils and snow/ice affecting the atmospheric concentrations of POPs. The obtained results show that the concentrations of polychlorinated biphenyls (PCBs) and hexachlorocyclohexanes (HCH) have decreased in the Southern ocean over the last decade with estimated half-lives of about four years, presumably due to a reduction of primary sources. The measurements done at land show that soils and snow are secondary sources of POPs during the austral summer, inducing higher levels of atmospheric POPs regionally. Conversely, the southern ocean is a net sink with air-to-water net deposition flux for most compounds and stations. For polychlorinated biphenyls, the air-water fugacity ratios are higher for the more hydrophobic compounds, and concentrations in phytoplankton decrease in the regions with higher phytoplankton biomass. These trends support the role of the biological pump as a key process driving the oceanic sink of POPs. Conversely, for other compounds that can be subject of bacterial degradation, such as HCHs, the results and model estimations suggest that degradation may deplete water column concentrations and enhance the atmospheric deposition of these compounds. Therefore, the cycling in the water column can affect the deposition fluxes, and thus the atmospheric transport potential of POPs. Similarities and differences between the Arctic and Southern Oceans in terms of POP cycling will be commented.

672 Atmospheric Deposition and Exchange of Persistent Chemicals in Bothnian Bay, Northern Baltic Sea

T.F. Bidleman, Umea University / Department of Chemistry; S. Newton, Stockholm University / Department of Applied Environmental Science (ITM); K. Wiberg, Swedish University of Agricultural Sciences (SLU) / Department of Aquatic Sciences and Assessment; K. Hansson, E. Brorstrom-Lunden, Swedish Environmental Research Institute (IVL); M. Ripszam, P. Haglund, Umea University / Department of Chemistry; K. Agosta, Umea University; O. Nygren, Umea University / Department of Chemistry

Air-sea exchange of persistent chemicals was investigated in Bothnian Bay (BB), northern Baltic Sea, to estimate current loadings and the impact of climate-induced changes in the ecosystem. Target compounds were persistent organic pollutants (POPs): hexachlorocyclohexanes, hexachlorobenzene, chlordanes, dieldrin, and endosulfans; current-use pesticides: chlorpyrifos and dacthal (chlorthal dimethyl) and naturally occurring brominated compounds: 2,4-dibromoanisole and 2,4,6-tribromoanisole. Levels of these compounds in BB air and water ranged between those reported in the Arctic and North American Great Lakes. Gas exchange direction was estimated from air and water concentrations (the latter corrected for binding to dissolved organic matter) and temperature-dependent Henry's law constants. Bromoanisoles were greatly oversaturated in surface water and volatilising. Net volatilisation or air-water equilibrium was estimated for most POPs during spring-summer, except for endosulfans which were undergoing deposition. It is not clear why most POPs were volatilising. Because BB is shallow and has a water residence time of only about 3 years, the water column cannot be a large historical reservoir like the Great Lakes or the Arctic Ocean. Gas exchange fluxes were estimated using the two-film model and compared to loadings derived from bulk deposition collection (precipitation plus dry particle deposition). Gas exchange dominated loadings to BB itself, but bulk deposition to the BB catchment followed by riverine transport could be comparable in magnitude. This source may supply additional POPs to be revolatilised during summer. A large uncertainty is the fraction of catchment loadings that are delivered to BB by rivers.

Modeling and Interpreting Effects of Metals Mixtures

673 Effects of metal mixtures to larval and adult Pimephales promelas: additive and non additive mechanisms

N. Lynch, Loyola University Chicago / Department of Chemistry; T.C. Hoang, Loyola University Chicago / Department of Environmental Science, CUERP

Much research has been conducted to assess the toxicity of metals on aquatic organisms. However, most of the research has focused on the toxicity of individual metals, although metals are usually present as mixtures in contaminated environments. The literature review indicates that metal mixtures may be additive, synergistic, or antagonistic to freshwater species. The goal of this research is to use a systematic experimental design to characterize the toxicity of Cu, Zn, Cd and Ni mixtures to *P. promelas*. Standard 96h toxicity tests were conducted with larval *P. promelas* based on the USEPA methods to determine metal mixture effects. All experiments were conducted in synthetic moderately hard water at neutral pH. For experiments with *P. promelas*, Cu and Zn, and Cu and Ni metal bi-mixtures appear to be synergistic. These results suggest a joint mechanism of toxicity of Cu and Zn, and Cu and Ni metal bi-mixtures in larval fathead minnows. However, the toxicity of Zn and Ni metal mixtures was additive to larval fathead minnows. Cd and Zn mixtures were antagonistic to larval fathead minnows up to Zn concentrations of 200µg/L, and additive up to 800µg/L Zn. These tests were repeated with identical water quality criteria using adult fathead minnows aged 3-5 months. Using adult fish would allow us to determine tissue concentration more accurately. Similar results were observed for Cu and Zn bi-mixtures. However, Cd and Zn mixtures were strictly additive to adult fathead minnows. Upon test termination, adults were collected and dissected to remove the gills. Gills were digested separately from the rest of the body to be analyzed for tissue metal concentrations. Whole body metal concentrations will be compared to concentration at the gills to see whether metals are accumulated at the gill site. Results of this study are important for the development of a Biotic Ligand Model for metal mixtures.

674 Modeling metal mixture effects related to soils contaminated with Cu, Ni and Zn

D.M. Schwertfeger, McGill University / Dept. of Natural Resource Sciences; M. Boudreau, McGill University / Natural Resource Sciences; W.H. Hendershot, McGill University / Dept. of Natural Resource Sciences

Much of the available ecotoxicity data is based on single-metal experiments; however, trace metals are rarely found alone in soils. The bioavailability and toxicity of metal mixtures needs to be understood in order to determine safe levels of metals in the environment. This research project was undertaken to develop best management practices, based on sound, scientifically defensible theory that can be used to minimize negative environmental or human health impacts of soils contaminated with mixtures of trace metals. To this end, an experiment was designed to compare the toxicity of binary and tertiary mixtures of Cu, Ni and Zn relative to concentrations of the individual metals giving similar toxic responses. For each of the three test soils varying in soil properties (pH 5.8 to 7.2), dose-response curves were established for each metal individually using 14-d plant growth assays conducted with Hordeum vulgare L. In addition to the single metal additions, three binary metal mixture amendments were included for which soils were spiked with approximately ½ EC50 of each metal, and one tertiary mixture was included for which soils were spiked with 1/3 EC50 of each metal. Metal-spiked soil samples were leached until leachate electrical conductivity values stabilized in order to ensure the "salt-effect" had been minimized. Plant bioassays were performed in controlled environmental chambers under standard conditions. Soil solutions were extracted post plant bioassay using a column leaching method and were analyzed for pH, total dissolved metals, dissolved

organic carbon, and Cu2+, Ni2+ and Zn2+ using ion exchange technique (IET). Results showed that the observed mixture toxicity was always significantly lower ($\alpha = 0.05$) than toxicity predicted by the concentration additive model. In testing the response additive model, the observed toxicity was in some cases greater than, and in other cases less than, the toxicity predicted by the model, depending on soil type. Metal free ion activities were used to calibrate a terrestrial biotic ligand model (TBLM) which better predicts the toxicity of the metal mixtures to barley root elongation. These data are expected to help risk assessors evaluate contaminated sites affected by mixtures of trace metals.

675 Modeling uptake and chronic toxicity of metal mixtures to plants, Daphnia and zebrafish

K. De Schamphelaere, Ghent University / Environmental Toxicology and Aquatic Ecology; C. Nys, University of Ghent / Environmental Toxicology and Aquatic Ecology; L. Versieren, KULeuven; P. Van Den Plas, Universiteit Antwerpen / Systemic Physiological and Ecotoxicological Research, Department of Biology; R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; E.E. Smolders, Katholieke Universiteit Leuven

In the context of a large-scale project on metal mixtures, we are using stable isotope and chronic ecotoxicity assays to determine combined and interactive effects between metals on uptake and/or chronic toxicity in organisms from three different trophic levels, i.e., *Hordeum vulgare* tested in hydroponic solutions (plant), *Daphnia magna* (invertebrate) and *Danio rerio* (zebrafish). Experiments with zebrafish have so far clearly demonstrated that the uptake rate of one metal (e.g., Cd) can be clearly affected by the presence or absence of another metal (e.g., Cd). Initial dynamic modeling shows promise in explaining and predicting these kinds of interactions. Full-factorial binary mixture experiments (Zn-Ni mixture) with *Daphnia magna* suggest that Zn and Ni may act 'synergistically' (by which we mean 'more toxic than expected based on independent action model predictions based on measured metal concentrations in the water'). However, this only appears to occur at concentrations that exceed the EC20 of each metal individually. At lower concentrations, the Zn-Ni mixture behaves non-interactively. Combining the existing chronic BLMs for Ni and Zn with the independent action model and extending this with model terms describing these 'synergistic' interactions at high concentrations yields a Ni-Zn *Daphnia* mixture BLM with reasonable predictive capacity, although further validation for a range of physico-chemistries is still required. Experiments with Cu-Zn mixtures with *H. vulgare* at three different Ca concentrations learned that Cu and Zn acted 'antagonistically' (based on metal cocnentrations measured in the water) at low Ca cocnentration but not (or less so) at high Ca concentration. However, BLM modeling learned that this 'antagonism' could be explained by competitive effects of Cu and Ca on the Zn-BL-site and of Zn and Ca on the Cu-BL-site. Taken all together, our results so far in the project suggest that interactions between metals do occur when performing analysis based on external metal concentrations (measured in the water), but that dynamic uptake models and/or 'mixture'-BLMs can in most cases help to explain these 'interactions'. Therefore, these kind of models will ultimately also be useful in predicting metal uptake and toxicity of metals occuring in metal mixtures. However, there is a clear further need to mechanistically (e.g., physiologically) understand some of the observed interactions, rather than just trying to understand them from a modeling perspective.

676 Dynamics of metal mixture accumulation and toxicity in aquatic organisms: a modeling framework and experimental results

R. Blust, University of Antwerp / Systemic Physiological and Ecotoxicological Research, Department of Biology; P. Van den Plas, University of Antwerp / Biology; V. Mubiana, S. Joosen, University of Antwerp

The accumulation and toxicological effects of metals are kinetic processes in which exposure scenario, dose and time are key factors. Both the physicochemistry of the environment as the physiology of the organism determine the response profile and how this develops as function of exposure level and time. Since chemical speciation and bioavailability can vary orders of magnitude and organisms have developed various strategies to handle both essential and non-essential metals it can be expected that also the toxicity of metals varies orders of magnitude even under otherwise similar exposure scenarios across species. Using a dynamic modelling approach that distinguishes bioactive and bioinactive metal pools (BIM-BAM) in the external and internal environment of an organism we demonstrate how this large variation can be explained. At the same time we also show how equilibrium models such as the biotic ligand model (BLM) can be a surrogate for the more complex dynamic models for application in risk assessment. Since metal exposure is more often a case of multiple rather than single exposure both dynamic and equilibrium models have to consider mixture exposure conditions and how the biological machinery responds to such situations in the different options that are available. Results of the dynamic mixture uptake and toxicity models are compared with results of single and multiple metals exposure experiments using existing and newly generated data on invertebrates and fish as examples (e.g., daphnids, zebrafish). Analysis of the kinetic uptake and toxicity data shows that both antagonistic and synergistic effects are obtained depending on the exposure scenario and how the dynamic modelling approach describes and explains these observations.

677 Modelling aquatic metal mixture toxicity with WHAM-FTOX

E. Tipping, Centre for Ecology and Hydrology; S. Lofts, Centre for Ecology & Hydrology / Shore Section

If metal toxicity occurs in the natural environment, it is probably due to a mixture of metals, and also protons in some cases. The WHAM-F_{TOX} model is designed to take mixtures into account, while at the same time dealing with "medium effects", i.e., variation in toxicity with water chemistry. The exposure of an organism to potentially-toxic cations is expressed by their "active body burdens" (n_i mol g^{-1}). Toxicity is quantified empirically through the product of the active body burden and a toxicity coefficient, α_i, providing a straightforward means of combining the toxic effects of different cations, including H^+. Key to the calculations is the use of the WHAM chemical speciation model to quantify the competitive reactions of cations with living organisms, thereby estimating the values of n_i. Toxicity is related to the variable F_{TOX}, given by $\Sigma\alpha_i n_i$. We have gathered data describing measured metal accumulation by living organisms, ranging from bacteria to mussels, exposed to different solutions, and compared them with predictions of metal binding to humic acid in the same solutions, using the chemical speciation model WHAM. After calibration to account for differences in binding site densities, a strong correlation ($r^2 = 0.89$) between the measured and predicted metal loadings is obtained. These findings are taken to justify the use of WHAM to estimate metabolically-available metal associated with living organisms. Application of the model to laboratory data from toxicity experiments with organisms ranging from bacteria to fish successfully accounts for the toxic effects of mixtures. In most cases, the data are insufficient to provide a stringent model test, so that the fits with WHAM-F_{TOX} are only slightly better overall than those with a conventional concentration-addition model that does not take bioavailability into account. However, results published by Mebane and colleagues for Zn-Cd-Pb toxicity to trout provide a stern test, and in this case WHAM-F_{TOX} is clearly superior. The results show that when exposure is quantified in terms of bound cations with F_{TOX}, the toxic response occurs over a much narrower range than when solution concentrations are used. This arises because changes in bound metal are associated with large changes in solution metal, following the Law of Mass Action, and also because of the high degree of heterogeneity in the binding sites.

678 Predicting the Toxicity of Metal Mixtures

L. Balistrieri, US Geological Survey, University of Washington / School of Oceanography; C. Mebane, US Geological Survey / NOAA Fisheries Liaison

The toxicity of single and multiple metal solutions to fish is predicted using an approach that combines calculations of: (1) solution speciation; (2) competition and accumulation of cations (H, Ca, Mg, Na, Cd, Cu, Pb, and Zn) on two types of biotic ligand sites; (3) a toxicity function that accounts for accumulation and potency of individual toxicants (H, Cd, Cu, Pb, and Zn); and (4) biological response. The approach is evaluated by examining water composition from single metal toxicity tests of trout at 50% mortality, results of theoretical calculations of metal accumulation on fish gills and associated mortality for single and multiple metal (Cd, Cu, Pb, and Zn) solutions, and predictions for a field site impacted by acid rock drainage. These evaluations indicate that predictions of metal mixture toxicity are multi-dimensional and not intuitive. Toxicity of a solution depends on the relative affinity and potency of toxicants for a given aquatic organism, suites of metals in the mixture, dissolved metal concentrations and ratios, and background solution composition (temperature, pH, and concentrations of major ions and dissolved organic carbon). A composite function that

includes solution composition, affinity and competition of cations for biotic ligands, and potency of hydrogen and individual metals is proposed as a tool to evaluate potential toxicity of environmental solutions to fish.

679 A Comparative Evaluation of Modeling Approaches for Metal Mixture Toxicity

K.J. Farley, Manhattan College / Civil and Environmental Engineering; J.S. Meyer, Arcadis US, Inc., ARCADIS

Although chemical speciation-based models [e.g., Biotic Ligand Models (BLMs)] have been developed to explicitly consider the modifying effects of Ca^{2+}, Mg^{2+}, Na^{+}, and H^{+} competition on metal toxicity, the models have largely been applied to single metals. Several modeling groups however are currently working to extend chemical speciation-based models to metal mixtures. Proposed models cover a wide range of approaches from single-site models with all metals exhibiting similar potency on the biotic ligand to multi-site BLMs with competition of all metals on the various binding sites. As part of this presentation, we compare and contrast four of the proposed modeling approaches based on their solution-chemistry calculation, their biotic ligand site characteristics, their assignment of metal potency on the biotic ligand, and ultimately their ability to describe toxic responses in metal-mixture studies. Results of this evaluation are then used to examine the following three modeling issues: Should metal binding to biotic ligands follow the binding sequence of metals to other ligands? How complicated do chemical speciation-based models need to be to adequately describe the toxic effects of metal mixtures? Are the available data sufficient in developing a defensible model for metal mixtures?

680 The Effects of Metals Mixtures on Metal Uptake in Rainbow Trout: Implications for Development of a Multi-Metal Biotic Ligand Model

K.V. Brix, University of British Columbia / Biology, University of Miami, RSMAS; M. Tellis, McMaster University / Biology; C.M. Wood, McMaster University / Department of Biology

Single metal Biotic Ligand Models (BLMs) have now been developed for a number of metals and model organisms. While these BLMs significantly improve our ability to regulate metals in the aquatic environment, in reality, organisms are often simultaneously exposed to metal mixtures, potentially resulting in metal interactions which are not accounted for in current BLMs. To begin developing a multi-metal framework, standard 3-h gill metal binding assays with Ag, Cd, Cu, Pb, Ni, and Zn were performed *in vivo* on freshwater rainbow trout. Fish were exposed across a range of concentrations to characterize uptake kinetics for single metals and all possible binary combinations. We hypothesized that metals with a similar mode of action (e.g., Na^{+} antagonists or Ca^{2+} antagonists) would interact in a competitive manner for uptake at the gill while no interaction would be observed for metals with dissimilar modes of action. Surprisingly, we found this was not the case with examples of both competitive and non-competitive inhibition observed, in addition to examples of stimulated metal uptake in the presence of mixtures. The underlying mechanisms for these unexpected interactions are unclear and suggest careful characterization of additional metal mixture combinations may be necessary before a reliable multi-metal BLM can be developed (Acknowledgements: Environment Canada MITHE Program, CDA, ICA, ILZRO, NiPERA, Rio Tinto).

Harmful Algal Bloom Toxins in Inland Waters: Environmental Contaminants of Emerging Concern

MP001 Establishing variability in the toxins produced by Prymnesium parvum exposed to variable culturing and toxicity inducing conditions

A. Parsons-White, Marshall University; M.Y. Armstead, Marshall University / Integrated Science & Technology; M. Wilson, Marshall University

Despite fish kills worldwide, little is known about *Prymnesium parvum* or the triggers and mechanisms for release of secondary metabolites the algae produces. Variability in toxins produced is attributed to environmental and genetic variability, but the differences in clonal cultures are not characterized with respect to the cellular selection of toxin synthesis. The variance of a single organism must be evaluated with respect to toxins produced before inconsistencies can be attributed to genetic diversity. Toxins produced by a clonal culture under varying salt concentrations and exposed to different toxicity induction methods were characterized in this study. Culture media will have a conductivity of 2000µS/cm, an average conductivity in streams where toxic blooms occur. Toxicity was induced by pH, divalent ion concentration, and temperature change. Toxicity was confirmed using larval fathead minnow and sheep red blood cell bioassays. Toxins were analyzed using Liquid Chromatography Mass Spectrometry to quantify each of the molecules produced under the varying induction methods. Resulting spectra was compared to a control spectra obtained from un-manipulated cell culture. By including endo- and exotoxin measurements in the evaluations, this multi-factorial design can be expanded to benefit our knowledge of the organism's ability to vary toxin production under multiple environmental scenarios.

MP002 Evaluation of a novel control method for Prymnesium parvum and its produced toxins

M.Y. Armstead, Marshall University / Integrated Science & Technology; A. White, M. Wilson, Marshall University / Integrated Science and Technology

Golden algae, *Prymnesium parvum*, is a toxin producing Haptophyte that has caused massive fish kills around the world. Historically, *P. parvum* blooms have occurred in brackish waters but recent blooms have shown the euryhaline organism to be moving inland with the range of tolerable conditions still undefined. Blooms of the golden algae result in fish loss in both hatchery and sport fishery settings each year. Engineering and management control measures have been implemented to curb losses from the blooms including ultra violet radiation treatment in hatcheries and managing sport fishery stockings to reduce the spread of the organisms. Evaluation of a novel method of controlling the organisms is presented herein. With modification, this method, which employs USEPA approved methodology for controlling fecal coliform bacteria, could be implemented in a hatchery or field setting. Success of the treatment in killing the organism and sequestering the toxins will be discussed.

MP003 Tissue detection of Anatoxin-A as biomarkers of exposure for veterinary diagnostic applications

W.K. Rumbeiha, P. Imerman, S. Ensley, D. Snider, Iowa State University / VDPAM; D. Schrunk, Iowa State University

Harmful algal blooms (cyanobacteria) are increasing in intensity, frequency and severity all over the world. Cyanobacteria are bacteria with photosynthetic abilities and they produced a wide variety of environmental toxins which affect wildlife, animals, and humans. Among these are potent bicyclic alkaloids Anatoxin-A, Homo-, Dihydro-, and Epoxy-anatoxin-A. Anatoxin-A is at least 10 times more potent than nicotine in stimulating nicotinic acetylcholine receptors, causing a depolarizing blockade of neuromuscular junctions. These alkaloids are produced by a wide range of fresh water cyanobacteria, including Anabaena, Aphanizomenon, Cylindrospermopsis, and Oscillatoria. Anatoxin-A, formerly called "Fast Death Factor" causes acute neurological signs in animals including rigidity, muscle tremors, paralysis, and death from respiratory paralysis occurs within minutes to hours post-exposure. Diagnosis of cyanotoxin intoxications still heavily depends water analysis for algae and toxins. But this approach is insufficient in confirming animal intoxications particularly if toxins do not cause tissue morphological changes. Here we report results of assay development for biomarkers of exposure utilizing samples from a dog with a history of possible exposure to cyabobacteria. For analysis, 5 g of gastrointestinal contents were added to 10 mL of double distilled deionized water and subjected to 5 freeze/thaw cycles with 10 minutes sonication between cycles. The extract was diluted 1:1 with methanol and a fraction of the diluent was injected onto an LC/MS/MS. For the liver sample, 5 g was added to 2 mL of water and extract diluted 1:1 with methanol before injection onto an LC/MS/MS. All samples were analyzed in duplicates. Anatoxin-A concentration was 146 + 26 ppb in the stomach contents, and 0.8 ppb in the liver. Additional diagnostic workup will include identification and quantitation of Anatoxin-A congeners in biological samples and development of cheaper and rapid diagnostic tests for Anatoxin-A in biological matrices is ongoing.

Chemical Analysis for Pesticides and Their Metabolite or Degradation Products: New and Updated Methods

MP004 Laboratory and field evaluation of three organic pollutant sampling methods: passive samplers, water extractions, and live oyster deployment

K. Raub, University of Connecticut / Department of Marine Sciences; P. Vlahos, University of Connecticut / Departments of Marine Sciences and Chemistry

Three methods for the in situ detection of organic contaminants were evaluated. The accuracy and precision of each method was determined for their ability to detect aquatic pesticide concentrations in a controlled laboratory study and field trials in the Housatonic River. This study focuses on six legacy and currently used pesticides; atrazine, terbutryn (prebane), dieldrin, metolachlor, prometon, and diazinon. Water extractions were conducted using solid-phase extraction (SPE, 47mm ENVI™). Thin film equilibrium passive samplers based on an ethylene vinyl acetate film and live Eastern oysters (*Crassostrea viginica*) were used as proxies for alternative indicators of contaminant concentrations. A controlled laboratory study provided a comparison of sampling variables for each method by testing the extraction efficiency with known concentrations of each pesticide. Eastern oysters were held in spiked seawater for 4 hours and a decrease in particulate matter (as measured by Coulter Counter) was used to determine variance in filtration rate between individual oysters. The results of the laboratory study were used to evaluate the results from the field study in which water grab samples were taken along an estuarine transect and passive samplers were deployed alongside bags of Eastern oysters. The goal of this study is to determine advantages and limitations of equilibrium passive samplers as a practical alternative to cumbersome field extractions.

MP005 Distributions of Fipronil and Its Metabolites in San Francisco Estuary Sediments

D. Yee, San Francisco Estuary Institute; E. Agus, East Bay Municipal Utility District; M.D. Sedlak, San Francisco Estuary Institute

Fipronil is a slow-acting broad-spectrum fluorinated phenylpryazole insecticide used for a variety of applications, including structural pest control (e.g., for ants and termites) and topical pet pest treatments (for fleas and ticks), among other uses. With increased attention on the toxicity of pyrethroid insecticides on non-target organisms in the environment, there has been a gradual shift in the market to alternative insecticides such as fipronil. The Regional Monitoring Program for Water Quality in San Francisco Estuary has monitored a variety of pesticides in the region since 1993, including some fipronil and its metabolite compounds first in 2002-2003, and again since 2009. Sediment samples collected from locations distributed throughout the estuary were analyzed for fipronil, fipronil desulfinyl, fipronil sulfide, and fipronil sulfone. Fipronil was usually found in lower concentrations than its metabolites, with fipronil sulfone typically occurring at the highest concentrations in any given sample. In the earlier monitoring period, concentrations of these compounds were found at low concentrations, typically 0.06 ug/kg dry weight or lower. In the latter monitoring period, concentrations of all the fipronil compounds had increased, with maximum concentrations up to 0.56 ug/kg dry weight. These increases are in line with sales and use trends in California over the same period. Spatial distribution of fipronil and its metabolites also generally followed expected patterns, with the greatest concentrations found in Lower South Bay, which has relatively low flushing rates and accumulates fine sediments. There are not yet sediment toxicity thresholds for many estuarine species, but calculated porewater concentrations for the sites with the highest concentrations approached levels that may show effects in the most sensitive species. With monitoring of current-use pesticides as patterns in their usage change, attention can be directed to the environmental fate and impacts the most prevalent compounds, and management actions to reduce or control their usage can be taken, with benefits of those actions later observed.

MP006 Biotic and abiotic degradation of S-metolachlor and its commercial product Mercantor Gold® in aqueous media

L. Gutowski, Leuphana Universitat Luneburg; O. Olsson, K. Kuemmerer, Leuphana University Lüneburg

S-metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(1S)-2-methoxy-1-methylethyl] acetamide) is a selective chloroacetanilide herbicide intensively used for annual grassy weeds, corn, soybeans, peanuts, and other crops. In recent years more effective and risk-reduced S enantiomer has replaced the existing rac-metolachlor in the worldwide market since 1976. Mercantor Gold® is a mixture of S-metolachlor (86%) with mixture of aromatic hydrocarbons (14%). Once the compound is released into the environment it undergoes both biotic and non-biotic processes. As large amounts of pesticide are introduced to the environment, it is of great importance to investigate the biodegradation and photodegradation in the natural environment. The present study investigates the environmental pathways of S-Metolachlor and its commercial product Mercantor Gold® by Syngenta. Two tests of different bacterial density from OECD series were applied to test the biodegradation: the Closed Bottle Test (CBT, OECD 301D) and Manometric Respiratory Test (MRT, OECD 301 F). Photodegradation in aqueous media under simulated solar irradiation was performed using a UV/VIS xenon lamp (TXE 150, UV consulting Peschl, Mainz), which emits spectra similar to natural sun light (300-800nm). Samples after 8h photodegradation were administered the CBT and MRT biodegradation tests. The primary elimination of parent compound was monitored at 220 nm by high performance liquid chromatography (HPLC-UV). The formed transformation products (TP's) were analyzed by liquid chromatography coupled with multiple stage ion trap mass-spectrometry (LC-Ms). S-metolachlor was efficiently photo-degraded under laboratory conditions. Analyses by HPLC-UV showed a 69% elimination of pure compound and a 74% elimination of commercial product, both dissolved in water. Abundant photoproducts were detected by LC-Ms. No biodegradation has been observed for two compounds in the CBT and MRT tests as well as in the samples after photo-treatment. However, analyses through the MRT test revealed new TP's in the samples after photodegradation. This study obtained results which show that S-metolachlor as well as its commercial product Mercantor Gold® efficiently undergo abiotic primary elimination in aqueous media, however their photo-TP's are not biodegradable in performed tests and therefore might be persistent in the environment or exhibit further transformations.

MP007 Development of Multi-residue Method for 8 Benzoylurea Insecticides in Crops Using HPLC and LC-Tandem Mass Chromatography

J. Kim, Korea Institute of Toxicology / Analytical & Environmental Chemistry; J. Seo, J. Kim, Korea Institute of Toxicology; E. Kim, J. Kim, Seoul National University / Department of Agricultural Biotechnology

Multi-residue method of 8 benzoylurea insecticides (8BUs; diflubenzuron, triflubenzuron, hexaflumuron, teflubenzuron, novaluron, lufenuron, flufenoxuron and chlorfluazuron) in chinese cabbage, green pepper and bean were developed with high performance liquid chromatography and liquid chromatography-tandem mass spectrometry. Using standard solutions, extraction with acetone and partition system with *n*-hexane gave reasonable recovery of >90 %, and clean-up with florisil-glass column resulted in good efficiency of >94%. Recovery test of 8BUs in crop samples was conducted by employing the established method to give recovery of 80%-110%, and method limit of quantitation (MLOQ) was 0.02 mg/kg, respectively. Relative standard deviation (RSD) was low enough for each matrix to confirm the precision of analytical procedure at two different concentrations. Excellent sensitivity and selectivity were obtained with liquid chromatography-tandem mass spectrometry-ESI(-) even with the samples of no clean-up, suggesting liquid chromatography-tandem mass spectrometry can be used for the fast multi-residue method of 8BUs in chinese cabbage, green pepper and bean.

MP008 Aqueous Degradation of Pesticides with Contact Glow Discharge Electrolysis: Degradation Behavior and Kinetic

S. Hong, National Academy of Agricultural Science / Chemical Safety; C. Mok, Gachon University; H. Kwon, T. Kim, D. Kim, National Academy of Agricultural Science

Whether pesticides are degraded or not when glow discharge electrolysis is applied to the water where fruits and vegetables are being washed. The degradation of pesticides, fenothiocarb and imidacloprid in aqueous solution by Contact Glow Discharge Electrolysis (CGDE) was investigated. Pesticides spiked into distilled water were treated with CGDE. The results demonstrated that the aqueous degradation of fenothiocarb and imidacloprid by CGDE were 57.8% and 43.2%. The degradation of both pesticides was further enhanced at pH 3.0 using citric or hydrochloric acid. Both types of acid enhanced the degradation rate and amount. Fenothiocarb degradation was achieved to77.14% at pH 3 with citric acid and to 100% with HCl. Similarly, the degradation of imidacloprid was 70.18% and 93.02% with citric acid and HCl respectively. It seems that pH 3.0 condition is favorable for production of $^{\bullet}OH$ radicals or favorable for attacking organic compounds by $^{\bullet}OH$. However, the degradation rate and amount of both pesticides were significantly reduced at the presence of methanol. The decrease is that there is the competition between pesticides and MeOH for $^{\bullet}OH$. Because of non-selective behavior of $^{\bullet}OH$, the degradation of pesticides was not completely inhibited in the present experiment. The degradation of both pesticides by CGDE completely obeys first-order rate law with high regression coefficient values (R^2 > 0.99 in all cases).

Deepwater Horizon/MC 252 Well Incident Update

MP009 Combined effects of salinity, temperature and PAH exposure on the development of the early-life stages of the oyster Crassostrea virginica

N. Martin, Florida Gulf Coast University / Department of Marine and Ecological Sciences, College of Arts and Sciences; A. Volety, J. Vignier, Florida Gulf Coast University / Department of Marine and Ecological Sciences; J.M. Devine, Florida Gulf Coast University; E. Nickols, Florida Gulf Coast University / Department of Marine and Ecological Sciences, College of Arts and Sciences; L. Haynes, Florida Gulf Coast University; P. Soudant, University of Brest; F. Chu, Virginia Institute of Marine Science; J. Morris, M. Carney, J. Lipton, D. Cacela, M. Krasnec, Stratus Consulting Inc

The 2010 Deepwater Horizon Oil Spill released oil into the Gulf of Mexico. Many of the polycyclic aromatic hydrocarbons (PAHs) found in oil are toxic. The timing of the oil spill coincided with the spawning season of the Eastern oyster (*Crassostrea virginica*), an environmentally and commercially important shellfish species. Response actions during the spill event included major releases of freshwater from the Mississippi River diversion structures that caused salinities within the estuaries to be lower than average conditions at the critical spawning times. Therefore, the objective of this study was to examine the effects of multiple stressors – salinity, temperature and oil on the early life stages (gametes, embryos, and larvae) of *C. virginica.* We exposed early life stage oysters to varying temperatures (25° and 30 °C), salinities (5 and 25 ppt) and oil concentrations (High Energy Water Accommodated Fractions) using static exposure over four days. Results indicate fertilization success decreased, developmental abnormalities increased, and larval growth and survival decreased in a dose-dependent manner as a result of SPAH (sum of 50 analytes) exposure. Further, 5 ppt salinity negatively impacted gamete fertilization, embryo and larval development and survival compared to 25 ppt treatments. Results from individual and combined salinity, temperature, and PAH tests will be presented.

MP010 Sublethal effects of deepwater horizon oil on Crassostrea virginica larvae using dietary pathways

J. Vignier, R. Ketover, J. Devine, A. Rolton, A.K. Volety, Florida Gulf Coast University / Department of Marine and Ecological Sciences; P. Soudant, Universite de Bretagne Occidentale / LEMAR; F.E. Chu, Virginia Institute of Marine Sciences / Department of Environmental and Aquatic Animal Health; J.M. Morris, M.W. Carney, J. Lipton, D. Cacela, M. Krasnec, Stratus Consulting Inc.

The eastern oyster, *Crassostrea virginica*, is one of the most commercially and ecologically important shellfish species in coastal region of the northern Gulf of Mexico. The long planktonic nature (14 to 21 days) of oyster larvae and their micro algal diet make them vulnerable to acute exposure to contaminants bound to suspended sediment, adsorbed onto algal and other particles, and in solution. Following the explosion of the Deepwater Horizon (DWH) oil platform off the coast of Louisiana on the 20th April 2010, crude oil was spilled in the Gulf of Mexico coincident with the spawning season of oysters. PAHs and oil droplets, based on their lipophilic properties, can adsorb onto phytoplankton and be ingested by *C. virginica* larvae. We simulated this exposure pathway in the laboratory by exposing oyster D-larvae to solutions of a commercial species of microalgae, *Isochrysis galbana*, that were gently stirred with four different concentrations of DWH oil. Oysters were continuously exposed to oil adsorbed onto algae cells for 14 days,

in a static renewal system. Growth, feeding behavior and mortality were measured through the exposure, and PAH content in the larval tissue as well as survival were assessed at the end of the experiment. GC-MS/MS analysis of the larval tissue demonstrated that larvae accumulated PAHs by ingestion of oil droplets and contaminated algae. Survival and growth were adversely affected with PAH exposure in a dose-dependent manner.

MP011 Correlating OPAH concentrations with zebrafish toxicity of Gulf of Mexico samples around the Deepwater Horizon oil spill: a bottom-up approach

C.E. Donald, S.G. O'Connell, Oregon State University / Environmental and Molecular Toxicology; R.L. Tanguay, K.A. Anderson, Oregon State University / Environmental and Molecular Toxicology

The 2010 Deepwater Horizon oil spill introduced a wide range of bioavailable contaminants in the Gulf of Mexico, including PAHs and OPAHs. Previous research suggests that PAHs are not the primary driver of toxicity in some contaminated sites. We observed unique OPAH signatures at each of four sites in the Gulf of Mexico during and after shoreline oiling, with concentrations of OPAHs in passive sampling device extracts varying up to 100-fold across sites and sampling locations. By utilizing an *in vivo* developmental model with embryonic zebrafish (*Danio rerio*), we will simulate exposures OPAH concentrations detected in near-shore water in each Louisiana, Mississippi, Alabama, and Florida during the oil spill and in the following year. This bottom-up approach will provide potential insight into specific drivers of toxicity and will be useful in developing a model to predict toxicity in resident organisms with passive sampling devices.

MP012 Transgenerational Phototoxicity in Fiddler Crabs Exposed to Deepwater Horizon Oil

L. Taylor; M.M. Alloy, University of North Texas / Department of Biological Sciences; I. Palmer, J.A. Stoeckel, Auburn University; H. Pace, Stratus Consulting / grad student; J.M. Morris, Stratus Consulting; J. Lipton, Stratus Consulting, Inc.; A.P. Roberts, University of North Texas

The 2010 Deepwater Horizon (DWH) oil spill resulted in a large release of polycyclic aromatic hydrocarbons (PAHs) into the Gulf of Mexico. PAHs exert a variety of toxic effects on aquatic organisms with early lifestages being particularly sensitive. The goal of this research was to expose reproductively active adult fiddler crabs (*Uca longisignalis*) to oiled sediments and examine the effects on adult reproduction and survival of offspring. Adult crabs were collected from a reference location and transported to Auburn University where they were exposed to sediments spiked with one of four levels of DWH oil. Crabs were allowed to burrow and breed in the contaminated sediments for up to 10 days. Gravid females were moved to clean water AHAB rearing systems and larvae collected following hatch. Larval production and survival were monitored. A subset of larvae were also used in UV phototoxicity experiments in the absence of any additional PAH exposure. Adult reproduction was not significantly reduced by PAH exposure at the tested concentrations. Survival of larvae post-hatch that were not exposed to UV was also not reduced significantly. However, when exposed to UV in clean water, larval mortality increased significantly in a manner positively correlated with the sediment PAH concentration to which they were exposed as embryos. These data demonstrate the importance of also considering combined effects of non-chemical and chemical stressors in ecological risk assessment and the potential for trans-generational effects after exposure to PAHs.

MP013 Photoenhanced PAH Toxicity of Deepwater Horizon Oil to Early Lifestage Oyster and Mahi-mahi

M.M. Alloy, University of North Texas / Department of Biological Sciences; T. Curran, University of North Texas; J.T. Oris, Miami University / Department of Biology; M. Grosell, University of Miami / Division of Marine Biology and Fisheries; A. Volety, Florida Gulf Coast University / Department of Marine and Ecological Sciences; A.P. Roberts, University of North Texas

The 2010 Deepwater Horizon Oil Spill resulted in a large release of polycyclic aromatic hydrocarbons (PAH) into the Gulf of Mexico. Photoenhanced toxicity is a well described phenomenon in which molecules of certain PAHs interact synergistically with ultraviolet radiation (UV). This interaction generates reactive oxygen species resulting in oxidative stress and acute toxicity. Translucent, early lifestages of aquatic organisms are generally more susceptible to photoenhanced PAH toxicity than juvenile and adult stages. The purpose of this study was to assess the sensitivity of early lifestages (gametes, embryos, larvae) of a Gulf of Mexico invertebrate (eastern oyster; *Crassostrea virginica*) and vertebrate (mahi-mahi; *Coryphaena hippurus*) to photoenhanced PAH toxicity. Exposure media consisted of PAHs in water accommodated fractions (WAF) prepared using slick oil mixed with a high energy method (HEWAF) or chemical dispersants (CEWAF). Variable UV intensities were achieved using an outdoor microcosm exposure system to simulate high UV intensity light conditions and an UV filter plastic/neutral density filters to achieve low UV intensity light conditions. Phototoxic effects on fertilization, survival, and hatching were observed in both a UV and PAH concentration-dependent manner.

MP014 Polycyclic aromatic hydrocarbon biomarkers suggest continued exposure in sharks after the Deepwater Horizon Oil Spill

A.E. Leary, J. Gelsleichter, University of North Florida; D. Grubbs, Florida State University Marine and Coastal Lab

The Deepwater Horizon Oil Spill (DHOS) released large quantities of liquid petroleum into the Gulf of Mexico. This is the largest spill in US history and the depth that it occurred creates a unique yet challenging research opportunity. It is vital to determine the effects on Gulf wildlife from oil-related pollutants, particularly the polycyclic aromatic hydrocarbons (PAHs), which are the most toxic components of oil. PAHs are readily metabolism once an organism is exposed; therefore biomarkers for PAHs have been used to determine degree of exposure. This study evaluated PAH biomarkers such as Phase I and II detoxification enzymes, which create a more water soluble metabolites for excretion; these metabolites were also measured. Sharks were collected from oil impacted areas in the Gulf of Mexico from 2011-2013. The specific enzymes that were measured in liver were cytochrome P450a1a (Cpy1a1) and glutathione-S-transferase. Additionally petrogenic fluorescent aromatic compounds (FACs) were measured in bile. Thus far results suggest continued exposure with several species indicating significant increases in GST activity and FAC concentration from 2011 to 2012. This increase likely represents the redistribution of PAHs settle in sediment.

Great Lakes Binational Monitoring and Surveillance of Critical Substances and Priority Toxics

MP015 Monitoring of legacy and emerging organic contaminants in tributaries of the Great Lakes

D.A. Alvarez, USGS-CERC, USGS Columbia Environmental Research Center; S. Perkins, USGS Columbia Environmental Research Center; E.T. Furlong, US Geological Survey / National Water Quality Laboratory; D. Sullivan, USGS Wisconsin Water Science Center

In recent years, the emphasis on monitoring has begun to shift from so-called legacy pollutants to a wide array of new chemicals being discovered in the environment that is often lumped collectively into a group referred to as "contaminants of emerging concern". While it has been known for several decades that compounds such as pesticides, detergents, personal care products, and pharmaceuticals enter the environment, improvements in the instrumentation and analytical methodology for detecting chemical substances in various environmental media (air, water, sediment, biota) have brought increased awareness and concern over the presence and potential risk that these chemicals may pose. In the drainage basin for the Great Lakes, large areas of inland forests and wetlands that once served to regulate the quantity and quality of water flowing into tributaries have been lost due to urbanization. As a result, tributaries pass on their pollutant loads from agriculture, industry, and urban development directly to the lakes. In the fall of 2010, passive sampling devices were deployed at 58 sites in tributaries along the US side of the Great Lakes to provide information on the occurrence and distribution of the CECs and legacy contaminants. The passive samplers used were the semipermeable membrane device (SPMD) for hydrophobic organics and the polar organic chemical integrative sampler (POCIS) for the hydrophilic organics. Representative chemicals or chemical classes detected at greater than 20% of the sites include: atrazine, DEET, fragrances, phosphate-based flame retardants, PAHs, PCBs, chlordanes, endosulfan, and PBDE-47. The estimated time weighted average water concentrations for many of these chemicals ranged from low nanogram per liter to low microgram per liter levels. In addition to the chemical analyses, POCIS extracts were screened to the presence of estrogenic chemicals using the Yeast Estrogen Screen. This screening revealed that 80% of the field sites contained chemicals which were weakly estrogenic.

MP016 PAHs, Nitro-PAHs and Petroleum Biomarkers in Sediments of the Southern Lake Michigan

L. Huang, S. Batterman, S. Chernyak, University of Michigan

Polycyclic aromatic hydrocarbons (PAHs) in the Great Lakes basin are of concern due to their significant atmospheric deposition and persistence in bottom sediments. The nitro derivatives of PAHs, nitro-PAHs (NPAHs), which can have stronger carcinogenic and mutagenic activity than parent PAHs, may follow similar transport routes and be accumulated in the sediments. However, the current distribution of these contaminants in Great Lakes sediments is not well known. This study characterizes PAHs and NPAHs, as well as petroleum biomarkers (hopanes and steranes), in sediments collected at 24 offshore sites in southern Lake Michigan. The Σ_{15}PAH concentrations varied from 213 to 1291 ng/g dry weight, 2 to 10 times lower than levels reported during 1980s and 1990s. The Σ_{11}NPAH concentrations ranged from 2.9 to 18.6 ng/g dry weight, and included several carcinogenic compounds (1-nitropyrene and 6-nitrochrysene); earlier NPAH measurements are unavailable. Σ_5Hopane and Σ_6Sterane concentrations ranged from 97.7 to 355 ng/g and 6.2 to 36 ng/g dry weight, respectively. SVOC concentrations increased with elevated organic carbon content, and two sites consistently showed high levels of all target SVOCs. The relative abundances of PAHs, NPAHs, hopanes and steranes were similar across sites. Based on the measured compositions, the major contributors of PAHs and NPAHs in sediments are combustion sources, dominated by coal, wood and biomass, while the petroleum biomarkers indicate both petrogenic and biogenic sources. We believe that this is the first report of NPAHs levels in sediments in the Great Lakes. The detection of carcinogenic NPAH compounds at relatively high concentrations indicates potential risks to aquatic organisms, and a need for further assessment.

MP017 Forensic Discoveries, Trends and Sources of Targeted Emerged, Emerging and/or Re-merging Halogenated Substances in Great Lakes Herring Gulls

R.J. Letcher, Environment Canada / Science and Technology Branch, Ecotoxicology and Wildlife Health Division

The Great Lakes Herring Gull Monitoring Program (GLHGMP) has been ongoing in Canada for decades with annually collected eggs, and in some cases tissues, of herring gulls (*Larus argentatus*) from fifteen Canadian and US colony sites across the Great Lakes. Targeted analytical screening using archived and recently collected samples of this bio-sentinel continues to be ongoing for priority and previously unknown, bioaccumulative halogenated substances. The present study reports on recent and new forensic discoveries of chlorinated, brominated and fluorinated contaminants, and in cases where egg levels are in the parts-per-billion range or higher, the comparative examination of their retrospective spatiotemporal trends, (aquatic and terrestrial) ecosystem bioaccumulation, sources and fate. Examples of priority and new targeted substances that have been screened for and present in herring gull eggs and/or tissue include flame retardants (FRs) such as organophosphates, and larger and more heavily brominated FRs resembling decabromodiphenyl ether (BDE-209). Also, brominated substances that are not FRs but rather degradation products of replacement brominated FRs. New fluorinated substances are also reported including mono- and di-perfluoroalkyl phosphates (PAPs) or varying fluoroalkyl chain lengths, which are known sources of fluorotelomer alcohols that in turn are precursors for bioaccumulative perfluorinated carboxylic acids. Understanding the complexity of source origins of these new substances, and the ecosystem and dietary accumulation and fate pathways to herring gulls and their eggs, are also discussed by examination of, e.g., retrospective spatiotemporal trends, comparison to dietary chemical tracers such as stable isotopes of carbon and nitrogen, and gull tissue distribution and metabolism.

MP018 Air-water exchange of legacy PBTs in the Great Lakes

R. Lohmann, University of Rhode Island / Graduate School of Oceanography; Z. Ruge, URI Graduate School of Oceanography; M. Khairy, University of Rhode Island; P.A. Helm, Ontario Ministry of the Environment / Environmental Monitoring and Reporting Branch; D.C. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division

Passive polyethylene samplers were deployed from April – October 2011 in lower atmosphere and surface water of Lakes Erie, Ontario and Superior to (i) enhance the measurements of the spatial variability of atmospheric concentrations of persistent bioaccumulative toxics (PBTs) around the Lake; (ii) assess whether the lakes are volatilizing or absorbing gas-phase PBTs to derive fluxes and loading to the lakes; and (iii) detect emerging contaminants of concern. Initially, we focused on polycyclic aromatic hydrocarbons and polychlorinated biphenyls. A total of ~125 samplers were deployed along the US and Canadian shore and in-lake in paired air-water deployments. Strong west-to-east gradients were observed for PAHs and PCBs, wich eastern sites cleaner in Lake Ontario than in Lake Erie. By using the same sampling matrix (in our case polyethylene, PE) in air and water, activity gradients across the air-water interface were derived. Air-water exchange ratios indicated heterogeneous results across Lake Ontario, but favored net volatilization for most OCPs and PCBs in Lakes Erie and Superior. Results were more varied for PAHs.

MP019 Screening Great Lakes Trout for emerging contaminants using Atmospheric Pressure Gas Chromatography Quadrupole Mass Spectrometry

B. Crimmins, X. Xia, D. Delach, Clarkson University; M. Milligan, SUNY Fredonia; J. Pagano, SUNY Oswego; P. Hopke, T. Holsen, Clarkson University

Recent advances in gas chromatography and mass spectrometry have provided powerful tools for the monitoring and discovery of chemicals of concern in the Great Lakes. One advanced technique is Atmospheric Pressure Gas Chromatography Quadrupole Time of Flight mass spectrometry (APGC-QToF). Unlike conventional GC-MS ionization sources, the APGC charge transfer process is performed by N2 plasma at atmospheric pressure. The softer ionization lowers fragmentation and increases the molecular ion abundance. At >10,000 resolution and the collection of a full mass range (< 1000 m/z), the QToF provides accurate mass measurements of targeted species and searchable spectra to be mined in the future for new compounds of interest. This technique is currently being evaluated as a confirmation and screening tool for trout in the Great Lakes. Preliminary results suggest fg sensitivity for halogenated species and the use this technique as a comprehensive, targeted and non-targeted, screening tool for semi-volatile compounds. A complete evaluation of this technique will be presented including; analysis in biological matrices, matrix affects, linearity and the use of this instrument as confirmation tool for comprehensive gas chromatography (GCxGC) experiments.

MP020 Trends of POPs in Plasma of Nestling Bald Eagles from the Great Lakes

W.W. Bowerman, University of Maryland / Department of Environmental Science & Technology; R.R. Yarwood, L. Fuentes, University of Maryland / Environmental Science and Technology; H.T. Pittman, University of Arkansas / Arkansas Fish & Wildlife Research Coop Unit; K.L. Simon, University of Maryland / Department of Environmental Science & Technology

The apex predator of the aquatic ecosystem of the Great Lakes is the bald eagle (*Haliaeetus leucocephalus*). Tissues from dead eagles, unhatched eggs, and blood plasma from individuals, have been analyzed since the 1960s for compounds that biomagnify to monitor spatial and temporal trends. The eagle is one of the most studied bird species in North America. The effects of many stressors have been determined for reproduction, developmental deformities, and other biological endpoints. Many monitoring programs across the Great Lakes have measured these compounds in tissues of bald eagles. We will focus primarily on comparisons of blood plasma concentrations for the periods 1986-1991, and 1999-2008, focusing on areas sampled under the Michigan Biosentinel Program and at Voyageurs National Park. The trends for POPs have shown decreases in concentrations of some chemicals including p,p'-DDE and PCBs, and have recently shown an increase in dieldrin at Voyageurs National Park. The background rate of developmental deformities encountered in hatched young is 1 in 20,000 hatchlings. The incidence rate of these deformities is another causal relationship that is associated with exposure to environmental pollutants. The spatial and temporal trends in these deformities, adjusted for sampling effort will also be presented. Deformities in wildlife is one of the Beneficial Use Impairments under the Great Lakes Water Quality Agreement for Areas of Concern. The International Joint Commission, under the new Great Lakes Water Quality Agreement, is considering apex indicators to measure progress under the agreement. One of the 16 indicators recently selected is "fish-eating birds", specifically, the herring gull (*Larus argentatus*) and the bald eagle. While the populations of bald eagles has continued to grow in the basin, recent analysis of shoreline nests has shown "sink-source" dynamics. Because of the known causal relationships to effects on reproduction, the eagle is a good indicator of the chemical, physical and biological health of the Great Lakes.

MP021 Passive Sampling and High Resolution Screening Techniques for Monitoring and Surveillance of Contaminants in Nearshore Waters of the Great Lakes

P.A. Helm, Ontario Ministry of the Environment / Environmental Monitoring and Reporting Branch; A. Muscalu, Ontario Ministry of the Environment; M. Robson, Brock University; E. Reiner, Ontario Ministry of the Environment; L. Shen, Brock University; K. Jobst, T. Kolic, A. Tencic, A. Boden, K. MacPherson, D. Morse, Ontario Ministry of the Environment; C. Marvin, Environment Canada; I. Brindle, Brock University

The quality of nearshore waters of the Great Lakes are strongly influenced by inputs from adjacent watersheds, agricultural runoff, urban stormwater, and discharges of treated municipal wastewater. Accompanying these inputs are a wide variety of environmental chemical contaminants arising from industrial, commercial, and consumer use of chemicals in the marketplace, legacy contaminants from previous industrial and agricultural use, by-products of combustion and degradation of current-use and legacy chemicals within the environment. Tracking the presence, sources, and fate of such a complex mix of chemical contaminants in the Great Lakes represents a formidable challenge. Of the approaches to address this challenge, passive sampling combined with targeted and non-targeted analytical approaches offer efficient and effective means of conducting monitoring and surveillance for aquatic contaminants across the Great Lakes basin. This presentation highlights results of monitoring activities using polyethylene passive samplers in urban impacted nearshore waters for traditional persistent, bioaccumulative, toxic compounds such as the PCBs, OC pesticides, PAHs, and PBDEs. Polyethylene (PE) samplers were deployed in selected rivers and streams and nearshore waters in the Lake Ontario and Lake Erie/Lake St. Clair basins, and in nearshore Lake Superior waters, including several sites influenced by municipal wastewater treatment plant discharges. Concentrations of PCBs, PAHs, and PBDEs were generally greater in watersheds and nearshore areas more strongly influenced by urban and industrial development. Multi-dimensional gas chromatography (GCxGC)-electron capture detection (ECD) was used as a non-targeted analytical screening tool for additional halogenated compounds. The GCxGC screening indicated the presence of a considerable number of compound classes at several stations, including chlorinated paraffins, chlorinated dibenzo-p-dioxins and dibenzofurans, halogenated PAHs, and several unknown halogenated substances. The greatest concentrations, and number and diversity of substances, were observed in PE samplers deployed at locations more influenced by municipal wastewater and urban/industrial development. Monitoring and surveillance in the aquatic environment using passive sampling and analytical screening techniques provided a more comprehensive picture of contamination by hydrophobic substances in the Great Lakes basin than historical monitoring approaches.

Use of Freshwater Mollusk Toxicity Data for Improved Conservation of Water and Sediment Quality

MP022 Development of a standard protocol for using Lymnea stagnalis eggs for the assessment of mixture toxicity in freshwater molluscs

K.L. Pedersen, H. Holbech, University of Southern Denmark / Department of Biology; L. Nielsen, University of Southern Denmark / Institute of Biology; P. Bjerregaard, University of Southern Denmark / Department of Biology

Synergistic effects of chemicals has been observed when a compound (called a chemosensitizer) inhibits the function of multidrug resistance pumps (MXR). As a consequence other compounds present may no longer be pumped out and may reach toxic levels. Systems for analysing pump inhibition has prevously been developed using fluorescense measurements in sea urching eggs challenged with combinations of chemicals. In search for a model system for freshwater molluscs we have developed a protocol for eggs from a laboratory culture of *Lymnea stagnalis* grown under an OECD standard protocol. The exposed eggs were kept separate in the wells of a 384 well microtiter plate and the pump activity was followed on a microtiter fluorescense plate reader. Two fluorescent compounds rhodamin B and calcein-AM were compared as markers of pump function and age of the eggs, exposure periods and various physical-chemical parameters were optimized. The system has many advantages such as easy access to genetically similar eggs, the possibility of handling many replicates and exposure groups simultaneously and the short exposure and measurement periods involved in the assay. As might be expected given the long evolutionary distance between sea urchins and gastropods differences have been observed; i.e. with triclosan which had a much lesser inhibitory effect on snail eggs than on sea urchin eggs. However, the inhibition of the pumps by the standard inhibitor verapamil confirmed that abc pumps are also active in snail eggs. Further, compounds like organic and inorganic mercury displayed the same stimulation of pump activity as previously seen in other species. The viability of the translucent *Lymnea* embryos can easily be observed visually using a video camera and the format therefore offers the possibility to relate measurements of pump activity directly to studies of changes in acute toxicity of mixtures.

MP023 Effects of glochidia age on metamorphosis and juvenile condition in freshwater mussels

A. Maynard, C. Barnhart, Missouri State University / Biology

The brooding period of glochidia of *Lampsilis* can be extended to well over a year by holding females at low temperature (10 °C), providing great flexibility in timing of captive propagation. An important question is whether aging of the glochidia affects the quality of the juveniles that develop from those larvae. Previous studies indicate that immature glochidia yield poor juvenile survival. We compared juveniles of fatmucket (*Lampsilis siliquoidea*) derived from mature glochidia differing in age by 1 year. We measured larval viability, attachment success, and metamorphosis success on host fish (*Micropterus salmoides*). We then observed the time to 50% mortality (LT50) of the newly metamorphosed juveniles during starvation as a measure of their condition. Two experiments were performed, each with 3 young (4 or 6 mo) and 3 old (16 or 18 mo) broods. In experiment #1 the juveniles were tested in water only. In experiment #2 they were tested in water and in 6 concentrations of NaCl as a stressor. In both experiments, young and old viable larvae were equally able to attach to the host, but old larvae were less likely to successfully metamorphose. In experiment #1, LT50 of starved juveniles was similar at 26 days for juveniles from both 6-mo and 18-mo old larvae. In experiment #2, LT50 in water only was 26 and 24 days for juveniles from 4-mo old and 16-mo old larvae, respectively. The 21-day LC50 of NaCl was 5.5 g/L for juveniles from 4-mo old larvae and 4.5 g/L for juveniles from 16-mo old larvae. These differences were statistically significant. Interestingly, NaCl lengthened juvenile survival in starvation at concentrations less than 4.5 g/L and shortened survival at higher levels. These data suggest that aging of *Lampsilis* glochidia over 1 year can affect juvenile quality for propagation and toxicity testing.

MP024 Effects of larval age and duration of parasitism on the sensitivity of newly metamorphosed freshwater mussels to NaCl

G. Bolton, A. Linan, C. Barnhart, Missouri State University / Biology

The use of captive-cultured freshwater mussels in toxicology is increasing, and it is important to identify factors that might affect the quality of test organisms. Mussel larvae (glochidia) are parasites that attach to a host fish and metamorphose into the juvenile stage, which is generally preferred for toxicology. We are studying the effects of the age of the glochidia and the duration of their parasitism on the quality of the resulting juveniles. Female mussels brood the fertilized eggs within their marsupial gills. In many species the brooded glochidia normally overwinter and can remain infective for over one year if the female is kept at low temperature. The duration of parasitism is temperature-dependent. We tested glochidia of fatmucket, *Lampsilis siliquoidea,* that had been brooded for 4 months (=young) or 16 months (=old). The glochidia were placed on host fish (largemouth bass) and the period of parasitism was varied by placing the fish at 10C for 0, 2, or 4 weeks before warming them to 23C so that development could continue. The mean durations of parasitism that were obtained were 14, 28 and 42 days. The newly metamorphosed juveniles were exposed for 24 hours to 7 concentrations of NaCl and control in reconstituted water at 20C. After exposure the mussels were returned to water without added NaCl and mortality was assessed 24 hours later. Juveniles derived from young larvae tolerated higher salt concentrations than those derived from old larvae. Salt tolerance also increased with longer duration of parasitism, and the difference in salt tolerance between juveniles from old and young larvae was reduced in juveniles that spent more time on the host. For juveniles with parasitic period of 14, 28, and 42 days, mean survival in 7.5 g/L NaCl was 20%, 33%, and 53% for juveniles from young larvae, versus 0%, 20%, and 60% for juveniles from old larvae. Control survival was 100% for all groups. These results suggest that both the age of mussel larvae and the duration of the parasitic period can affect juvenile quality for toxicology.

MP025 The influence of culturing conditions on the performance and sensitivity of the freshwater mollusc Sphaerium corneum in sediment toxicity tests

L.T. Nguyen, Ghent University / Applied Ecology and Environmental Biology; M.B. Vandegehuchte, Ghent University / Applied Ecology & Environment Bio; M. Vangheluwe, ARCHE; L. Bervoets, University of Antwerp / Systemic Physiological and Ecotoxicological Research; E.R. Garman, NiPERA / Ecotoxicologist; C.E. Schlekat, NiPERA; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology

The European freshwater mollusc *Sphaerium corneum* (fingernail clam) lives in the sediment and feeds on organic matter at the sediment surface as well as on particles filtered from the water column. This species has a potential to be used for sediment toxicity tests, which would add bivalves as a new taxonomic group in sediment tests for risk assessment. Yet, knowledge on maintenance conditions to obtain healthy organisms for toxicity testing is lacking. In the present study, the impact of laboratory culture conditions (i.e., substrate and food types) on the performance of *S. corneum* and its sensitivity to Ni was investigated. A set of four culture conditions differing in food types (i.e., yeast – *Urtica*- trout chow, YUT; and YUT together with TetraMin; YUT+TM) and in the presence or absence of sediment, were offered in a 2x2 design to molluscs of 3.01 ± 0.21 mm initial length (mean ± SE). Two culture conditions were also tested for larger clams (5.69 ± 0.06 mm initial length). After one month, survival, growth and reproduction were determined. The results show that for the small size molluscs, survival (71.2 ± 7.8% to 84.6 ± 6.1%) was not affected by the culture conditions. Two-way ANOVA revealed a significant interaction effect of food type and substrate on growth. No reproduction was noted for the molluscs of this size. For the larger clams, survival (86.8 ± 5.7% to 94.1 ±3.2%) and growth (0.34 ± 0.08 mm per organism to 0.41 ± 0.12 mm per organism) were not affected by the culture conditions. Reproduction of molluscs cultured in the sediment and fed YUT, however, was significantly higher (1.3 ± 0.18 larvae per organism) than that of the molluscs cultured in water only and fed YUT+TM (0.76 ± 0.16 larvae per organism). Our study demonstrates that the performance of *S. corneum* was influenced by the culture conditions. Culture system with sediment as bottom substrate and YUT as food appeared to be favourable for the fitness of the molluscs. To examine the influence of culture conditions on *S. corneum's* sensitivity to toxicants, molluscs originating from each of the cultures were simultaneously exposed for 5 days to one sediment type spiked with different concentrations of Ni. The results show a range of LC50 (95% confidence limits) from 811 (27-24,343) mg Ni/kg dry wt. to 1,300 (610-2,772) mg Ni/kg dry wt.

MP026 Statistical refinements for data analysis of mollusc reproduction tests: an example with Lymnaea stagnalis

S. Charles, University Lyon 1 / Laboratory of Biometry and Evolutive Biology; V. Ducrot, INRA / Ecotoxicology and Quality of Aquatic Ecosystems; H. Holbech, University of Southern Denmark / Department of Biology; T. Hutchinson, School of Biological Sciences, Plymouth University; L.L. Lagadic, INRA / Ecotox & Quality of Aquatic Environ; J. Oehlmann, Johann Wolfgang Goethe-Universitat Frankfurt am / Aquatic Ecotoxicology; L. Weltje, BASF SE / Agricultural Centre; M. Delignette-Muller, VetAgro Sup

Since 2012 European experts work towards the development and validation of an OECD test guideline for mollusc reproductive toxicity with the freshwater gastropod *Lymnaea stagnalis*. A ring-test involving six laboratories allowed studying reproducibility of results, based on survival and reproduction data of snails monitored over 56 days exposure to cadmium. A classical statistical analysis of data was initially conducted by hypothesis tests and fit of parametric concentration-response models. However, as mortality occurred in exposed snails, these analyses require to be refined, particularly in avoiding bias that exists when the number of clutches/eggs is analysed without accounting for mortality, or when replicates where mortality occurred are excluded; in the latter case, a number of organisms are discarded and valuable data can be lost. In this context, the purpose of our statistical study was twofold. First, we refined the statistical analyses of reproduction data accounting for mortality all along the test period. The variable number of clutches/eggs produced per individual-day was used for EC_x modelling, as classically done in epidemiology in order to account for the time-contribution of each individual to the measured response. Furthermore, the combination of a Gamma-Poisson stochastic part with a Weibull concentration-response model allowed accounting for the inter-replicate variability. Second, we checked for the possibility of optimizing the initial experimental design through the reduction of exposure duration and/or number of replicates. Based on the six datasets we show that using the per individual-day unit in ecotoxicology avoids the exclusion of data (as a consequence the ECx could be not estimated from remaining data) and ensures an unbiased reproduction data analysis when mortality occurs in exposed animals. We also show that the experimental design may be optimized, depending on what should be prioritized. Even if further studies would be necessary with other kinds of compounds, we illustrate the fact that, in the case of cadmium, and if 6 replicates are kept, a 35-day exposure duration would be sufficient to characterize the toxicity. In the same way, 3-4 replicates appear sufficient if the exposure duration stays at 56 days. However, before the reproduction test with *L. stagnalis* can be standardized, other works are necessary to furtherrefine the experimental design, e.g., by regarding exposure duration and replication simultaneously.

MP027 Evaluation of yolk protein as biomarker for endocrine disruption in molluscs

J.E. Morthorst, H. Holbech, K.L. Pedersen, K.L. Kinnberg, P. Bjerregaard, University of Southern Denmark / Department of Biology

During recent years invertebrates and especially molluscs have received increasing attention in the field of endocrine disruption and development of OECD test guidelines to assess the effects of endocrine disrupting compounds (EDCs) in molluscs is under development. The development of standardized tests to detect effects of EDCs in molluscs has proved cumbersome due to lack of specific biomarkers and endpoints for endocrine effects. Intersex (presence of oocytes in the testis) and induction of vitellogenin (the yolk protein precursor in oviparous vertebrates) have been used as biomarkers for EDCs in fish for decades. Vitellogenin (vtg) is mainly present in females however vtg synthesis can be induced by estrogens and EDCs in juveniles and males. During the last decade yolk protein has been used as biomarker in bivalve studies and alkali-labile phosphate (ALP) has been the applied method to indirectly estimate vtg levels. ALP was developed as an indirect method for determination of vtg in fish before more reliable and specific methods like ELISA (Enzyme-Linked Immuno Sorbent Assay) were developed. The use of yolk protein as biomarker in molluscs is based on the assumptions that vtg synthesis is also regulated by estrogens in molluscs even though it still remains unknown if and where vertebrate steroids are synthesized in molluscs and regulation of the endocrine system in molluscs is also unknown. By using our newly developed ELISA the present work investigates if yolk protein is a suitable biomarker for estrogenic exposure in molluscs by exposing the freshwater bivalve *U. tumidus* to 17β-estradiol (E2) (57, 164 and 512 ng/L) for eight weeks during their reproductive phase. Histological examination of the gonads revealed that E2 did not cause intersex and the ELISA revealed that the normal sex specific concentration distribution seen in fish was not seen in hemolymph of unexposed *U. tumidus*. The concentration of the protein did not differ among the sexes and was approximately 10000 times higher in male *U. tumidus* than in male fish. The results from the ELISA and ALP showed good correlation and the sites of yolk protein synthesis were investigated by immunohistochemistry. Based on the results we do not support the general use of yolk protein as biomarker for estrogenic exposure in bivalves because the yolk protein levels in unexposed males are high suggesting that yolk protein might have additional functions in *U. tumidus*.

MP028 The invasive Asian clam (Corbicula fluminea) as a bioindicator of water quality

S. Flower, University of Tennessee / Geology; M. McKinney, Geology

The Asian clam, or *Corbicula fluminea*, was introduced into the United States in the early 1900's and has since spread widely throughout much of North America. As this invasive species is abundant in many types of habitat, ranging from pristine to very polluted water bodies, it is increasingly used as way to monitor human impacts on water quality. To examine water quality in urban streams, we collected clams in streams within and around Knoxville, Tennessee. These streams vary significantly in water quality and therefore provide a way to examine the effectiveness of these clams as bioindicators. Stream water quality was measured at the time of sampling by recording the conductivity, pH, total dissolved solids, salinity, and water temperature. Live clams were briefly stored in a cooler with water and an aerator. The height, length, and width of the live specimens were measured to determine any differences in morphology between populations. The samples were cooked for 3 minutes in boiling water within two days of sampling. The visceral tissue was then placed in a convection oven for 5 days to

remove excess moisture and shells were left out to air dry. The weight of the shell and cooked tissue was then measured and the Mussel Condition Index (MCI) was determined through a ratio of cooked meat weight to the added weight of the cooked meat and shell weight. Finally, soft tissue and shell material were analyzed with an ICP-OES to determine heavy metal content. Our results support the use of these clams as bioindicators in several ways. The MCI shows statistically significant differences ($p < 0.05$) among streams with populations in more polluted streams having a reduced MCI. Specifically, increased conductivity correlated to a decrease in visceral mass relative to shell weight. Regarding morphology, there was also a correlation between less pollution and more elongated shells. The cause of this pattern is still unclear. Finally, we found several heavy metal trends that indicate significant differences among sites, almost certainly related to variations in anthropogenic activities among the streams.

MP030 Acute sensitivity of freshwater mollusks and commonly tested invertebrates to select chemicals with different toxic modes of action

N. Wang, USGS / Columbia Environmental Research Center; C.G. Ingersoll, US Geological Survey / Columbia Environmental Research Center; C.D. Ivey, USGS – CERC / Columbia Environmental Research Center / Toxicology; B.G. Brumbaugh, US Geological Survey / Columbia Environmental Research Center; J.M. Besser, USGS- Biological Resources Division / Columbia Environmental Research Center; E. Hammer, C. Bauer, USEPA; T. Augspurger, US Fish and Wildlife Service / Ecological Services; S. Raimondo, USEPA; B.K. Shephard, USEPA / Office of Env. Assessment (OEA-095); J.E. Bartoszek, US Fish and Wildlife Service / Division of Environmental Assessment and Restoration; C. Barnhart, Missouri State University; N. Eckert, USFWS

Previous studies indicate that freshwater mollusks are more sensitive than commonly tested organisms to some chemicals, such as copper and ammonia. Nevertheless, mollusks are generally under-represented in toxicity databases. Studies are needed to generate data with which to compare the sensitivity of mollusks to other invertebrates and to evaluate the adequacy of existing or proposed US Environmental Protection Agency (EPA) ambient water quality criteria (AWQC) for the protection of mollusks. The objectives of this ongoing study are to compare the sensitivity of six mussel and two snail species from different tribes or families, and three commonly tested invertebrates (amphipod *Hyalella azteca* and cladocerans *Ceriodaphnia dubia* and *Daphnia magna*) in acute water exposures with 10 chemicals representing different toxic modes of action, and to "screen" acute sensitivity to 10 additional chemicals with a commonly tested mussel species (fatmucket *Lampsilis siliquoidea*). The chemicals were chosen based on the interest of the EPA in developing or updating AWQC, the availability of toxicity data for non-mollusks, the sensitivity of other non-unionid mollusks to the chemicals, and toxic mode of action. Preliminary results of the tests conducted to date indicate that: (1) mussels representing different tribes or families have similar sensitivity to the tested chemicals; (2) mussels are sensitive to 9 of the 10 tested chemicals compared to the commonly tested species and other species in national databases; (3) the AWQC may not protect mussels from acute exposures of ammonia, chloride, or nickel; and (4) AWQC may need to be derived or updated for chloride, potassium, and sulfate to reflect the sensitivity of mussels to these ions.

MP031 Sensitivity of freshwater mussels at two life stages to acute or chronic effects of sodium chloride or potassium chloride

C.D. Ivey, USGS – CERC / Columbia Environmental Research Center / Toxicology; R.A. Consbrock, US Geological Survey, CERC / Toxicology, Columbia Environmental Research Center; J.L. Kunz, US Geological Survey / Columbia Environmental Reserach Center; C.G. Ingersoll, US Geological Survey / Columbia Environmental Research Center; N. Wang, USGS / Columbia Environmental Research Center; C. Hammer; C.R. Bauer, USEPA; T. Augspurger, USFWS; S. Raimondo, USEPA; C. Barnhart, Missouri State University / Biology; B.G. Brumbaugh, US Geological Survey / Columbia Environmental Research Center

Native freshwater mussels are in decline. Nearly 70% of the 300 species in North America are endangered, threatened, of special concern, or already extinct. The declines in the abundance and diversity of North American mussels have been attributed to a wide array of human activities that cause pollution, water-quality degradation, and habitat destruction and alteration. Limited studies indicate that freshwater mussels may be more sensitive to major ions such as chloride, sodium, and potassium than commonly tested organisms used to derive US Environmental Protection Agency (USEPA) ambient water quality criteria (AWQC). The objective of this study is to evaluate the sensitivity of fatmucket (*Lampsilis siliquoidea*) at two life stages (glochidia and juveniles) in acute or chronic exposures to sodium chloride (NaCl) or potassium chloride (KCl). We also tested the glochidia of fatmucket in 24-hour acute exposures in waters at different hardnesses ranging from 50 to 300 mg/L as $CaCO_3$. We tested newly transformed or about 2 month-old juveniles of up to 11 mussel species in 96-hour acute NaCl exposures and newly transformed juveniles of four mussel species in 96-hour KCl exposures at hardness 100 or 170 mg/L as $CaCO_3$. In our ongoing chronic 28-day exposures, we evaluated the effects of NaCl or KCl on juvenile fatmucket survival, weight and biomass. Acute EC50s from the glochidia tests increased from 674 mg NaCl/L at hardness 50 mg/L to 2692 mg NaCl/L at hardness 300 mg/L. Acute EC50s for newly transformed juveniles ranged from 2100 to 2600 mg NaCl/L and the EC50s for the 2-month-old juveniles ranged from 2500 to 5200 mg NaCl/L at a hardness of 100 mg/L. Most of these EC50s were below the final USEPA acute value in the acute AWQC (2834 mg NaCl/L or 1720 mg Cl/L). The 28-d NaCl EC20 for fatmucket based on biomass was 450 mg NaCl/L. Acute KCl EC50s for newly transformed juveniles of the four mussels ranged from 72 to 116 mg KCl/L, which makes these mussels the most sensitive species in a KCL toxicity database. Acute KCl exposure with glochidia and chronic KCl exposures with juvenile fatmucket are ongoing. The preliminary results indicate that mussels are generally more sensitive to NaCl and KCl compared to other species in national toxicity databases, and USEPA acute AWQC for Cl may not be protective of freshwater mussels.

MP032 Assessing the Sub-lethal Effects of Chloride, Potassium, and Ammonia on Adult Villosa iris Using Histological Evaluations of Vital Organs

J. Rogers, Virginia Polytechnic & State Uni / Department of Fish and Wildlife Conservation; W. Henley, Virginia Polytechnic Institute and State University / Fish and Wildlife Conservation; A. Graumann, Virginia Polytechnic Institute and State University; J.W. Jones, US Fish and Wildlife Service / Virginia Field Office; W. Cope, North Carolina State University / Department of Toxicology

Once supporting a diverse assemblage of more than 40 mussel species, the North Fork Holston River (NFHR) has seen drastic declines in its species diversity and abundance. Historical industrial activities at Saltville, VA, as well as continued pollution of the river from ongoing nearby sources, are believed to be significant causes of these declines. Seepage waters from the area add high concentrations of ammonia (NH_3) and various ions, including sodium (Na^+), chloride (Cl^-), and potassium (K^+) to the river and are suspected of negatively impacting mussel populations downstream of Saltville. The objective of this study was to assess the health of adult *Villosa iris* exposed for three months to environmentally relevant concentrations of Cl^-, K^+, and NH_3. Stock solutions were created using pond water and reagent grade NaCl, KCl, and NH_4Cl. The high level treatments (705 mg/L Cl^-, 8 mg/L K^+, and 0.15 mg/L NH_3) were determined from measurements taken in the NFHR around Saltville. The EPA Aquatic Life Chronic Criterion for Cl^- (230 mg/L) and NH_3 (0.014 mg/L) were used for the low level treatments in these systems. There is currently no chronic criterion for K^+, so the low level (4 mg/L K^+) was set at 50% of the high treatment. Six treatments plus one control each consisted of five replicate recirculating aquaculture systems (19 L downweller buckets) each containing eight adult *V. iris*. Each treatment consisted of only one chemical constituent (Cl^-, K^+, or NH_3) at either the low or high level. Five bucket systems with pond water with no additional ions were used as the control. Histological evaluations of vital organ tissues including gills, digestive glands, kidneys, and gonads assessed the non-lethal, chronic effects of the separate treatments on mussel tissues. Preliminary results for the majority of variables indicate no detectable differences among treatments and controls. Digestive gland diverticula cells with degraded cytoplasm was significantly higher ($p < 0.04$) in the low Cl^- treatment (27%) than in the high Cl^- treatment (15%); however, mortality in the high Cl^- treatment (35%) was significantly higher than the low Cl^- treatment (8%, $p=0.02$) and control(13%, $p=0.05$). No differences were observed in mortality among K^+ (20%) or NH_3 (23%) treatments and controls. Effects of captivity and mortality as well as toxicological mechanisms will be discussed as potential explanations for the histological evaluation results.

MP033 Assessing the effects of wastewater treatment plant effluents on freshwater mussels

S. Ciparis, Virginia Tech / Fish and Wildlife Conservation; L.R. Iwanowiicz, US Geological Survey / Leetown Science Center; S.E. Lingenfelser, US Fish and Wildlife Service / Virginia Field Office

Effluents produced by municipal wastewater treatment plants (WWTPs) contain several inorganic and organic compounds that can potentially affect the physiology of aquatic organisms. Freshwater mussels may be particularly sensitive to effects of contaminant mixtures in WWTP effluent due to their sessile nature and processing of large volumes of water through filter-feeding activities. The purpose of this study is to assess the effects of WWTP effluents on several aspects of freshwater mussel physiology, including: ion regulation, contaminant detoxification, nutrient acquisition and storage, and functioning of the immune and neuroendocrine systems. Mussels (*Lampsilis fasciola*) will be deployed upstream and downstream of WWTPs in the Clinch-Powell watershed, Virginia, USA, which is home to several imperiled freshwater mussel species. Mussels will be deployed for six weeks during low-flow conditions. At the end of the deployment, samples of hemolymph and tissue will be analyzed for reactive oxygen species, activities of enzymes, concentrations of ions, glucose, and neurotransmitters, and cellular structure. In addition to upstream and downstream site comparisons, effects of several other independent variables on mussel response variables will be considered, including: proportion of stream flow as effluent, receiving stream condition, watershed land use, and measured contaminant concentrations in water and sediment. Results will be discussed in terms of future regulation of WWTPs in watersheds containing endangered species and using suites of biomarkers to determine the effects of specific classes of contaminants on freshwater mussels.

MP034 Disrupting the Steroid Hormone Cascade: Effects of the Aromatase Inhibitor Fadrozole Hydrochloride on the Unionid Mussel Lampsilis fasciola

J. Leonard, NC State University / Department of Environmental and Molecular Toxicology; W.G. Cope, North Carolina State University / Department of Environmental and Molecular Toxicology; C. Barnhart, Missouri State University / Biology; R.B. Bringolf, University of Georgia / Interdisciplinary Toxicology Program

Androgenic effects of endocrine disrupting chemicals (EDC) may be seen less often than those caused by estrogenic compounds because of the relatively high substrate-specificity of androgen receptors compared to estrogen receptors. However, androgen-induced masculinization of females poses concerns for population stability. We evaluated the effects of sub-chronic exposure of fadrozole hydrochloride, a model aromatase (enzyme that converts testosterone to estradiol) inhibitor used as a therapeutic drug to treat breast cancer, on the condition, metabolism, and reproductive status of the unionid mussel *Lampsilis fasciola*. Mussels of both sexes were exposed to a control and 3 concentrations of fadrozole (2 µg/l, 20 µg/l, and 50 µg/l) and samples of gill tissue were taken on days 0, 4 and 12 for metabolomic analysis. Mussel behavior was observed daily, including mantle display, siphoning and larval (glochidia) releases. There were no significant differences in weight gain between sexes or after 4 d exposure, but most treatments showed increased weight gain relative to the control after 12 d exposure. The highest concentrations resulted in fewer instances of glochidia releases as well as higher larval mortality rates. Unlike our previous study with the synthetic estrogen 17α-ethinylestradiol, treatment concentrations did not appear to alter foot extension of either sex, but mantle displays of females were significantly different from the control at 2 µg/L and 50 µg/L. Metabolomic analysis revealed 240 known biochemicals in mussel gill tissue. Glycogen-related metabolites declined in exposed mussels, while both glucose-6-phosphate and fructose-6-phosphate increased, suggesting utilization of glycogen into glycolysis for energy during early exposure. Subtle, significant sex-specific differences were detected in aromatic and branched-chain amino acid metabolism, as well as ornithine metabolism. Our observations indicate female-specific metabolic effects at the lowest concentration (2 µg/L) after 4 d exposure, while males showed significant metabolic changes mostly at the highest concentration (50 µg/L). However, after 12 d, most metabolite concentrations returned to levels similar to the control in both sexes, suggesting recovery from fadrozole-induced physiological changes and few adverse impacts of fadrozole on natural mussel populations.

MP035 Assessment of burrowing behavior of freshwater juvenile mussels in sediment

J.P. Hughes, Columbia Environmental Research Center; C.G. Ingersoll, US Geological Survey / Columbia Environmental Research Center; N.E. Kemble, USGS – Biological Resources Division / Columbia Environmental Research Center

Standard sediment toxicity assessment methods have been adapted for conducting whole-sediment toxicity tests with juvenile freshwater mussels. Juvenile mussels reportedly burrow in sediment for the first 2 to 4 years of life, but may also inhabit the sediment-water interface. The objective of this study was to evaluate mussel burrowing behavior in three control sediments (silica sand [Granusil #4030, Unimin Corporation], West Bearskin fine grained at 3% total organic carbon [TOC], and Spring River at 1% TOC) with various species and ages of juvenile mussels. Exposures were conducted in 60-ml plastic syringes (1 inch diameter) with the top of the syringe removed. 40 mm of sediment and 20 mm of overlying water were used in each syringe. Up to 5 mussels were introduced into the syringe for 4 to 24 hours. At the end of the test, mussels were isolated by siphoning off overlying water then pressing the syringe plunger until 1.7 mm of sediment was exposed at the top of the syringe. The extruded sediment was scraped off the top of the syringe with a spatula into a dish for counting mussels in that layer. The process was repeated until all mussels were recovered. Species tested to date include fatmucket *(Lampsilis siliquoidea*; size 0.4-0.6 mm [about 3 weeks post transformation] and 2-4 mm [about 10 weeks post transformation]), notched rainbow mussels (*Villosa constricta;* size 6-8mm [about 20 weeks post transformation]), and washboard mussels (*Megalonaias nervosa*; size 1.0-1.5 mm [about 12 weeks post transformation]). Greater than 90% of the mussels burrowed into sediment within 15 minutes and none of the mussels were observed at the sediment-water interface. No consistent differences were observed in sediment burrowing behavior between species, life stage, sediment type, or time in sediment. Most of the mussels were isolated from the upper layers of sediment (typically >80% at sediment depths of 0-1.7 or 1.7-3.4 mm) with no mussels isolated from a sediment depth >6.8 mm. Based on species and age of mussels tested to date, juvenile mussels up to an age of 4-months and a up to a size of 4 mm readily burrow into sediment and would likely be exposed to contaminants in whole-sediment and associated pore water throughout a 28-day sediment toxicity test. Additional studies are planned comparing burrowing behavior of older juveniles of different species to determine if and when juvenile mussels might inhabit the sediment-water interface and begin filtering overlying water.

MP036 Direct Sediment Toxicity Bioassay of Bis (Tributyltin) Oxide (TBTO) to Periwinkle Tympanotonus fuscatus var radula

E.T. Ogbomida, National Centre for Energy and Environment / Ecotoxicology and Environmental Forensics; L.I. Ezemonye, University of Benin / Animal and Environmental Biology

Acute and sublethal effects of bis (tributyltin) oxide (TBTO) the active compound of antifouling biocides used to prevent encrustation of organisms on submerged structures was conducted to evaluate the response of periwinkle, *Tympanotonus fuscatus var radula* an important shellfish in the Niger Delta. The study employed direct sediment toxicity assessment of the Organization for Economic Development and Cooperation (OECD) # 218 protocol. T. fuscatus var radula were exposed to varying concentrations (20, 30, 40 and 50µg/l) of TBTO for acute test with particular reference to survival. The acute toxicity results showed increased mean percentage mortality of periwinkles with increase concentrations and exposure duration. The 10-day estimated lethal concentration (LC50) and derived safe concentration were 27.98µg/l and 2.8µg/l respectively. Tissue serum analysis of the Periwinkles snailafter 10, 20 and 30days exposure to varying sublethal TBTO concentrations (0.1, 1.0, 10 and 20µg/L) showed increased significantly ($p < 0.05$) free testosterone levels with increased concentration and exposure durations. TBT induced elevated testosterone in snails may be indicative of imposex expression an irreversible sexual abnormality in female molluscs. The observations in this study showed that *T. fuscatus var radula* are susceptible to imposex development and may affect their reproductive ability which may lead to population decline. Therefore constant monitoring and assessment of TBT is necessary for protecting sensitive benthic invertebrates representing major proportion of the diet of many species in the Niger Delta ecological zone.

MP037 Relation of contaminants in sediment and water to unexplained declines of freshwater mussels in the Clinch River

C.M. Bergeron, North Carolina State University / Department of Environmental and Molecular Toxicology; J. Rogers, Virginia Polytechnic & State Uni / Department of Fish and Wildlife Conservation; A. White, P.R. Lazaro, North Carolina State University / Department of Biology; J.W. Jones, US Fish and Wildlife Service / Virginia Field Office; B. Beaty, The Nature Conservancy; D. Shea, North Carolina State University / Department of Biology; B. Evans, US Fish and Wildlife Service; S.R. Alexander, US Fish & Wildlife Service; W. Cope, North Carolina State University / Department of Toxicology

The Clinch River watershed of Virginia and Tennessee supports one of the nation's greatest concentrations of freshwater biodiversity, but agricultural and mining practices, development, spills, and other anthropogenic activities have degraded habitat and water quality. Mussel populations in this watershed have declined in both species richness and abundance, and there is a critical need to investigate the effects of chemical alterations to water and sediment quality. The aim of this research is to understand the potential causes of ongoing declines by characterizing contaminants (metals and organics) at 8 mainstem and 4 tributary sites. We deployed juvenile Rainbow mussels (*Villosa iris,* 24 mm mean total length) in enclosures (cages in sediment and silos above the sediment) at sites for 5 months, which spanned the range of population impacts. Throughout the mussel deployment period (June-October), we collected samples for contaminant analyses in surface water, sediment, and adult resident mussel tissue. After retrieval of mussels from enclosures, survival, growth, and contaminant body burdens were determined. Passive sampling devices (PSDs) were analyzed for 48 current use pesticides, 28 chlorinated pesticides, 21 polychlorinated biphenyls (PCBs), and 50 polycyclic aromatic hydrocarbons (PAHs). Concentrations of analytes detected were generally near or below the quantitation limits. Water samples from the mainstem and tributary sites indicated differences throughout the watershed. Notably, elevated water quality parameters in two of the tributary sites provide preliminary evidence that these streams are sources of contaminants to the Clinch River. Juvenile mussel survival ranged from 50-95% in cages and from 88-99% in silos. There were no differences among sites in survival in either cages or silos; however, at two sites in the zone of mussel decline, survival in cages was significantly less than in silos, which may be related to sediment contaminant exposure. In addition, mussels at most sites increased in length over the study, but mussels from healthy populations downstream of the zone of decline were larger than mussels from upstream sites in both cages and silos. These data on contaminant analyses in water, sediment, and mussel tissue will be used to inform toxicity tests with juvenile mussels in 2013.

MP038 Estrogens, Gametogenesis, and Field Investigations for Several Unionid Species including the federally endangered, Pleurobema clava

D. Sovic, Ohio State University; R.P. Lanno, Ohio State University / Department of Evolution, Ecology, and Organismal Biology

Evidence of a functional role for vertebrate steroids has been demonstrated in a number of invertebrate species, including several molluscs. This knowledge, in turn, has generated interest and research into the possibility of invertebrate endocrine disruption due to exposure to both exogenous natural steroid hormones and xenobiotics that have the potential to mimic the action of these compounds. Exposure to the natural vertebrate estrogen, 17β-estradiol (E2), for example, has been shown to induce accelerated gamete development in multiple mollusc species.Little information is available, however, for the freshwater Unionid bivalves, a group for which much concern over declining populations and loss of species currently exists. Here, we report findings of two separate field studies on gametogenesis in *Elliptio complanata* and the federally endangered clubshell, *Pleurobema clava* as they relate to seasonal estrogenicity of extracts from Polar Organic Compound Integrative Samplers (POCIS) as determined using the Yeast Estrogen Screen (YES) assay. In order to specifically investigate effects of E2 exposure on gamete maturation and viability in freshwater Unionids, *Elliptio insulsa*, were dosed at one of three exposure levels in October, 2012. Effects on ova and sperm development were determined on biopsies collected 10 days and 6 months post-exposure and biopsy-generated data were compared with histological sections of vicera collected immediately following final biopsy collection. Comparisons of data collected via biopsy and traditional histological techniques provided data to evaluate the potential for utilizing non-lethal biopsy sampling to assess Unionid gametogenesis.

MP039 Assessing the impact of complex urban-derived contaminants on freshwater mussels using indicators spanning multiple levels of biological organization

P.L. Gillis, Environment Canada / Aquatic Contaminants Research Division; R. McInnis, Environment Canada; F. Gagne, Environment Canada / Emerging Methods; S. Higgins, Environment Canada; S.R. de Solla, Environment Canada / Wildlife Toxicology Research Section; T. Hooey, Environment Canada; C.D. Metcalfe, Trent University / Environmental & Resource Studies

The Grand River (ON, Canada) watershed has historically supported a diverse population of freshwater mussels, but recent studies indicate that 30-50% of resident species have been lost, losses which have been attributed at least in part to 'pollution', although no direct examination of cause has been conducted. Water quality is impaired in the urbanized region of this watershed with some metals, chloride, dissolved oxygen, and ammonia, failing to meet Water Quality Guidelines, at various times and locations. This study examined the effect of a complex mixture of contaminants to both chronically (>10 yr) exposed wild mussels (*Lasmigona costata*), and mussels caged (1 month) directly up and downstream of a large municipal wastewater treatment plant (MWWTP) that employs secondary treatment technology. A wide range of pharmaceutical and personal care products (PCPPs) and metals found in the mussel's tissues demonstrates that these compounds are bioavailable to mussels. Wild mussels collected downstream of the urbanized area exhibited effects across multiple levels of organization, including significantly elevated levels of lipid peroxidation and metal-binding proteins, as well as significantly reduced condition factor and mean age (compared to mussels collected upstream of the urban area). In addition, a survey of the wild mussel population revealed a complete absence of live mussels for many kilometers downstream of the wastewater treatment plant outfall, even though mussels, including Species at Risk, were found directly upstream of the MWWTP. In the caging study, PCPPs accumulated by passive sampling devices confirmed that mussels were deployed in the MWWTP effluent plume. Mussels caged downstream of the MWWTP exhibited significantly higher levels of lipid peroxidation, glutathione S-transferase activity, as well alternations in immune function (increased plasma lysozyme activity and hemocyte viability) compared to upstream mussels confirming that MWWTP effluent exposure at least contributed to the effects observed in wild mussels. These results indicate that chronic exposure to a complex mixture of contaminants negatively impacts mussels and corroborates previous assumptions that 'pollution' contributed to the decline of the freshwater mussel population in this watershed. Given the complex nature of the exposure, the specific chemical(s) and/or water quality parameter(s) responsible for the observed effects has not yet been identified.

MP040 Effects of the Kingston coal ash spill on heavy metal content of the Asian clam (Corbicula fluminea)

H. Johnson, University of Tennessee / Geology; M. McKinney, Geology

On December 22, 2008, the coal ash impoundment of the Tennessee Valley Authority (TVA) Kingston Fossil Plant near Harriman, Tennessee ruptured, resulting in the release of over 4.1 million cubic meters of coal ash slurry into the adjacent area. An estimated 25% of the ash flowed directly into the Emory River with such force that it was able to travel 3.2 kilometers upstream. The confluence of the Emory River and the Clinch River is 3.2 kilometers downstream of the spill, and the Clinch River flows into Watts Bar Lake of the Tennessee River another 6.4 river kilometers downstream. The long-term impacts of this event on nearby aquatic ecosystems remain poorly understood. Because the coal ash is now incorporated into the sediment of the Emory, Clinch & Tennessee rivers, the aqueous concentrations of metals may increase in the future as ash particles are mobilized or transformed by rain events, dredging, or biological activity. As many trace metals in the coal ash bioaccumulate in the food web, analysis of animal tissue offers a more accurate assessment of the amount of bioavailable toxins over time than analysis of surface waters or sediment. Our work focused on the accumulation of heavy metals in the Asian clam, *Corbicula fluminea*, which was introduced into the United States in the early 1900s and has since spread widely throughout much of North America. As this invasive species is abundant in many types of habitat, ranging from pristine to very polluted water bodies, it is increasingly used as way to monitor human impacts on water quality. In this case, there were extant populations of Asian clams that occurred throughout the water bodies near the ash spill. We collected specimens from 5 populations of *Corbicula* and examined the trace element content of both shell and soft tissue. Concentrations of 25 elements were measured by inductively coupled plasma optical emission spectrometry

(ICP-OES). Using multivariate methods, we found that several metal concentrations, such as copper, vary significantly among sites. Importantly, these metallic variations are related to the distance and location of the populations from the ash spill. We find little to no correlation between metal content of shell and soft tissue.

Screening Techniques in Environmental Mass Spectrometry

MP041 High-throughput Analysis of Nicotine and Nicotine Metabolites in Urine

J. Feng, B. Wei, I.J. Rehmani, J.E. McGuffey, L. Wang, CDC/NCEH / Division of Laboratory Sciences

Tobacco use is a leading cause of preventable death in the USA. Tobacco users' behavior impacts exposure to tobacco and the toxic chemicals that are present. Nicotine is the major addictive component of tobacco, thus its rate of metabolism may affect the frequency and intensity tobacco use. Information on nicotine metabolite profile will help in identifying individuals at high risk for developing diseases related to tobacco use as well as those amenable to tobacco cessation programs. In order to more rigorously assess tobacco-related exposures, we developed a stable-isotope dilution LC/MS/MS method for quantification of urinary nicotine and its major known metabolites. Glucuronide metabolites for nicotine, cotinine and *trans*-3´-hydroxy-cotinine were hydrolyzed with β-glucuronidase, and total amounts of these compounds along with nicotine-*N*-oxide, cotinine-*N*-oxide, nornicotine, norcotinine and 4-hydroxy-4-(3-pyridyl)butanoic acid were quantified. Sample preparation of this method is fully automated on a custom-designed robotic system to enable high throughput analysis. Mass spectrometric parameters for these metabolites were adjusted so that all of them can be measured with one analysis, even though the typical concentrations of these metabolites vary dramatically in urine samples. To evaluate its performance, the method is applied to 91 urine samples from tobacco users. The lowest detection rate for any of these metabolites is 87% in these samples. Our method analyzes more than 95% of total nicotine metabolites in human urine, and thus provides the profile to establish nicotine metabolic phenotypes by comparison of individual metabolite and total nicotine concentrations. This method will be applied to large population studies like PATH and NHANES to help establish the relationships between nicotine exposure and metabolic phenotype. Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not constitute endorsement by the Public Health Service, or by the US Department of Health and Human Service. This study was funded through an interagency agreement by the US Food and Drug Administration Center for Tobacco Products.

MP042 The Use of Mass Defect for Compound Discovery by Gas Chromatography/High Resolution Time-of-Flight Mass Spectrometry

J.D. Byer, LECO Corporation; G. Pacepavicius, Environment Canada; M. Alaee; P.V. Hodson, Queen's University / School of Environmental Studies; D. Alonso, J. Binkley, LECO Corporation

The difference between a compound's nominal mass and exact mass is called its mass defect. The use of mass defect in mass spectrometry has increased in recent years as mass analyzers become increasingly more accurate in their mass measurements. Modern advances in time-of-flight mass spectrometers (TOFMS) have enabled them to achieve more than 25 k resolving power routinely, and up to 50 k in select cases. We characterized a number of Atlantic anguillid eel (*Anguillid* sp.) samples chemically for legacy chlorinated and brominated persistent organic pollutants (POPs). In this study, these whole fish homogenates were pooled by location and their extracts were injected on a Leco Pegasus GC-HRT to identify halogenated POPs not measured previously. Identification was achieved by leveraging the hydrogen substituted for chlorine (H/Cl) scaled mass defect with the scaling factor of 34/33.96102, for the ratio between the nominal and exact mass of Cl-H. The most prevalent features in the plot of H/Cl Mass Defect vs Nominal Mass corresponded to legacy compounds such as polychlorinated biphenyls, organochlorine pesticides (i.e., DDT metabolites), and polybrominated diphenyl ethers. A series of non-legacy chlorinated styrenes, as well as, brominated aromatics that were formerly unknown were also identified in these samples. This presentation will highlight several examples that showcase the utility of using mass defects for interrogating data acquired with high resolution TOFMS for the identification of non-target compounds.

MP043 New Detection Methods for Emerging Environmental Pollutants using LC-MS/MS

A. Schreiber, P. Winkler, AB SCIEX

Pharmaceuticals and Personal Care Products (PPCP) are environmental contaminants of growing concern. In order to properly assess the effects of such compounds on our environment, especially their disruption of endocrine function in mammals and fish, it is necessary to accurately monitor their presence in the environment. The diversity of chemical properties of these compounds makes method development challenging. Here we present results of PPCP findings in water samples collected in different geographies and from different type of water. All samples were analyzed by direct injection Liquid Chromatography coupled to tandem Mass Spectrometry (LC/MS/MS). Two analytical methods were used. A method which enables the quantitation of PPCP at low ppt levels using Multiple Reaction Monitoring (MRM) and identification using full scan MS/MS with mass spectral library searching. In addition, a high resolution and accurate mass QqTOF system was used to further explore collected samples for unexpected analytes. Data processing turned out to be the bottleneck of the general unknown screening methodology. New and advanced data processing tools where used to automatically identify unexpected and unknown pollutants.

MP044 Identification of phase II pharmaceutical metabolites in reclaimed water using Orbitrap mass spectrometry: towards an achievable mass balance

J. Wang, Florida International University / SERC and Chemistry; V. Panditi, FIU; P.R. Gardinali, Florida International University / Department of Chemistry & Biochemistry and SERC

This study described an analytical method for the identification of common phase II pharmaceutical metabolites in reclaimed water using liquid chromatography high resolution Orbitrap mass spectrometry after solid phase extraction (SPE). The qExactive mass spectrometer was operated at resolution of 70,000 in MS mode and 35,000 in data-dependent MS/MS mode, without using lock mass. Parent drugs and their metabolites were tentatively identified based on accurate mass measurements using a mass tolerance of 5 ppm. A detailed examination of extracted ion chromatograms (XICs) for all potential metabolites revealed the presence of 2 phase II metabolites of sulfamethoxazole: acetylsulfamethoxazole and sulfamethoxazole glucuronide, and erythromycin with its bio-active metabolite desmethyl-erythromycin in reclaimed water. The high resolution data-dependent MS/MS spectra of each compound were further investigated using metabolic profiling software. After comparing characteristic ions obtained in MS/MS mode with those predicted by the software and reported in previous studies, the metabolites were positively identified. Further analysis of reclaimed waters over 30 days revealed the presence of all the metabolites in 100% of the samples. The metabolites were then quantified based on the response factors obtained for their parent drugs. As a result, averaged concentrations of sulfamethoxazole, acetylsulfamethoxazole and sulfamethoxazole glucuronide were calculated at 2848 ± 1367 ng/L, 1980 ± 1410 ng/L, and 2859 ± 1526 ng/L, respectively. The two metabolites represented in average 54 %, of the total sulfamethoxazole present in reclaimed water. Desmethyl-anhydroerythromycin represented 13% of the total input of erythromycin in reclaimed water. To our knowledge, this is the first known report of sulfamethoxazole glucuronide surviving intact through wastewater treatment plants and occurring in environmental water samples.

MP045 Using otolith microchemistry to reconstruct mercury exposure histories in freshwater drum captured near coal-fired power plants

L. Friedrich, Stantec Consultants Ltd.; R.J. Reash, American Electric Power; N. Halden, University of Manitoba; V.P. Palace, Department of Fisheries & Oceans Canada, Stantec Consultants Ltd.

Concern has been expressed regarding increasing mercury levels in muscle tissue of fish captured near coal-fired power plants on the Ohio River. To examine this issue, an American Electric Power – Electric Power Research Institute (AEP-EPRI) study was commissioned to evaluate the mercury and selenium content of fillet tissue and assess the levels against the United States' Environmental Protection Agency's human health fish tissues criterion. Such chemical analysis of visceral tissues can provide information on recent exposure; however, depuration, metabolic transformation, and tissue

redistributions prevent any historical data from being recovered. Otoliths, the calcified structures in the inner ear of teleost fish, are considered to be metabolically stable and, therefore, may serve as continuous recorders of exposure to trace elements in the environment. They form by the deposition of layers of calcium carbonate in a protein matrix deposited annually throughout the lifetime of the fish, thus providing a time scale to the chemical data. Otolith microchemistry has increasingly been used as a tool for assessing the exposure history of fish in mining-impacted sites. We have specifically used this tool to determine exposure to selenium downstream from active coal mines. The present study examines the possibility of using otolith microchemistry to reconstruct exposure Se and Hg histories of freshwater drum (*Aplodinotus grunniens*) to effluents from the coal-fired power plants. Mercury and selenium concentrations in the otoliths were determined using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), and will be used to determine the relationship between measured tissue levels and the most recent levels of these trace elements recorded in the otolith.

MP046 Applying differential mobility spectrometry with unique gas-phase separations to the analysis of naphthenic acids

R. Kern, T. Sukuma, A. Schreiber, P. Winkler, AB SCIEX; K. Peru, Environment Canada

Naphthenic acids (NA) from crude oils and water samples have been the subject of many environmental studies. These compounds, which are carboxylic acids containing one or more saturated ring structures, have the generic formula of CnH2n+zO2 (for monocarboxylic acids) where z is and even, negative integer marking the "hydrogen deficiency." The definition of NAs has expanded recently to include S, N and aromatic species that occur in oil sands processing waters. While much research has been done with accurate mass spectrometry and conventional chromatography, the potential for gas-phase separations of NAs remains an untapped resource. Here, we present preliminary data that demonstrates the gas-phase separation of naphthenic acid components using differential mobility spectrometry (DMS) with volatile chemical modifiers.

Implementing Ecological Risk Assessment Weight-of-Evidence Approaches that Contribute to Decision-Making

MP047 Linking a Weight-of-Evidence Analysis to Restoration in a Landscape-scale Terrestrial Ecological Risk Assessment

R.N. Hull, Intrinsik Environmental Sciences Inc.; M. Machmer, Pandion Ecological Research Ltd.

A landscape-scale terrestrial ecological risk assessment (ERA) was completed for an area that had been affected by decades of industrial emissions. The risk assessment used a weight of evidence analysis that included analysis of chemical concentrations in soil, plant community analysis (e.g., plant species diversity and richness, presence of sensitive or tolerant plant species, plant seral stage, and percent cover of trees, shrubs, herbs), statistical analysis of plant community parameters relative to soil, chemical and topographic characteristics, wildlife habitat suitability evaluation, wildlife risk modeling, and other lines of evidence. The conclusion was that risks to plant communities could not be ruled out for a portion of the study area lands, and that wildlife communities were influenced by the changes to habitats. A plan is under development to identify options for remediation and restoration of impacted areas. The data needed to make these recommendations are not always the same as those collected for the ERA. In addition, the plan will be looking at ways to effectively address issues related to ecosystem function, biodiversity, habitat connectivity and suitability for a wider range of species than those assessed in the ERA. Initial progress will be highlighted in this talk, and the topic will be explored further at a joint SETAC/Society for Ecosystem Restoration Workshop planned for June 2014 on Restoration of Impaired Ecosystems.

MP048 Quantitative Weight-of-Evidence Risk Assessment for the former Kelly AFB, San Antonio, Texas

C.J. McCarthy, CH2M Hill / Environmental Services

A site-wide ERA was conducted for the former Kelly Air Force Base (AFB) in San Antonio, Texas using the Texas Commission on Environmental Quality's (TCEQ) ecological risk assessment (ERA) guidance (2001). Risks to ecological communities were characterized within a 3.5-mile reach of the 45-mile long Leon Creek that flows through the former AFB. Greater than 10 years of previously collected compliance monitoring data from multiple third-party investigations, which were not designed for risk assessment purposes, were integrated into a Tier 3 weight-of-evidence (WOE). Data included four different single-species toxicity tests, FETAX, fish and benthic community surveys, habitat indices, fish and herptile tissue chemistry, and co-located surface water and sediment chemistry. These data were used along with modeling of stormwater and groundwater inputs to Leon Creek to develop multiple lines of evidence for Tier 3 measurement endpoints. The WOE approach from Menzie et al., 1996 was employed to provide a quantitative means of evaluating the risk to communities within Leon Creek. Results of the WOE helped provide perspective to a complicated site and minimized the need to design and collect costly site specific Tier 3 data. Final results of the WOE within the ERA were incorporated into the risk management decision process to bring the 30 waste management units tied to the Leon Creek to Regulatory Closure.

MP049 Application of a Practical WOE Framework in Case Study ERAs in British Columbia, Canada

C.E. Mackintosh, R.A. Hill, Azimuth Consulting Group; P.J. Allard, Azimuth Consulting Group Partnership; G.S. Mann, Azimuth Consulting Group Inc.

Application of WOE approaches to ERA evaluations has become the standard of practice in ERA over the past several years. There are several WOE frameworks available in the literature or provided by regulatory bodies to guide risk assessment practitioners. These frameworks differ in their approaches and degree of quantification. We have opted for an intermediate degree of quantification in our WOE approach that maintains transparency and rigor, but is also practical to implement and easy for clients, stakeholders and regulators to understand. Overall, the WOE approach we follow is consistent with Environment Canada guidance (2012), which is the default method for federal contaminated sites in Canada. In our experience, this semi-quantitative approach has been appropriate for all contaminated sites evaluated in our practice. The WOE approach gives advance consideration of the merits and limitations of LOEs during selection in the Problem Formulation stage, provides criteria and a simple rating method for evaluating LOEs during risk characterization, and integrates multiple LOEs by assessment endpoint based primarily on narrative rationale to reach risk conclusions. We present this WOE framework and case study ERA applications from contaminated sites in British Columbia, Canada.

MP050 Relating Uncertainty, Weight of Evidence, and Precaution in the Regulatory Assessment of Chemicals in Canada

M.A. Bonnell, Environment Canada / Ecological Assessment Division

The Canadian Environmental Protection Act (CEPA) 1999 states that the Ministers of the Environment and of Health shall apply a *weight-of-evidence approach* and the *precautionary principle* when (1) conducting and interpreting the results of a screening assessment, (2) reviewing a decision of another jurisdiction to prohibit or restrict the use of a substance for environmental or health reasons, and (3) during an assessment of whether a substance specified on the Government's priority chemical list is toxic or capable of becoming toxic. However, CEPA, perhaps rightly so, does not specify how this should be done likely because application of such principles is very context dependent. Both weight of evidence and precaution are influenced by uncertainty, thus all three concepts must be considered together when making assessment decisions, whether this is for a specific assessment endpoint or characterizing overall risk. As a result, chemical assessment programs have had to develop internal approaches to communicate how decisions are made that incorporate the above three concepts. Here we discuss the importance of data quality and quantity as it affects uncertainty and how uncertainty can be propagated through to the weighing of evidence and the degree to which precaution is applied when making assessment decisions. We also discuss how evidence can be weighed by considering scientific and regulatory relevance as well as "strength of inference" and how appropriate levels of precaution can be applied. Two examples will be used to demonstrate the above concepts. The first will use the hypothesis "is the substance bioaccumulative according to international regulatory criteria of BCF or BAF >5000 ?" The second will use the hypothesis "does the substance have the potential to cause adverse effects" for the scenario where near and far-field exposure to a persistence and bioaccumulative substance is likely.

MP051 A Weight of Evidence Framework for Assessing the Endocrine Activity of Acetone: Lessons from the USEPA EDSP

E.M. Mihaich, Environmental and Regulatory Resources; C.M. Holmes, BASF ArgoResearch / Ecotoxicology; R. Waites, S. Dimond, SABIC; J. Busch, American Chemistry Council

Acetone is a widely-used organic solvent found naturally in the environment. It is also present in the body from the breakdown of fat. Acetone's use in pesticide formulations, however, earned it a place on the exposure-based List 1 of the USEPA Endocrine Disruptor Screening Program. The EDSP includes 11 validated Tier 1 *in vitro* and *in vivo* screens that evaluate a chemical's potential to interact with estrogen, androgen, and thyroid pathways. Tier 1 is intended to be interpreted as a battery, not as individual assays. Given that the EDSP screens were chosen to have redundancy across the battery, a transparent hypothesis-based weight of evidence (WoE) procedure can be employed to make sense of the many mechanistic and apical endpoints. For acetone, the mechanistic *in vitro* estrogen and androgen receptor binding, estrogen receptor transactivation, steroidogenesis, and aromatase screens were all negative. Additionally, the results from two of the *in vivo* mammalian assays, the uterotrophic and Hershberger, were also negative. In the two remaining *in vivo* screens, there were minor, random effects on uterine weights and clinical chemistry parameters in the female pubertal and body weight and testosterone changes in the male pubertal that were not considered treatment-related. Similarly, in the two *in vivo* ecotoxicology assays, several effects on apical endpoints were noted. In the fish short-term reproduction assay, fecundity was reduced in the mid-dose, while fertility was reduced in both the mid and the high dose. Although statistically significant, fertility still met the control fertility criteria of >95% in all doses and was not considered a treatment-related adverse effect. Lastly, hind limb length was increased at the high dose in the amphibian metamorphosis assay, however, only when it was normalized to snout-vent length. Given there were no other corroborating findings in thyroid histopathology or in thyroid parameters in the pubertal screens, it is unlikely that the effect on normalized hind limb length translates to thyroid disruption by acetone. Consistency across endpoints, as well as ranking them based on their relevance for the hypothesis is important, particularly when there are both mechanistic and apical endpoints being evaluated. Employing a transparent WoE procedure for the Tier 1 screens, along with existing data in the open literature, it can be concluded with a high level of confidence that acetone is not an endocrine active compound.

Emerging Halogenated Phenolic Chemicals in Biotic and Abiotic Environments

MP052 Metabolism of Polybrominated Diphenyl Ethers; What the literature tells us

S. Petropoulou, California Environmental Protection Agency / Department of Toxic Substances Control, Environmental Chemistry Laboratory; W. Duong, DTSC\ECL / Berkeley; M. Petreas, J. Park, DTSC\ECL

Several studies report ubiquitous levels of polybrominated diphenyl ethers (PBDEs) in human tissues and biological fluids. A 2003 review paper on the metabolism of these compounds reported PBDE half-lives in humans that vary between 6.5 to 12 years depending on the levels of exposure and human body burdens, while it documented that the concentrations of many PBDE congeners in human serum were low or absent suggesting metabolism or lack of accumulation. Since then, a number of laboratory animal exposure studies have found significant species-specific differences in uptake, absorption, kinetics, metabolism, and disposition of several PBDEs confirming that the metabolism of PBDEs might need further investigation, since many of the oxidative metabolites have been proven more toxic than the parent congeners. In addition, several studies, over the last five years, document the presence of several hydroxylated and methoxylated metabolites in human serum. In this work, we present a literature survey that aims to give an overview of the metabolites that have been identified in vitro and in vivo studies. We compare these studies and their results in order to identify similarities and differences on the identified congeners in order to further understand the metabolism of PBDEs in humans. The literature search was based on the databases of Web of Knowledge and Google Scholar using the following search terms: "polybrominated biphenyls ethers/PBDEs and metabolism," "PBDEs and oxidation," "Hydroxylated and or Methoxylated polybrominated biphenyls and serum." Disclaimer: The views expressed herein are those of the authors and do not necessarily reflect those of the Department of Toxic Substances Control, California Environmental Protection Agency.

MP053 Solid phase extraction of hydroxylated brominated Diphenyl ethers (HO-PBDEs) from human serum and quantitative analysis by mix mode fast HPLC-MS/MS

S. Petropoulou, California Environmental Protection Agency / Department of Toxic Substances Control, Environmental Chemistry Laboratory; W. Duong, DTSC\ECL; Z. Smith, University of California / Department of Chemistry; M. Petreas, DTSC\ECL / Berkeley; J. Park, DTSC\ECL

Several reports document the bioactivation of polybrominated biphenyl ethers (PBDEs) by hydroxylation and the accumulation of several hydroxylated metabolites of PBDE congeners (OH-BDEs) in human serum. OH-BDEs have attracted particular interest due to their potency. Recent in-vitro studies have shown that several OH-BDEs act as ERα and ERβ agonists, while they have been also found to bind to transthyretin, controlling the transportation and release of thyroxine in the bloodstream. The same studies show that even though the metabolites are more polar than the PBDEs, their excretion rates are not higher, leading to high accumulation levels. These facts underline the need for their accurate measurement and determination especially in human fluids. We are presenting a novel analytical technique for their determination based on a high throughput solid phase extraction for sample cleanup using only 250 uL of serum. The serum extracts are analyzed using a LC-MS/MS chromatographic method, on a mix mode analytical column for the separation of all the stereoisomeric congeners in a single run (15 min). The method determined all the major HO-BDEs of the most detected PBDE congeners (i.e., BDE47, BDE99 and BDE100) in humans. The limits of detection (LOD) for most are < 8 pg/mL while for 4-OH-BDE90 and 6'-OH-BDE99 the LOD is 60 pg/mL. The recoveries range from 63.4-95% while the accuracy, precision, stability and reproducibility of the method confirm that this method can be used to further investigate the metabolism of PBDEs in humans and their relation to human health effects. Disclaimer: The views expressed herein are those of the authors and do not necessarily reflect those of the Department of Toxic Substances Control, California Environmental Protection Agency.

MP054 Simultaneous Determination of Hydroxylated Polychlorinated Biphenyls in the Liver and Brain Using Gas Chromatography/Mass Spectrometry

A. Eguchi, National Institute for Environmental Studies / · Center for Environmental Health Sciences; K. Nomiyama, Center for Marine Environmental Studies (CMES), Ehime University; M. Ochiai, Center for Marine Environmental Studies (CMES); H. Mizukawa, Hokkaido University / Graduate School of Veterinary Medicine; Y. Nagano, Center for Marine Environmental Studies (CMES), Ehime University; K. Nakagawa, K. Tanaka, H. Miyagawa, Shimadzu Corporation; S. Tanabe, Ehime University / Center for Marine Environmental Studies (CMES)

Analysis of hydroxylated polychlorinated biphenyls (OH-PCBs) and their homologs in critical organs, such as the brain and liver, will be increasingly important in the future for ecotoxicological assessments. Recent studies suggested that the levels and profiles of OH-PCBs in animal blood vary by species and several animals may be at a risk from these metabolites including congeners which are not found in human at present. However, because of difficulties in detecting low-chlorinated (e.g., 3–4 chlorine atoms) OH-PCBs in biological tissues, comprehensive investigations on the levels of various OH-PCB homologs in the brain and liver are still limited. In this study, we developed a comprehensive, highly sensitive, and robust method for determining 3-8 chlorinated OH-PCBs in liver and brain samples by using isotope dilution gas chromatography (GC) coupled with electron capture negative ionization mass spectrometry (ECNI-MS) These results were compared with those from GC coupled with electron ionization high-resolution mass spectrometry (EI-HRMS). Clean-up procedures for analysis of OH-PCBs homologs in liver and brain samples involve a pretreatment step consisting of acetonitrile partition and 5% hydrated silica-gel chromatography before derivatization. The method detection limits of OH-PCBs obtained by GC/ECNI-MS and GC/EI-HRMS were 0.58–2.6 pg g^{-1} and 0.36–1.6 pg g^{-1} wet wt, respectively. The recovery rates of OH-PCB congeners in spike tests (10 and 50 pg) were 64.7–117% (RSD: 4.7–14%) and 70.4–120% (RSD: 2.3–12%) for GC/ECNI-MS and GC/EI-HRMS, respectively. The peaks of 15 and 29 OH-PCB congeners were identified in the brain of finless porpoises and the liver of Baikal seals, respectively.

This analytical method may enable the simultaneous detection of various OH-PCBs from complex tissue matrices. In addition to the tri- and tetra-chlorinated OH-PCBs, some unknown congeners were also discovered in the brain of finless porpoises and liver of Baikal seals. Since they might have a high transfer potential into the brain and livers, the identification of these predominant unknown OH–PCBs is needed. Furthermore, this method allows more comprehensive assessment of the biological effects of OH-PCB exposure on critical organs.

MP055 Halogenated phenolic compounds in cetacean brain: blood-brain transfer and distribution in eight brain regions

M. Ochiai, Center for Marine Environmental Studies / Center for Marine Environmental Studies (CMES); K. Nomiyama, Center for Marine Environmental Studies (CMES), Eh; T. Isobe, Ehime University / Center for Marine Environmental Studies (CMES); T.K. Yamada, National Museum of Nature and Science, Japan / Department of Zoology; Y. Tajima, National Museum of Nature and Science, Japan; M. Makara, National Museum of Nature and Science, Japan / Department of Zoology; M. Amano, Nagasaki University / Graduate school of fisheries science and environmental studies; S. Tanabe, Ehime University / Center for Marine Environmental Studies (CMES)

Recent studies reported that halogenated phenolic compounds such as hydroxylated polychlorinated biphenyls (OH-PCBs) and hydroxylated polybrominated diphenyl ethers (OH-PBDEs) could competitively bind to thyroid hormone (TH) transport protein transthyretin, TTR, and inhibit TH transport. THs are essential for neuronal development, and *in vitro* studies have shown that low doses of OH-PCBs suppressed the TH-dependent gene transcription and development of mouse Purkinje cells. It is reported that cetaceans accumulate higher levels of persistent organic pollutants such as PCBs and PBDEs, and their hydroxylated metabolic products, OH-PCBs and OH-PBDEs, which have also been detected in their plasma. However, there are only few reports on the levels of the hydroxylated metabolites in cetacean brain. In this study, residual levels and accumulation features of OH-PCBs and OH-PBDEs in the brain of finless porpoises (*Neophocaena phocaenoides*), melon-headed whales (*Peponocephala electra*) and striped dolphin (*Stenella coeruleoalba*) stranded or bycaught along Japanese coasts were determined. In addition, the present study investigated accumulation features of OH-PCBs and OH-PBDEs in the eight regions of the brain: frontal lobe, occipital lobe, limbic system including hippocampus, hypothalamus, pituitary gland, cerebellum, pons and medulla oblongata with an objective to study the link between the distribution in the brain and its toxicological implications. OH-PCBs and OH-PBDEs were found in all the regions of the brain. The median level of OH-PCBs was comparable between the brain and blood, whereas the levels of OH-PBDEs were significantly lower in the brain than in blood. Variation in the levels of OH-PCBs and OH-PBDEs was found among the eight regions of the brain, with localized accumulation in the pituitary gland. This result indicates potential localization and region-specific effects of halogenated phenolic compounds in cetacean brain.

MP056 Early Exposure to PBDE Metabolites Disrupts Development in Zebrafish

L.J. Macaulay, Nicholas School of the Environment; L. Dishaw, W. Dong, K. Rock, Duke University / Nicholas School of the Environment; A. Oliveri, Duke University / Department of Pharmacology and Cancer Biology; E. Levin, Duke University / Department of Psychiatry, Department of Pharmacology and Cancer Biology, Nicholas School of the Environment; H.M. Stapleton, Duke University / Nicholas School of the Environment

Polybrominated diphenyl ethers (PBDE) and polychlorinated biphenyls (PCB) are oxidatively biotransformed by mammals into halogenated phenolic compounds (OH-BDEs and OH-PCBs). These metabolites are often not considered in risk assessments, despite the fact that they often have greater endocrine disrupting effects than the parent chemicals. To better understand the toxicological risks these compounds pose, we examined the effects of ten different OH-BDE, OH-PCB, and halogenated phenolic compounds during embryonic development. Zebrafish were exposed from 4 hours post fertilization (hpf) to 6 days post fertilization (dpf) at a range of concentrations to determine overt toxicity. LC50 values ranged from a high of 7.83μM for 2,4,6 Trichlorophenol to 134 nM for 6-OH-BDE 47, which was the most toxic compound tested. Severe developmental delays were observed (decreased pigmentation, spine/tail deformities, pericardial and abdominal edema) in fish treated at doses below the overt toxicity threshold. Significant changes were observed in embryo morphology, using the Otic Vesicle Length (OVL), Head Trunk Angle (HTA), and retinal pigmentation as staging metrics. The average HTA declined in 6-OH-BDE-47 treated fish from 82° to 68°. OVL increased from 2.92 in the control animals to 7.14 with exposure to 6-OH-BDE-47. A 60% reduction in retinal pigmentation was also observed. All of these morphological changes are indicative of developmental delays, and may be mediated by disruption of thyroid hormones which regulate these developmental processes. We also used the thyroid hormone T3, as a positive control and observed similar effects, although at lower doses. The exposed zebrafish and their controls are also undergoing behavioral assessment to evaluate the early and persisting effects of early life exposure to PBDE metabolites. All behavioral exposures were performed at doses below the overt toxicity threshold, which were found to have no gross morphological effects on development. Short-term effects in larvae are assessed for swimming activity in response to light-dark transitions. Persistent neurotoxicity is determined by tests of sensorimotor plasticity, predatory escape responses and spatial learning. These results could indicate that exposure to low levels of OH-BDEs can alter zebrafish embryonic development. The observed effects may be related to disruption of the thyroid system, specifically deiodinase enzymes.

MP057 Biotransformation of BDE-100 to Potentially Toxic Metabolites: Predominant Role of Human CYP2B6

M.S. Gross, D.M. Butryn, University at Buffalo / Department of Chemistry; B.P. McGarrigle, J.R. Olson, University at Buffalo / Department of pharmacology and toxicology; D.S. Aga, University at Buffalo / Department of Chemistry

Polybrominated diphenyl ethers (PBDEs) are additive flame-retardants used in numerous consumer products, resulting in their presence as ubiquitous environmental contaminants. Animal and human studies have supported an association between PBDE exposure and neurotoxic and endocrine disruption effects. Furthermore, recent studies have found that hydroxylated metabolites/analogs of PBDEs (OH-BDEs) accumulate in human serum at levels similar to or greater than their parent PBDEs and that OH-BDEs show an increased toxicity. Although human exposure to OH-BDEs may result from diet and natural sources, another source for the occurrence of OH-BDEs in humans is through the biotransformation of parent PBDEs. However, insufficient data exists on the metabolism of PBDEs in humans. The objectives of this study were to qualitatively and quantitatively characterize the *in vitro* metabolism of 2,2´,4,4´,6-pentabromodiphenyl ether (BDE-100), one of the most abundant PBDEs found in humans, by recombinant human cytochrome P450s (CYPs) and pooled human liver microsomes (HLMs). Individual incubations of BDE-100 with recombinant human CYPs (1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) showed that CYP2B6 was predominantly responsible for the metabolism of BDE-100 into its monohydroxylated and dihydroxylated analogs. Kinetic studies of BDE-100 metabolism (0-60 μM) suggest that CYP2B6 has a high affinity for the formation of monohydroxylated-BDEs. Derivatization of OH-BDE metabolites into their methoxylated equivalents (MeO-BDEs) allowed for analysis to be completed using gas chromatography tandem mass spectrometry (GC-MS/MS). Our results will ultimately better inform future mechanistic and epidemiological studies investigating the potential of PBDEs and their metabolites to produce neurotoxic and endocrine disrupting effects. (Supported in part by NIEHS, grant #ES021554)

MP058 Longitudinal study of hydroxylated PCBs (OH-PCBs) in serum of school children and their mothers living in East Chicago, IN and Columbus Junction, IA

W. Koh, The University of Iowa / Interdisciplinary Graduate Program in Human Toxicology; R. Marek, The University of Iowa / Department of Civil and Environmental Engineering; P. Thorne, J. DeWall, The University of Iowa / Department of Occupational and Environmental Health; K. Hornbuckle, The University of Iowa / Department of Civil and Environmental Engineering

Polychlorinated biphenyls (PCBs) tend to biotransform into various types of metabolites with hydroxylated metabolites (OH-PCBs) the most common form. Studies have shown that some OH-PCBs are more toxic than their parent PCBs. As part of Airborne Exposure to Semi-volatile Organic Pollutants (AESOP) study, we studied both PCBs and OH-PCBs in serum from two communities living in East Chicago, IN and Columbus Junction, IA in United States. We have collected blood from junior high school-aged children and their mothers since 2008. All 209 PCB congeners and 65 OH-PCBs congeners were evaluated. Samples were analyzed using gas

chromatography with tandem mass spectrometry (GC-MS/MS) for both PCBs and OH-PCBs after a series of extraction and clean-up procedures. In this study, we compare the level of OH-PCBs between two communities, study the association of PCBs and OH-PCBs as well as investigate the trend of OH-PCBs in these two communities and in each individual from year to year. Our results showed that both communities have similar levels of OH-PCBs and the concentration of OH-PCBs and their parent PCBs were associated. We also report the pattern of OH-PCBs in each individual, between the generation, within the community and between the communities.

MP059 The CID MS/MS analysis of bisphenol A analogues and derivatives

W. Zou, CDPH – ELAP; A. Aditham, J. She, California Department of Public Health

We use an Agilent 1290 UHPLC coupled with an Agilent 6460 triple quadruple mass spectrometer (MS). We optimize electrospray ionization (ESI) source parameters and compound parameters to maximize sensitivity on MS. Flow injection optimization (FIA) and on-column optimization using Kinetex phenylhexyl column (150 x 2 mm, 2.1 micron) are conducted to study fragmentation pattern of each compound. Sciex QTrap 5500 MS is used for ion tree spectral analysis and Thermo Exactive Plus Orbitrap MS is used to acquire accurate mass of the molecular ion and fragmented ions (in source fragmentation). Fragmentation patterns of BPA, BPAF, BPB, BPF, BPS, BADGE, BFDGE, and other BPA analogues and derivatives are presented. For example, under the current condition, the major fragments of BPA are m/z 212 (loss of CH_3) and m/z 134 (loss C_6H_6O), the major fragments of BPAF are m/z 267 (loss of CF_3) and m/z 67 (CF_3^-), the major fragments of BPB are m/z 212 (loss of CH_2CH_3) and 148 (loss of C_6H_6O), the major fragments of BPS are m/z 156 (loss of C_6H_6O) and m/2 92 ($C_6H_6O^-$). The results of CID MS/MS analysis are applied to construct MRM transitions of each compound for quantitation purpose. Initial demonstration of capability, in-house validation using control charts of 20 batches QC samples, and third-party validation using certified reference material and proficiency tests will be conducted. Furthermore, MRM library on MS/MS and MS^2 accurate mass library will be built for comprehensive screening of BPA derivatives in human urine.

MP060 Bisphenol A, 2,4-Dibromophenol, 2,4,6-Tribromophenol and Tetrabisphenol A in human serum samples by LC-MS/MS using off-line high throughput SPE

S. Petropoulou, California Environmental Protection Agency / Department of Toxic Substances Control, Environmental Chemistry Laboratory; M. Petreas, J. Park, DTSC\ECL

Several studies use older techniques to determine BPA levels in serum (such as elisa), but these techniques have been proven unsuccessful cause they lack sensitivity. Similarly reports on human health effects of Tetrabromobisphenol-A (TBBPA) support a low bioavailability and a rapid excretion in mammals with levels in non-occupational human serum samples < 0.1ng/mL. Recent toxicokinetic studies show slower excretion rates as initially reported and indicate the lack of information on its toxicity to humans and animals relative the environmental concentrations. 2,4-Dibromophenol (2.4-DBP) and 2,4,6-tribromophenol (2,4,6-TBP) have been identified as degradation products of the BPA and TBBPA, but they have also been used independently as flame retardants. Currently there are no studies on their toxicity and human health effects, probably due to the lack of an accurate and sensitive technique for their measurement. In this work we report a new validated LC-MS/MS method using isotopic dilution for the determination of the above mentioned compounds in human serum. Samples were processed using formic acid with an enzymatic hydrolysis of the glucuronides/sulfatase conjugates followed by an off-line high throughput solid phase extraction procedure using HLB cartridges by Waters. The recoveries for all the compounds were from 80-112% with standard deviation less than 5%. The LODs are 0.1 ng/mL for BPA and 2.4-DBP and 0.01 ng/mL for TBBPA and 2.4.6-TBPin 100uL serum. The calibration curves are linear, while the accuracy, precision, stability and reproducibility confirm that this method can be used as adequately for biomonitoring purposes and lower level toxicity studies. Disclaimer: The views expressed herein are those of the authors and do not necessarily reflect those of the Department of Toxic Substances Control, California Environmental Protection Agency.

MP061 PBDEs Disrupt Thyroid Hormone Homeostasis and Deiodinase Activity in Human Placental Tissues

C. Leonetti; C.M. Butt, Duke University; H.M. Stapleton, Duke University / Nicholas School of the Environment

Due to their structural similarities to thyroid hormones, polybrominated diphenyl ethers (PBDEs) interfere with thyroid hormone homeostasis and regulation. Thyroid hormones are key signaling molecules orchestrating fetal organ development and are essential for proper neurodevelopment. Inappropriate levels of maternal and/or fetal thyroid hormones during pregnancy can result in growth retardation, fetal malformations, or abnormal brain development that can lead to cognitive deficiencies and learning disabilities later in life. Maintenance of thyroid hormones in peripheral tissues is partially governed by a class of selenoprotein enzymes, deiodinases (DIs), of which there are three different isoforms that differ in their tissue distribution and catalytic capacities. Type 3 DI is predominantly expressed in placenta tissue and catalyzes inner ring deiodination (IRD), converting T4 to its biologically inactive form, rT3. In our previous research, we found that PBDEs and other halogenated organic contaminants inhibit type 1 DI activity in human hepatic tissues; however, it is unclear whether similar effects would be observed on Type 3 DI activity in placental tissues. In the present study, we investigated the associations between PBDEs, thyroid hormone levels, and Type 3 DI activity in human placental tissues. We also investigate the correlation between PBDEs in 3rd trimester serum samples and PBDEs in placental tissues collected at birth. Using our previously developed method for Type 1 activity, we measured Type 3 DI activity in microsomal fractions of 100 human placental tissues. Type 3 DI activity rates measured ranged from 0.06 to 3.86 pmoles of rT3/min/mg protein. Preliminary findings indicate that placental Type 3 DI activity is negatively associated with maternal serum BDE 47 (r=-0.4478, p=0.0545), suggesting that PBDEs may affect thyroid hormone regulation via DI enzyme inhibition. Further research will be conducted to measure endogenous thyroid hormone concentrations in the placental tissues and PBDE concentrations in placental tissues and multivariate models will be used to determine the strongest variables affecting thyroid hormone concentrations and birth outcomes.

MP062 Immunoanalytical method for the sensitive and specific detection of BDE-47

C.S. Bever, University of California – Davis / Entomology; Z. Majkova, University of California – Davis; Y. Wang, Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences; J. Dechant, S. Gee, B. Hammock, University of California – Davis

Polybrominated diphenyl ethers (PBDEs) are a class of compounds that have been used as flame retardant additives since the 1970s. They have been widely used in electronics, furniture foam, and plastics. PBDEs have been found extensively in environmental and human samples. The human health effects from PBDEs at these levels are unknown, but they are suspected to have effects on brain development and endocrine disruption. Human and environmental monitoring programs are often limited by the cost and complexity of sample testing. From previous monitoring work, one specific congener, 2,2',4,4'-tetraBDE (BDE-47) is often the major PBDE congener present and that which is the most frequently detected. Because of this, and based on our previous work developing successful polyclonal antibodies that specifically recognize BDE-47, we aimed to develop a more stable and sustainable source of antibodies specific for BDE-47. These antibodies, single domain antibodies (sdAbs), are more heat and chemically stable, and unlike conventional antibodies allow for cost-effective and unlimited production. Antibody-based assays can complement conventional analytical methods, because they are faster, cheaper and exhibit comparable sensitivities. Here we present an antibody-based analytical method for the rapid detection of BDE-47. Alpacas were immunized with a surrogate molecule of BDE-47 covalently attached to a carrier protein. The resulting mRNA coding for the heavy chain antibodies were isolated, transcribed to cDNA, and cloned into a phagemid vector for phage display library construction. Selection of sdAbs recognizing free BDE-47 was achieved by panning under carefully modified conditions. Sensitivity of the final assay format could detect BDE-47 down to ppb (ug/L) level. Cross-reactivity analyses confirmed that this method was specific for BDE-47, with < 15% activity towards BDE-99 and BDE-100. After being heated to 75°C for 1 hour, the sdAbs retained >75% activity, while the previously developed polyclonal antibodies retained < 25% activity. This heat stability of the sdAbs combined with the ability to produce vast quantities in prokaryote cultures makes it an ideal reagent for immunoassays. Testing of this assay with human samples will also be presented.

These sdAb-based assays could be used for routine biomonitoring efforts to perform screening analyses of samples before more costly follow-up methods are completed.

MP063 Determination of PBDEs and HBCDs in indoor dust and biological material using APPI-LC-MS/MS

R. Kern, AB SCIEX; B. Rut, Lublin University of Technology; A. Schreiber, AB SCIEX

Application of LC-MS/MS with Atmospheric Pressure Photo Ionization (APPI) for the determination of PBDE target congeners and HBCD stereoisomers in indoor dust samples and biological material. Instrumental detection limits (IDL) are included and range from 0.07 ppb to 0.24 ppb for selected PBDE congeners and from 0.12 to 0.32 ppb for HBCD stereoisomers. In recent years polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) have emerged as a subject of great concern because of their increasing levels in the human body, causing disturbance of the thyroid hormone homeostasis and chronic neurotoxicity, and because of their ubiquity in the environment, especially indoors. Indoor dust and biological material have become a repository for PBDEs and HBCDs, resulting in developments of sampling strategies and analytical methodology for determination of these chemicals. Traditionally, GC-MS has been employed for the analysis of PBDEs and HBCDs in environmental samples, but this technique causes thermal degradation of higher brominated PBDE congeners and interconversion of HBCDs. Hence, liquid chromatography coupled with tandem mass spectrometry (LCMS/ MS) has more recently been used for the determination of PBDEs and HBCDs.

MP064 Determination of Isoprostanes in Human Serum by Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS)

W. Guo, DTSC-ECL; S.E. Petropoulou, California Department of Toxic Substances Control; N. Holland, School of Public Health, University of California at Berkeley; M. Petreas, California Department of Toxic Substances Control, California Environmental Protection Agency / Environmental Chemistry Laboratory; J. Park, California Environmental Protection Agency / Department of Toxic Substances Control, Environmental Chemistry Laboratory

F2-isoprostanes (IsoPs) are sensitive and reliable biomarkers for oxidative damage/stress and have been linked to cardiovascular disease, cancer, diabetes, cataract, atherosclerosis, neurodegenerative diseases and aging as well as exposures to cigarette smoking, cancer treatment therapies and environmental pollutants. However, given that IsoPs are a series of isomers with possibly different biological functions, it is still a challenge to accurately monitor their changes in biological samples simultaneously. The measurements are particularly difficult in human blood samples due to endogenous background, low abundance, and relatively small changes in quantities under stress conditions caused by internal/external conditions such as disease or exposure. In the past, gas chromatography tandem mass spectrometry (GC-MS/MS) and immunological approaches were used for isoprostane analyses. Although the GC-MS/MS is a sensitive method, it requires complicated sample preparation involving derivatization steps that may create false positives due to artifacts, while the immunological assay lacks specificity. In the present study, we developed a liquid chromatography tandem mass spectrometry (LC-MS/MS) method using isotopic dilution to simultaneously determine six F2-IsoPs (8-iso-$PGF_{2\alpha}$, 8-iso-15(R)-$PGF_{2\alpha}$, 15(R)-$PGF_{2\alpha}$, $PGF_{2\alpha}$, 11β-$PGF_{2\alpha}$, 5-i$PF_{2\alpha}$-VI) in human serum or plasma. The analytes were extracted from 200 uL of human serum using an Oasis®MAX cartridge (Waters) and measured on a Shimadzu 20AD LC coupled to an AB Sciex 5500 QTrap mass spectrometer system controlled by Analyst 1.5.1. The linearity of the method was demonstrated for the calibration ranges of 31-10,000 pg/mL for six IsoPs isomers with acceptable precision and accuracy. This method was validated in a pilot study (10 participants) and the 8-iso-$PGF_{2\alpha}$ levels were highly correlated with those of obtained by the GC method ($y=0.9928x-0.0316$, $R^2 = 0.7352$). To our knowledge, this is a novel LC-MS/MS approach to simultaneously measure the major isoprostane isomers in human serum. This method will be applied to both clinical and research settings to characterize and elucidate mechanistic pathways of oxidative stress. The views expressed herein are those of the authors and do not necessarily reflect those of the California Department of Toxic Substances Control.

Sustainability and Resource Use in Life Cycle Assessment

MP065 System Assessment of Water Systems Using Life-Cycle Assessment and Water Footprint Approaches

X. Xue, EPA; T. Hawkins, USEPA / Sustainable Technology Division, Systems Analysis Branch; N.J. Ashbolt, National Exposure Research Laboratory, USEPA

Centralized water and wastewater management systems are facing multiple economic and environmental challenges. The alternative water and wastewater systems offer the possibility of meeting current goals while improving environmental performance, and promoting sustainable economic development if a systems-view is considered. We utilized life-cycle assessment and novel water footprint approaches to assess environmental impacts and water requirements for three system configurations: 1) the current centralized drinking water (DW) and wastewater system, 2) centralized DW, but with household composting toilets and local graywater treatment and environmental release, and 3) blackwater-only pressure sewer for energy and nutrient recovery, with local graywater treatment and reuse, and rainwater harvesting and treatment to augment DW supply. We provide a comprehensive evaluation of the whole anthropogenic cycle from raw water extraction to wastewater treatment and reuse; and introduce novel energy, water, carbon, and nutrient inventories to address composting, urine-diversion toilets and blackwater options. The resulting analyses are aimed at facilitating stakeholder decision-making for the next generation of urban water systems, and are only illustrative of the sort of options and trade-offs a community may face. Preliminary results demonstrate that the two alternative systems require less energy and result in lower global warming potential, compared to business as usual with centralized urban water infrastructure. As described, System 3 presents the lowest energy requirement and global warming potential. For System 2 drinking water supply is the dominating element for the total energy consumption, with the composting toilet and graywater release via an existing septic tank contributing to less than 10% of total energy consumption. Hence, application of renewable energy (e.g., energy and heat recovery from PV solar panels, food waste, and others) to offset energy needs for rainwater pumping in System 3 (25% of total energy consumption) or graywater treatment (60% of total energy consumption) in System 3 are examples of stakeholder discussions and subsequent analyses that follow. The energy recovered from blackwater and food residuals could also significantly offset (by at least 45 %) the energy consumption of graywater and rain water treatment. In addition, System 3 provides the lowest eutrophication potential and virtual water requirement.

MP066 LCA as a Measurement Tool for Environmental Performance and Product Improvement in Salmon Aquaculture: Benefits of Innovation using Copper Alloy Meshes

R. Dwyer, International Copper Association / Health, Environment and Sustainable Development Program; N. Ayer, S. Martin, EarthShift, LLC; L. Gace, International Cpper Association, Ltd. / Technology Developemnt & Transfer Program

Aquaculture of Atlantic salmon (*Salmo salar*) and other marine salmonid species is expanding rapidly to meet global demand, with production currently centered in Norway, Scotland, Chile and British Columbia. The industry has been criticized because of several environmental impacts of concern. Changes in the industry are being implemented slowly, in response to recommendations of multistakeholder discussions like the WWF's Salmon Aquaculture Dialogue. However, measurement and demonstration of the effectiveness of improvements has been inconsistent, due to the range of capabilities among aquaculture companies, regional differences, and other factors. Life Cycle Assessment (LCA), adhering to ISO standards, holds promise as a consistent set of metrics to compare operations and measure the effectiveness of implementing infrastructure and process improvements in decreasing the environmental impacts of the industry. In this example, LCA is being used to assess the environmental and other performance benefits of replacing antifoulant-coated nylon mesh nets with similarly-sized mesh woven from copper alloy wire. The copper alloy mesh (CAM) is stronger, more durable, and fully recyclable at end of life. LCA was used to compare a 16-month salmon grow-out in a pilot installation of CAM enclosures in Chile to the 2012 average environmental performance of the nylon net-pen production industry, and also with a grow-out harvest from a "best-in-class" nylon-pen farm. Results of the study showed that use of CAM net-pens reduced contributions to marine aquatic ecotoxicity by up to 50%. Further, the CAM net-pen systems showed statistically significant improvements over the conventional nylon net-pen systems for three of the

other six environmental indicators assessed, and comparable performance for the remaining indicators. The results of these comparative LCAs in Chile identified infrastructure and operational attributes of the CAM net systems where improvements were possible. CAM net pens were installed recently in British Columbia and Scotland, and the earlier LCA results are being used to optimize infrastructure design and operations in the new grow-out cycles, set for harvest in late 2014. These new installations will also be used to gather more LCI data so that the LCA modeling can be updated and continue to be used to optimize design, installation and operational decisions.

MP067 Life Cycle Assessment of Drinking Water Disinfection

T. Hawkins, USEPA; S. Cashman, L. Weiss, Eastern Research Group; N.J. Ashbolt, National Exposure Research Laboratory, USEPA

In January 2011, the US Environmental Protection Agency partnered with the US Small Business Administration and a coalition of private and public sector leaders from the Cincinnati region to form the Water Technology Innovation Cluster (WTIC) in order to develop and commercialize innovative water technologies that solve environmental challenges and spur sustainable economic development in the region. One theme within the cluster has been the development of alternative drinking water disinfection technologies. In the US, roughly 17 trillion gallons of public drinking water are supplied each year. While chlorine is the disinfectant most commonly used, alternatives are being considered to minimize disinfection by-products (which have recently been regulated), improve operator safety, and generally advance sustainability goals. Here we describe a model for estimating the life-cycle human health and environmental impacts and costs associated with a base case drinking water treatment process based on a mid-west US city. While disinfection generally represents a small part of total impacts, the entire process is considered in order to provide an appropriate context within which results for disinfection should be understood. The model was augmented from the base case to represent systems employing alternatives including ferrate, microfiltration, and ultraviolet disinfection. The life cycle assessment (LCA) model was developed in the OpenLCA software platform, using a combination of US LCI and EcoInvent background processes, and with impacts calculated using the TRACI method. Results are presented for the reference system including TRACI impact categories, in particular including human health, global warming potential, and energy demand as well as cost and metrics pertaining to pathogens and disinfection by-products. Preliminary results for disinfection alternatives are also presented. Analysis is performed to identify key parameters affecting outcomes associated with the disinfection process. Moving forward, the intent is that the model developed here will serve as the basis for assessment of additional drinking water treatment alternatives and changes to municipal water systems more broadly.

MP068 Centralized vs. distributed waste water treatment: implications for nutrient impacts

A.D. Henderson, University of Michigan / Environmental Health Science, School of Public Health, University of Texas School of Public Health / Environmental and Occupational Health Sciences

Conventional wastewater treatment plants (WWTPs) are known to have high energy footprints, in addition to affecting receiving streams via nutrient and chemical emissions, and there are efforts to improve their performance, making them resource recovery facilities. There are also efforts to re-think traditional wastewater infrastructure, with a focus on areas that lack such infrastructure: solar outhouses, solar concentrators (www.sanivation.com), and composting toilets. Life cycle assessments of the latter have not been conducted, but centralized WWTPs have been studied, identifying nutrients and metals associated with sludge disposal as strong contributors to normalized impacts. These results should be taken with a grain of (nonmetallic) salt, as characterization factors (CFs) for metals are still under development, and CFs for nutrients are often constants, when recent work suggests strong spatial variation. There are many unknowns regarding the economic and social feasibility of alternative wastewater treatment systems. From an environmental perspective, though, one of the main drivers for comparing traditional WWTPs with any sort of distributed system will be the fate of nutrients. Centralized WWTPs with anaerobic digestion may reduce nutrient loading to some degree, but these systems always discharge directly to waterways. Both dispose of nutrient-rich sludge, though distributed systems will tend to have a lower application rate over a larger area. The study addresses the question of how latter may affect some of the nutrient impacts identified in previous LCAs. In this study, the potential nutrient losses in the soil-sludge-water system will be investigated through the use of the process-based models EPIC / APEX and Manure-DNDC. These analyses will ultimately form the foundation for improved characterization factors for soil-based nutrient emissions in LCA, complementing the new factors for freshwater emissions. A sensitivity analysis will examine the potential benefits of distributed sludge application/disposal, identify best practices for such techniques, and provide insight into the sustainability of centralized vs. distributed waste water management.

MP069 Biochar benefits to water resource recovery facilities: a life cycle assessment perspective for land applications

L. Miller-Robbie, B. Ulrich, D. Ramey, S. Herzog, T. Cath, C. Higgins, Colorado School of Mines / Civil and Environmental Engineering

The long-term sustainability of conventional wastewater treatment is potentially threatened by risks associated with biosolids disposal. Land application is a popular means of disposal and provides the opportunity for nutrient recycling. However, environmental contamination is a concern due to synthetic organic chemicals in biosolids. Biochar, a "charcoal" type product made from pyrolysis of agricultural wastes, may sorb such chemicals and nutrients, keeping them in the soil. Many other benefits of biochar have been shown such as sequestration of carbon, reduced greenhouse gas emissions, and increased soil fertility. Although the benefits and costs of biosolids and biochar have been described separately, their combination has not been investigated. This study uses life cycle assessment (LCA) to evaluate the sustainability of a proposed strategy, in which yard waste is delivered to a biochar plant co-located with a wastewater treatment plant, and upon completion, the two products are mixed and transported for land application. The study boundaries included all materials and energy inputs and outputs, as well as the energy to produce and transport all inputs and outputs. Processes related to biosolids where only included following anaerobic digestion. Three scenarios were evaluated: no biochar added (current case), combined biosolids and biochar, and no land application allowed (as is being considered by many European countries). Life cycle impact categories of energy use (EU), global warming (GWP), eutrophication (EP), and acidification potential (AP) were quantified using process-based LCA methods. Preliminary results show that life cycle inputs for biochar production (at a mixture rate of 9% biochar to biosolids) contributes 4% of EU, 1% of GWP, 5% of AP, and 14% of EP to the total for the combined system. Mixing and transporting biochar and biosolids for land application contributed no more than 8% to any impact category. Therefore, biosolids dewatering processes contributed the majority of all impact categories. Notably, an estimated 76% of the system-wide life cycle GWP could be offset by the carbon stabilized within the biochar. Potential on-site uses for byproducts of biochar production (waste heat, syngas, and bio-oil) were also investigated. This study is a first-step in evaluating the sustainability of a combined biosolids and biochar system for land application. Related human and environmental impacts are also explored in this study.

MP070 Life Cycle Assessment application and sustainable practices for the cardboard production. Case study: Smurfit Kappa Mexico

J.C. Carpio, Smurfit Carton y Papel de Mexico S.A. de C.V. / Process Improvement; L.P. Guereca, UNAM / Environmental Engineering

Given the pressure and demands of the market, the efforts done by some companies to fit their operation with the environmental regulations need to be recognized. This is the case of the application of the Life Cycle Assessment tool (LCA) by Smurfit Kappa Mexico, which belongs to the industry of cardboard and paper production. This sector is incentivized for using various instruments, such as tools for the environmental impacts evaluation to comply with national and international environmental standards During the interpretation phase of the LCA application, from a direct projection of 100 years of the Greenhouse Effect impact, it is noticed that the production of containerboard with virgin fibers has a negative contribution of 30% over the production using recycled fibers. The higher contribution of the production from virgin fibers can be explained by the additional processes implied in the generation of the pulp which requires a bigger amount of resources as water and energy. Due to the importance that today has acquire the production of containerboard with recycled fibers in Mexico, it was decided to carry out a more sensitive interpretation of the results from the LCA application at this specific scenario. The results show that the production of the containerboard itself contributes with an 80% of the total in the Greenhouse Effect emissions, and a 14% is attributed to the transportation

required for the fibers collection. After the application of the LCA with inventory data of the company, official and non-official entities established that the company has to implement different action plans to extrapolate the benefits of using this tool for the improvement of the actual situation. For example, it was identified that one of the main contributions to the Greenhouse Effect is the energy used in the plants; by this issue, the company has decided to promote the use of an International standard (ISO 50001:2011) with the purpose of reaching a continuous improvement in a formal Energy Management System. Generally, different environmental impacts are identified in the containerboard production. For most of them there are policies and procedures. Most of the difficulties that are generated in the production of containerboard with recycled fibers could be decreased mostly with a major public-private interaction.

MP071 Life Cycle Assessment of social interest housing in Mexico

J. Chargoy; A. Sojo Benitez, N. Suppen Reynaga, Center for Life Cycle Assessment and Sustainable Design (CADIS); R. Reyes Mazzoco, Universidad de las Américas Puebla

Mexico has a complex housing problem, which includes social, economic and environmental aspects. This justifies the development of research projects that analyze the environmental impact throughout the life cycle of houses. The objective of this paper is to determine the environmental profile of different Mexican social houses with Life Cycle Assessment (ISO 14040) methodology. The functional unit for the study is: Inhabit a house with the minimum conditions of lighting, insulation and temperature, providing hydro-health services, electricity and gas, through a secure structure of 60 m^2 which allows resting, protection and family integration for 4.5 people for 60 years. Inventory analysis was performed with the collaboration of housing developers, and with literature research. The Impact Assessment was done with SimaPro 7.2, using the method Ecoindicador 99 (H). It was observed that the use phase contributes the most potential environmental impact on the life cycle of social housing, followed by obtaining materials.

MP072 Characterization and sustainable management of photovoltaic waste

K. Collins, Clemson University; K. Tisza, A. Anctil, Clemson University / Environmental Engineering and Earth Sciences

There is a general consensus about the need for photovoltaic modules to be recycled in order to be a truly green product but concrete actions are lacking in the US. The image of photovoltaic (PV) producing large amount of waste that will further aggravate the e-waste problem could have a negative impact on the popularity of PV as a green energy. So far most of the effort has been toward investigating the impacts from raw material production and use in manufacturing PV panels, but little has been done to quantify the impacts associated with their disposal. Various environmental organization have raised the issue of end-of-life and the need for recycling but little as been done in that way, except for few companies that have established voluntary take back programs, mainly to ensure their own future as their technology is based on scarce materials. The most desirable option for PV would be to recycle the whole module, which would result in no waste. By mass, most of the current modules are recyclable considering that more than 80% of the composition is glass (after removal of the frame for crystalline silicon modules). Glass is recyclable but most of the currently recycled glass is container glass and the flat glass used in PV, because of contamination has a much lower value and is usually not recycled as new flat glass but rather used for fiberglass applications or insulation products, as it doesn't meet the requirements for other applications. It is not clear that there is a sufficient market for all the glass generated from PV waste and therefore that recycling is the best option. This gap in knowledge will be addressed by conducting tests simulating disposal. Procedures will be developed for preparing samples for various extraction tests, including EPA Method 1311 and California WET, which will be performed. Characterization of the waste stream from PV will be useful to establish a sustainable pathway for either recycling of valuable material or safe disposal of waste. Life-cycle analysis from gate to grave for photovoltaic will be performed based on these specific experimental results and used to compare the impact of landfilling and recycling options from an environmental and economic point of view.

MP073 Life Cycle Impact Assessments of Photovoltaic Capacity Additions: The optimal rate of deployment with sensitivity to time-based GHG emissions

D. Triplican Ravikumar, M. Chester, T. Seager, M.P. Fraser, Arizona State University

Current (Photovoltaic) PV deployments are motivated by the need to increase long term energy security and reduce the environmental impacts of energy production. The environmental impact assessments of PV deployments are based on the assumption that the environmental benefits can be allocated at the time of installation. However, the environmental benefits of PV system do not accrue immediately after installation but accrue over the entire life cycle of the PV module. There is an inter-temporal trade-off which depends on the magnitude of upfront PV manufacturing GHG emissions and the year-on-year GHGs avoided when PV electricity displaces electricity generated from fossil fuels. Moreover, environmental impact assessments of PV systems preclude temporally sensitive technology factors which impact the environmental benefits allocated to a PV depending on the year of installation. These factors include: the varying rates of technology improvements for different PV technologies, electricity mixes of the deployment location and location where PV systems are manufactured. By not incorporating inter-temporal trade-off analysis and temporally sensitive technology factors that influence net environmental impacts of PV deployments, PV capacity additions can inadvertently become counter-productive by increasing net GHG emissions over the short term. Also, policy makers forgo an opportunity to optimize PV capacity additions for minimal short term GHG impacts. This project designs and implements an optimization model to minimize the short term environmental impacts of PV system deployments by incorporating the inter-temporal trade-offs involved. When integrated with PV LCAs, this model can help policy makers to minimize short term impacts along with fulfilling long term renewable energy policy goals. The results show that the optimal PV deployment strategy for the three states – California, Wyoming and Arizona – varies depending on the electricity mixes of these states. The optimal PV deployment strategy is sensitive to the state of technology and the choice of PV technology to fulfill the targets. Also, adopting a sub-optimal PV deployment strategy to meet California's PV policy targets by importing Silicon PV modules from China will increase the CO_2 emissions over the short term.

MP074 Dynamic Life Cycle Assessment of an optimised biodegradation of petroleum hydrocarbons by a diculture of Azotobacter vinelandii and Pseudomonas sp.

I.N. Onwurah, University of Nigeria, Nsukka / Dept of Biochemistry; O.A. Oje, University of Nigeria, Nsukka / Dept of Biochemistry

Introduction A sustainable approach in ameliorating the negative effect of oily wastewater in the environment can be achieved through an integrated environmental biotechnology. Some methods used in bioremediation of such oily waste water are not sustainable in terms of energy, material resources and soil fertility recovery. In this work, an optimised bioremediation strategy that utilizes a consortium of hydrocarbonoclactic and nitrogen fixing bacteriain limited nitrogen and oxygen medium, was compared with a generalised natural bioremediation method using life cycle assessment methodology. *Methods* The toxicities of the oily wastewater on soil microorganisms, vegetation/plants were used in calculating the impacts of the oily waste water discharges, using LCA methodologies. In this work, the calculation methods and rules for dynamic life cycle assessment in some studies were adapted, with the aim of comparing the ecological impact of petroleum hydrocarbons (PHCs) spill in an ecosystem and the discharge of oily waste water mixed with the consortium of *Azotobacter vinelandii* and *Pseudomonas* sp. before discharge into the environment. *Results* There are many issues in PHC bioremediation LCA, where different assumptions lead to different outcomes. Determining the functional unit, reference situations and system boundaries, as well as the impact calculation methods and kinetics of hydrocarbon degradation and natural attenuation, are important from this point of view. Determination of the natural attenuation in land farming technology for oily waste situation emerged as one of the critical points in the calculations. Time scale can strongly affect the final outcome where effects of long-term discharge of oily wastewater are considered. *Conclusions* The population and type of microorganisms in a given environment determine the attenuation of the PHCs discharged into it. Nitrogen has been noted to be the most limiting nutrient in the bioremediation of PHC contaminated environment. Fixed nitrogen mitigation requires quick actions, and uncertainties related to natural bioremediation are higher for

continuous application. However the bioremediation potential of oily waste water mixed with the consortium was optimised for each area that received the waste. The study showed that such compatible hydrocarbonoclactic and diazotrophic bacteria should be developed for sustainable treatment of oily waste before discharge into the environment or in bioremediation of crude oil polluted environment.

MP075 Effects of Co-products on the Life-cycle Impacts of Microalgal Diesel

K. Soratana, Arizona State University / School of Sustainable Engineering and The Built Environment; W.J. Barr, Arizona State University / School of Sustainable Engineering and The Built Environment, Arizona State University; C.L. Antaya, A.E. Landis, Arizona State University / School of Sustainable Engineering and The Built Environment

Microalgal diesel production has been investigated for decades, yet it is not commercially available. Part of the problem is that the production process is energy and chemical intensive due, in part, to the high portion of microalgal biomass left as residues. The lipid content makes up only 30-40% by weight of microalgal biomass. This study investigated cradle-to-gate life-cycle environmental impacts from six different scenarios of microalgal diesel and combination of its co-products. The co-products considered are land application or animal feeds produced from (1) lipid-extracted microalgae (LEA), (2) distillate residues and (3) simultaneous saccharification-fermentation (SSF) residues, bioethanol produced from LEA, biomethane produced from (1) LEA, (2) SSF residues and (3) glycerol, and land application from solid digestate and recycling nutrients from liquid digestate of anaerobic digestion process. Global warming potential, eutrophication potential and photochemical smog formation potential were the three environmental impact categories assessed using a life cycle assessment framework and the Tool for the Reduction and Assessment of Chemical and other environmental Impacts (TRACI). The results are presented to indicate processes with the major contribution in each environmental impact category, while simultaneously evaluating tradeoffs among different co-product scenarios. Net energy ratio of each scenario is also calculated. The main contributions of this process life cycle assessment are to inform the industry on the environmental impacts from different microalgal diesel co-product pathways and opportunities for production process improvement.

MP076 The Public Health Implications of Road Freight Transportation in the United States: A Life Cycle Assessment Approach

M.I. Elsaid, Extension School Sustainability and Environmental Management Program; R. Sanchez, Harvard University / Extension School Sustainability and Environmental Management Program

Road transportation of freight is a major source of criteria air pollutants, greenhouse gases (GHG) and air toxics. Meanwhile, current regulations address emissions from a production view with a sole tie of public health impacts of the road freight transportation process with emissions associated with the use phase (i.e., tailpipe). The life cycle assessment (LCA) of the road freight transportation process includes feedstock life cycle, truck life cycle (i.e., truck manufacture, use, maintenance and disposal), and roadway life cycle (roadway construction, maintenance, and disposal). All elements of the road freight transportation process have the potential to adversely impact public health. Accordingly, this research aimed to construct the first complete LCA model that captures all impacts associated with all life cycle elements of the road fright transportation in the United States. Subsequently, TRACI (2.0) and ReCiPe (2008) impact assessment methodologies were used to evaluate the public health burden of the constructed LCA model. The road freight life cycle climate change impacts were relatively high, with an overall score of 0.162 (kg CO_2 eq.)/ton-mile. Carbon dioxide accounted for 95.60% of global warming impacts, of which 87.4% originated from truck operation phase. The truck operation phase accounted for 81% of the aggregate life cycle respiratory effects score of 1.63E-04 (kg PM_{10} eq.)/ton-mile. Carcinogenic impacts were found to be 2.83E-09 CTUh/ton-mile, while the non-carcinogenic category scored 4.79E-08 CTUh/ton-mile. Nearly all carcinogenic and non-carcinogenic effects to human health (99.99%) were attributed to 24 air emissions, 13 emissions to soil, and 11 emissions to water.The feedstock life cycle accounted for 66% and 37% of full life cycle carcinogenic and non-carcinogenic impacts, respectively. ReCiPe end-point results were adjusted to reflect on TRACI's results. The human health end-point score of the life cycle was found to be 3.19847E-07 DALY/ton-mile, a 14.81% aggregate drop from ReCiPe's original model. The truck operation phase share of the life cycle's end-point score was 61.75%, while the feedstock was 28.48%. The public health burden of one ton-mile of road freight activities corresponded to a monetary value of $2.015E-03. The total road freight activities in 2007 accounted for 1.35 trillion ton-miles, which correspond to health burden cost of $2.72 billion.

MP077 An Interactive Display for Results of Food Life Cycle Assessments

A.J. Berardy, Arizona State University / School of Sustainability

Life cycle assessment is a useful tool in accounting for the inputs and outputs of a process or product all the way from resource extraction to end of life. LCA is one of the most common methods for environmental assessment, and has been applied to everything from solar panels to soy beans. One of the unique features that make LCA so versatile is the functional unit, which allows multiple alternatives serving the same purpose to be compared. However, the functional unit of a given food item is debatable, and the functional unit of a diet is even more difficult to define, despite there being several LCA studies comparing varying diets. The default move is to use weight or calories as a universal functional unit, as these are fairly neutral factors. This makes comparison of different types of food different. One kilogram of lettuce compared to one kilogram of beef is not a fair comparison to make. Likewise, 100 calories of orange juice cannot be fairly compared to 100 calories of rice. Instead, an alternative method to display results that can still utilize findings reported in this manner would be to report the impacts based on multiple different nutrients. Calories, protein, fat, carbohydrates, and more are all of concern to the average person. If the food is known, and the impacts per weight are known, the impacts can be converted based on a nutritional profile available from a national nutrition database. The problem then is that the dataset gets very large as each new food and nutrient type is added. The solution proposed here is to create an interactive display that allows the reader to delve into however much detail they want regarding the food's impacts based on multiple functional units. This graph would resemble those on the website "Gapminder" in that the axes would be variables that the user could change. The default could be calories on X versus GHG emissions on Y, but the X axis could change to any nutrient, while the Y axis could change to other environmental impacts such as land use or water use. This display should allow readers to get a far better idea of the entire picture when comparing multiple foods or diets by allowing them to find what is most meaningful to them, not just the researchers.

MP078 Considering use phase exposure to cosmetic chemicals

A. Ernstoff, University of Michigan / School of Public Health; P. Fantke, DTU Management Engineering / IER; A.D. Henderson, University of Michigan / Environmental Health Science, School of Public Health, University of Texas School of Public Health / Environmental and Occupational Health Sciences; O. Jolliet, University of Michigan / School of Public Health

For products used by consumers in daily life, such as cosmetics, use-phase exposures to chemical ingredients may exceed exposures due to far-field, upstream and downstream life cycle emissions. To date, use-phase exposures have not been considered in an LCA framework; though they have been considered in risk assessments, such exposures have been on a chemical by chemical basis. To scientifically support design or policy decisions, enhanced understanding of consumer use-phase exposures is needed. We use a shampoo case study to demonstrate a modeling framework for consistently evaluating use-phase and environmental exposure pathways for aqueous-phase cosmetics. Since many compounds used in cosmetics lack empirical dermal uptake data, we evaluate existing skin permeation models against in vitro data collected from the literature. This model comparison supports applying the ten Berge model ($R^2 \approx 0.65$, $GSD^2 \approx 23$). We compare environmental to use-phase exposure via three routes: first, inhalation of the gas phase; second and third, skin permeation by the aqueous and gas phases. The resulting intake fractions for five different chemicals in shampoo range from 0.0005 to 0.4 kg_{intake} / $kg_{in\ product}$, as a result of varying chemical properties, such as the octanol-water partition coefficient. Across these compounds, skin permeation is the dominant intake route. Use-phase aqueous dermal intake and inhalation, as well as environmental intake uncertainties, generally span around 3 orders of magnitude, with uncertainty on the former largely due to modeled skin permeation coefficients. The sum of use-phase intakes can be up to 4 orders of magnitudes greater than the sum of environmental intakes. As use-phase intake decreases, environmental intake tends to increase, and vice versa. We will discuss uncertainty associated with the skin permeation modeling as well as use characteristics. Nonetheless,

the model suggests relative magnitudes of exposure; coupled with toxicity information, this approach may lead to chemical prioritization and substitutions to promote consumer safety. Our results emphasize the importance of considering both use-phase and environmental pathways when assessing exposures across a product's life cycle.

Wildlife Exposure and Effects Relative to Emerging Anthropogenic Substances

MP079 Bobcats as biological indicators for brominated flame retardants

E. Jooste, Southern Illinois University Carbondale / Cooperative Wildlife Research Laboratory; D. Chen, Carleton University / Chemistry, Ecotoxicology and Wildlife Health Division, Wildlife and Landscape Directorate, Science and Technology Branch, Environment Canada, National Wildlife Research Centre, Carleton University / Department of Chemistry, Southern Illinois University Carbondale / Cooperative Wildlife Research Laboratory; C. Nielsen, Southern Illinois University / Cooperative Wildlife Research Laboratory

The monitoring of contaminants in the environment is essential to the health of humans and wildlife. To comprehend and compare spatial and temporal trends in these contaminants, it is essential that they are analyzed and reported in a standardized manner. Bioindicator species can be used to assess the status of ecosystems at risk, as well as monitor changes. Ideally, a biological indicator species should have a high trophic status, have a widespread distribution, have a well-known biology, and be present in numbers sufficient enough to be studied. Since mammalian wildlife are physiologically similar to humans, they are considered useful bioindicators for environmental monitoring in ecosystems, and high concentrations of contaminants in their tissues could therefore indicate a similar pattern in humans. Mammalian carnivores may be especially useful for evaluating toxin levels in ecosystems because they occupy high trophic levels and accumulate ingested substances, including emerging organic contaminants such as brominated flame retardants (BFRs). Bobcats (*Lynx rufus*) are widespread throughout North America and in the absence of larger carnivores, serve as the apex predator in many ecosystems. Here we evaluate the suitability of using bobcats as biological indicators to detect the contamination of a suite of BFRs in terrestrial ecosystems. Other persistent organic pollutants, such as polychlorinated biphenyls (PCBs) and organochlorinated pesticides and herbicides, will also be studied.

MP080 Examination of Brominated Flame Retardant Contamination in Illinois Waters through Fish Monitoring

M. Widelka, Southern Illinois University Carbondale; T. Hornshaw, Illinois Environmental Protection Agency; D. Chen, Southern Illinois University Carbondale

The Illinois Environmental Protection Agency employed the Fish Contaminant Monitoring Program to monitor heavy metal, pesticide, and PCB pollution within Illinois aquatic environments. However, this monitoring effort has not examined brominated flame retardant contamination. Brominated flame retardants are substances added to plastics and electronics to prevent spontaneous combustion. Due to their chemical nature, brominated flame retardants escape from products and enter the aquatic environment. Brominated flame retardants have shown to reach high levels within predator species of fish and mammals, and may effect thyroid hormone levels, neurodevelopment, and reproductive success. The Illinois Environmental Protection Agency has archived samples of fish ranging back to the 1980s. With the Agency's cooperation, archived and recent fish samples will be tested for nine brominated flame retardant compounds. Impaired river systems, as well as their sources of contamination, will be identified. Environmental and human health risks, as a result of the impaired river systems, will be determined. The concentrations of brominated flame retardants will also be compared against known thresholds to determine whether there are any current adverse effects on aquatic wildlife. This information may be used by the Environmental Protection Agency to regulate further monitoring projects or take actions to reduce the amount of contaminants within Illinois waters.

MP081 Combined Effects of Microplastics and Fluoranthene on Hyalella azteca

S. Au, Clemson University Institute of Environmental Toxicology; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX)

The contamination of aquatic ecosystems by plastic debris has received increasing global concern as worldwide annual production has reached approximately 280 million tons in 2011. The plastic types that account for about 70% of the total global demand include: polyolefins (i.e., polyethylene and polypropylene), polyvinyl chloride, polystyrene solid, expandable polystyrene, and polyethylene terephthalate. Packaging (39.4%) and building and construction (20.5%) are the main plastic applications. Depending on the plastic, microplastics can either be directly manufactured for the purpose of producing cosmetic and cleaning products or indirectly enter the environment via the photooxidation and physical abrasion of macro-sized plastics. In addition to being primary stressors to aquatic organisms, microplastics can adsorb other contaminants, including persistent organic pollutants (POPs). This study characterized the adsorption of a common organic contaminant, fluoranthene, to polyethylene (PE) microbeads. In addition, the bioavailability of the adsorbed fluoranthene was quantified. These microplastics may compose upwards of 85% of debris in sediments. Hence, this research focused on the toxicity of microplastics and adsorbed fluoranthene on the benthic invertebrate *Hyalella azteca*. Preliminary results suggest that fluoranthene adsorbs to PE and that together they are toxic to *H. azteca*. This poster will present results of both acute and chronic bioassays illustrating the effects on survival, growth and reproduction.

Integrated Effects and Exposure Assessment and Risk Management of Endocrine Active Compounds

MP082 Stability of Carp Vitellogenin Under Deleterious Shipping and Storage Conditions

S.E. Bartell, Normandale College Community College; B.L. Olson, H.I. Schoenfuss, St. Cloud State University

Vitellogenin (Vtg) is the most widely depended on biomarker of exposure to endocrine disrupting chemicals in oviparous vertebrates. However, there is a scarcity of published studies regarding the stability of this protein under conditions often experienced by scientists working at field sites or in studies that require samples to be shipped to laboratories. Field collections, laboratory protocols, transport and shipping expose Vtg to potential degradation and subsequent misinterpretation of measured Vtg concentrations. Lack of detailed knowledge concerning the stability of this protein is hampering the ability of researchers to design experiments appropriately and to accurately interpret data. In this study, we subjected plasma samples from six gravid female common carp (*Cyprinus carpio*) to a variety of temperature and storage regimes including most of the conditions likely experienced in field studies or during shipping of plasma samples. Furthermore, we tested the utility of stabilizing agents to prevent break down of the protein during inclement collection and storage conditions. Vitellogenin concentrations were measured at multiple time points via enzyme-linked immunosorbent assay (ELISA) using a species-validated antibody and purified carp Vtg to determine relative differences of measured Vtg concentrations between treatment conditions. Even under standard laboratory conditions, Vtg will begin to degrade within hours of blood collection. Several commonly used field methods resulted in the rapid degradation of Vtg in fish blood or plasma. Storage of frozen plasma and especially freeze – thaw cycles were detrimental to Vtg recovery. Interestingly, whole blood stored on wet ice performed better than plasma stored under similar conditions. Trehalose and glycerin stabilizers both improved recovery of Vtg after storage. Information obtained through these studies will inform logistic considerations especially for field conditions and will help researchers to more accurately incorporate Vtg data into their findings.

MP083 Alkylphenols as Case Studies for Determining the Relevance of the Endocrine Mode of Action in Human Health and Environmental Assessments

K.K. Coady, The Dow Chemical Company / Toxicology & Environmental Research and Consulting; C.A. Staples, Assessment Technologies, Inc.; T.G. Osimitz, Science Strategies, LLC / President; B. Losey, RegNet Environmental Services; J. Klapacz, The Dow Chemical Company / Toxicology & Environmental Research and Consulting

Efforts to develop approaches and guidelines for the assessment of endocrine active compounds are now underway in various regions. Nonylphenol (NP) and octylphenol (OP) are data rich chemicals that provide an opportunity to examine the relevance of the endocrine mode of action to human health and environmental assessments. Systematic assessment of the potential endocrine activity of NP and OP in well-conducted screening-level studies (*in vitro* and *in vivo*) confirm that these compounds exhibit weak estrogenic-like activity with potencies generally ranging from 1,000 to 1,000,000-fold weaker

than the endogenous estrogen, estradiol. However, multiple lines of evidence indicate that these alkylphenols have other relevant modes of action in biological systems apart from just weak endocrine activity. Closer assessment of the toxicological data sets for NP and OP demonstrate that other modes of action, apart from endocrine activity, are influential in both human health and environmental assessment of these endocrine active compounds. This is apparent when examining the effects in higher-tiered, definitive toxicity tests (chronic, multigenerational *in vivo* studies with apical endpoints) which indicate that the weak endocrine activity of NP and OP does not predict the entire suite of observed adverse effects in both mammalian and non-mammalian systems. Concentrations of these alkylphenols detected in human biomonitoring studies are orders of magnitude below levels of concern for human health, and the great majority of concentrations of NP and OP in the environment are below levels of concern for environmental organisms. These case studies with NP and OP illustrate the need to incorporate the concepts of potency, critical effect, exposure, and risk in decision-making regarding determinations of endocrine disruption and assessments of human health and environmental impacts.

MP084 Workshop on lessons learned, challenges, and opportunities: The US Endocrine Disruptor Screening Program

E.M. Mihaich, Environmental and Regulatory Resources; R. Becker, American Chemistry Council; S. Borghoff, ILS; W. Casey, NIEHS; M. Dourson, TERA; J. Fowle, Independent Consultant; T. Hartung, Johns Hopkins University; M. Holsapple, Battelle; B. Jones, ILS; D. Juberg, Dow Agrosciences; O. Kroner, TERA; J. Lamb, Exponent; S. Marty, Dow Chemical Company; L. Rinckle, ILS; G. Van Der Kraak, University of Guelph; M. Wade, Health Canada; C. Willett, Humane Society of the US; J. Patterson, TERA

Fifty-two chemicals were recently screened using some or all of the 11 USEPA Endocrine Disruptor Screening Program (EDSP) Tier 1 assays and the data has been submitted to the EPA for review. Over 240 scientists participated in a workshop on the EDSP in April 2013 to share scientific learnings and experiences with the EDSP and identify opportunities to inform ongoing and future efforts to evaluate the endocrine disruption potential of chemicals. The first session focused on the conduct and performance of the 11 Tier 1 assays. Speakers and workshop participants highlighted challenges in conducting the assays and solutions developed by the laboratories, as well as issues relevant to data interpretation. The second session focused on how to apply relevant information from the current Tier 1 battery to identify potential modes of action and the value of a weight of evidence (WoE) assessment for evaluating potential interactions with endocrine pathways. Presentations and discussions explored the development of critical systematic evaluation of existing data prior to implementation of Tier 2 testing, and application of alternative data to replace Tier 1 assays. The third session provided perspectives on the future of endocrine screening and the promise of *in vitro* high-throughput analyses, toxicity pathways, and prediction models. A number of common themes emerged from the extensive discussions, including that a critical review and update of current Tier 1 screening guidelines is needed, the use of biomonitoring data for exposure-based prioritization should be evaluated, reducing the number of animals used in testing should be a goal, and the use of a robust WoE approach to align available Tier 1 data with potency and exposure information to better inform decisions on Tier 2 testing is needed. The presentation will summarize the discussions and present recommendations for improvements in future screening and testing practices.

MP085 Toxicity of environmentally relevant (low µg/L) concentrations of nanosilver to fish

J.D. Martin, Trent University / Division of Analytical and Environmental Toxicology; L. Murray, M.D. Rennie, University of Manitoba; C.D. Metcalfe, Trent University / Environmental & Resource Studies

Nanomaterials are becoming increasingly popular in consumer products due to their unique physico-chemical properties. Nanosilver is found in a wide range of food packaging materials, textiles, electronics, household appliances, cosmetics, and medical devices for its antibacterial properties. As a consequence of increased use, there are concerns that nanosilver is entering waterways from municipal sewage systems in low (µg/L) concentrations. The risks of low concentrations of nanosilver to aquatic ecosystems and the actual mechanisms of toxicity in fish are largely unknown. The toxicity of nanosilver may be due to the nanoparticle itself, the release of silver ions, or a combination of both. The goal of this research was to determine if nanosilver is toxic to fish at environmentally relevant (low µg/L) concentrations. Juvenile yellow perch (*Perca flavescens*) and fathead minnows (*Pimephales promelas*) were exposed to waterborne dilutions of either a nanosilver suspension or silver nitrate ($AgNO_3$) in both acute (< 5 d) and chronic (>21 d) exposure regimes. Gill, liver, and muscle tissue were collected for measurements of biomarkers and oxidative stress responses including glutathione, lipid peroxidation, metallothionein, heat shock proteins, and cytochromes P450 1A1 and 3A4/5. Histopathological examination for thickening of epithelia gill tissue was completed and silver accumulation was measured in the muscle and kidney. Overall, the results of this research help elucidate whether short- and long-term exposures to environmentally relevant concentrations of nanosilver cause sublethal toxicity in fish.

Endocrine disrupting chemicals and Pharmaceuticals in the Environment

MP086 Development of high through-put mouse cell bioassay responsive to both glucocortocoid-like and progesterone-like compounds

A. Miles, University of California / Department of Animal Science; J. Zhao, University of California / Department of Environmental Toxicology; J.C. Brennan, Department of Environmental Toxicology; G. He, University of California; M.S. Denison, University of California / Department of Environmental Toxicology

Many environmental contaminants can cause alterations in behavior, reproduction, and endocrine homeostasis in humans and wildlife. These endocrine disrupting chemicals (EDCs) have been identified in a wide variety of environmental matrices, food/feed samples and commercial and consumer products, and the majority exert their effects through mimicking and/or inhibiting the action of steroid hormones. Glucortocoids are regulators of important physiological, immunological and stress responses, yet few techniques are currently available for detection of xenobiotics that impact the glucocorticoid receptor (GR) signaling pathway. Accordingly, we report the development, optimization, and application of a stably transfected recombinant mouse hepatoma cell line that responds to glucocorticoids through induction of firefly luciferase in a concentration, time, chemical and GR-specific manner. This mouse GR-CALUX (chemically-activated luciferase expression) cell bioassay is very sensitive (minimal detection limit and EC50 for the glucocorticoid dexamethasone (DEX) is 1 pM and 13 nM, respectively). We optimized the mouse GR-CALUX cell line by examining the performance in a variety of culture media and serums, incubation temperatures, and incubation times. Response to DEX was observed as early as four hours of incubation. The presence of phenol red, a reported weak GR agonist, in the culture media had no affect on GR-dependent reporter gene expression in the absence or presence of DEX. Additionally, in contrast to other hormone receptor-based cell bioassays, charcoal stripping of the fetal bovine serum did not affect GR activity. Luciferase reporter gene activity was significantly enhanced when cells were incubated with DEX at 33 ºC as compared to incubation at 37 ºC. Since the mouse GR-CALUX cell bioassay also responds to progesterone, but was not responsive to other hormones (i.e., testosterone, estrogen, or retinoic acid), it is a useful screening tool for detection of both GR/PR agonists/antagonists. The utility of the GR/PR-CALUX cell bioassay for high-throughput screening was demonstrated through rapid analysis of a collection of more than 150 pesticides and 30 industrial chemicals for GR/PR agonists/antagonists. This new bioassay system provides a relatively rapid and inexpensive approach for the detection and relative quantitation of chemicals, chemical mixtures and sample extracts that can activate and/or inhibit these key steroid hormone signaling pathways.

MP087 Chlorinated parabens in the water environments and their AhR potency

M. Terasaki, University of Shizuoka / Institute of Environmental Sciences; M. Makino, University of Shizuoka / Graduate School of Nutritional and Environmental Sciences

The aryl hydrocarbon receptor (AhR) potency of 24 compounds of chlorinated parabens (ClPBs), including parent parabens (PBs) and their degradation products found as pollutants in the aquatic environment, was measured using a yeast assay. We specifically focused on the AhR potency of PBs in water samples extracted from swimming pools and rivers that receive effluent from sewage treatment plants (STPs). Sixteen compounds exhibited AhR potency, with their relative potency to β-naphthoflavone (BNF)

ranging from 5×10^{-5} to 9×10^{-2}. All mono-ClPBs exhibited an AhR potency greater than their corresponding parent and dichlorinated parabens. Hydroxybenzoic acid derivatives (*i.e.*, the primary degradation products of PBs) did not elicit any significant response (at < 10^5 nM). Fourteen compounds were found in both swimming pool and river water when using gas chromatography–mass spectrometry, with their total concentrations ranging from 350 ng/L to 1100 ng/L and from 95 ng/L to 280 ng/L, respectively. All water samples exhibited AhR potency, with their BNF equivalents ranging from 4.0 pM to 330 pM. The contribution ratio of active compounds accounted for 0.035–5.4% of the observed potency of water samples. Dichlorinated propylparaben exhibited the highest contribution ratio among the active components, followed by propylparaben. This study provides the first confirmation of the AhR potency of PBs using a yeast assay. In conclusion, although the chlorination of PBs markedly enhances AhR potency in compounds, their impact on the aquatic environment remains minimal.

MP088 Verification of Fish Multi-Generation Test with the Estrone Using Japanese Medaka (Oryzias latipes)

N. Tatarazako, National Institute for Environmental Studies / Endocrine Disrupter Research Laboratory; A. Nakamura, University of Tokyo; H. Watanabe, National Institute for Environmental Studies / Center for Environmental Risk Research; I. Tamura, National Institute for Environmental Studies; H. Takanobu, National Institute for Environmental Studies / Center for Environmental Risk Research; T. Iguchi, National Institute for Basic Biology

Fish multi-generation test on development by United States and Japan is the 27 weeks test that expose target chemical to fish for three generation: F0 (adult period), F1 (embryo to adult period), and F2 generation (embryo to sub-adult period). Main endpoints are reproduction (F0, F1), hepatic vitellogenin concentration(F0, F1, F2), secondary sex, intersex and histopathological observation of gonad (F0, F1, F2). It is also positioned as final test on OECD framework testing for endocrine disrupters of vertebrate. On 2012, latest revision of the protocol was constructed on drafting group meeting. Thus we verified this protocol with Estrone as the positive control of estrogenic compounds using Japanese medaka (*Oryzias latipes*). As a result, on F0 generation, only weak increase of male hepatic vitellogenin concentration was observed as effect. In contrast, on F1 generation, decrease of fecundity and fertility, attenuation of male secondary sex, appearance of intersex, and abnormality of testis were observed. Similar effects except reproduction are also observed on F2 generation; disparity of these effects between F1 and F2 generation was not evidently.

MP089 Temporal trends of emission, environmental level and human exposure of POPs over last 10 years (1999-2010) in Korea: Implication to science and policy

S. Kim, BK-21, School of Earth and Environmental Sciences, Seoul National University / Department of Marine Science, College of Natural Sciences, Incheon National University; J. Yoon, Center for Chemical Safety Management, National Institute of Environmental Research

Despite the first comprehensive reviewing on POPs status in Korea, a previous review chapter (Departments in Environmental Science, Volume 7, Chapter 2) including including emission, contamination and exposure, fate and transport, and national management strategy could not discuss and evaluate the temporal trends and the effect of the efforts and policies invested in POPs control and management, since most data were based on individual research results of academic groups in which POPs could not be systematically monitored in terms of time and space. Recently, we have collected monitoring data long enough in time (over 10 years) and wide enough in space (covering various land-use patterns and the Korean peninsula), which were produced at national monitoring stations under the governmental programs. This study aimed to elucidate the temporal trends of POPs emissions, concentrations in multiple compartments (air, water, soil, sediment, organisms, and marine products), and human exposure. The chronological data available for all the subjects investigated were present only for PCDDs/DFs and coPCBs. Their emission reduction with half-lives of ca. 2 yrs was followed by contemporaneous decrease of contamination levels in inland compartments, while a considerably slow or slight reduction occurred in human exposure and its related compartments (fishes and shellfishes as foodstuffs consumed, and marine compartments). The findings prove that a lag-time is present for the efforts of emission reduction to be so much effective as to be reflected directly in human exposure, and such a lag-time can be related with the fates connecting inland and marine environments. PCBs showed faster reduction in human exposure than dioxin-like compounds. As for other POPs, chronological trends and half-lives could not be determined owing to low detection frequencies of PCBs and OCPs in environmental compartments, the absence of monitoring data for OCPs in human exposure, and data limitation for emerging POPs present in recent a few years. Monitoring strategies are also recommended based on this meta-analysis.

MP090 Distribution and bioconcentration of endocrine disrupting chemicals among water, suspended particulate matter, algae, and fish

J. Yang, H. Li, Guangzhou Institute of Geochemistry / State Key Laboratory of Organic Geochemistry; Y. Ran, Guangzhou Institute of Geochemistry / Chinese Academy of Sciences

Endocrine-disrupting chemicals (EDCs) were investigated in the water and bile samples of feral fish species collected from the Pearl River Delta. They were analyzed by derivatization with pentafluorobenzoyl chloride (PFBOCl), and gas chromatography mass spectrometry (GC-MS) with negative chemical ionization (NCI) technique. The concentrations of 4-*tert*-octylphenol (OP), 4-nonylphenol (NP), and bisphenol A (BPA) in the water samples are in the ranges of 1-14 ng/L, 117-865 ng/L, and 4-377 ng/L, and those in bile are in the ranges of 14-39 ng/g bile, 950-4648 ng/g bile, 70-1020 ng/g bile, respectively. Aqueous or particulate EDCs respectively show significant correlations with DOC, POC, and Chl a, suggesting that biological pumping effect regulates their distribution and bioaccumulation. The partitioning coefficients (log K_{oc}) estimated for EDCs are much higher than the empirical log K_{oc}-log K_{ow} correlation, which is related to the heterogeneity of organic matter. The bioconcentration facors (BCF) of OP, NP and BPA for the investigated carp bile are in the ranges of 1500-12960, 1648-11137 and 3583-14178 with a mean of 4731, 5170, and 9027, respectively. Log BCF values for the fish bile and algae are higher than the reported log BCF-log K_{ow} correlation, which is possibly related to the influence of eutrophication in the investigated water ecosystems. It is found that the calculated estrogen equivalents (EEQs) in the fish bile are significantly related to those in the water samples. This investigation for the first time illustrates that the analysis of fish bile could reflect the concentrations of EDCs in the water environment and the internal exposure of EDCs to the investigated fish species.

MP091 Pharmaceuticals and other contaminants of emerging concern (CECs) in source and treated drinking waters from 25 drinking water treatment plants

E.T. Furlong, US Geological Survey / National Water Quality Laboratory; A.L. Batt, USEPA Office of Research and Development / National Exposure Research Laboratory; S.T. Glassmeyer, USEPA / Office of Research and Development; D.W. Kolpin, USGS

The widespread presence and distribution of pharmaceuticals & anthropogenic waste indicators (AWIs) in surface and ground water has created substantial scientific and public interest in their corresponding presence and distribution in water supplies and potential consumer exposure in drinking water. Thus, a comprehensive assessment of a wide range of CEC classes is necessary to assess potential for exposure to mixtures of these compounds. In response, the US Geological Survey and the US Environmental Protection Agency jointly conducted a study of CECs in water from 25 drinking water treatment (DWTP) plants across the United States, sampling ground- and surface-water sources prior to and after treatment processes commonly used to produce drinking water. The DWTPs studied reflected diverse geographic locations, water sources, disinfectants, and plant sizes. Five complementary methods were used to determine 199 CECs, including 134 pharmaceuticals. Subsets of compounds common to two or more methods were used to verify detections. Pharmaceuticals were detected in both source and treated water samples, ranging in concentration from 2.5 to 940 ng/L, with more frequent and higher concentrations generally occurring in source water samples. Metformin, tramadol, and carbamazepine were the most frequently detected pharmaceuticals in source water samples at maximum concentrations of 730, 42, and 40 ng/L, respectively. Metformin, carbamazepine and cotinine were most frequently detected in treated water samples at maximum concentrations of 92, 17, and 16 ng/L, respectively. Concentrations of carbamazepine, frequently detected in source and treated waters and measured in four methods, were significantly correlated. The AWIs caffeine, N,N-diethyl-meta-toluamide (DEET) , and tri(2-butoxyethyl) phosphate were most frequently detected in source water at maximum concentrations of 130, 98, and 470 ng/L, respectively. Bromoform , caffeine, and DEET were the most commonly detected AWIs in treated water

samples at maximum concentrations of 3300, 75, and 25 ng/L, respectively. Frequent high concentrations of bromoform likely are due to formation as a disinfection by-product. These preliminary results suggest that multiple pharmaceuticals and AWIs are present at concentrations below 1000 ng/L in both source and treated drinking water. Treatment processes evaluated in this study reduce detections and maximum concentrations of many, but not all, measured CECs.

MP092 Antibiotics and non-steroidal anti-inflammatory drugs (NSAIDs) in a characteristic urban stream of Argentina

Y. Elorriaga, D.J. Marino, A.E. Ronco, Centro de Investigaciones del Medio Ambiente, Dto. Química, Facultad de Ciencias Exactas, UNLP, CONICET; P. Carriquiriborde, Faculty of Exacts Sciences, Universidad Nacional de La Plata

Pharmaceuticals are emerging pollutants that continuously are entering the aquatic environments via domestic discharges. In the present study the occurrence and concentration levels of commonly used antibiotics (cephalexin, clarithromycin, ciprofloxacin, levofloxacin, chloramphenicol) and NSAIDs (ibuprofen, diclofenac) were assessed in the "El Gato", a typical urban stream of the "Gran La Plata" area (Buenos Aires, Argentina) receiving several storm water, wastewater and industrial discharges. Nine sampling points were selected along the whole water course. The pharmaceuticals were analyzed in the dissolved fraction of the surface water samples by HPLC/MS/MS after SPE extraction. The presence of the studied pharmaceuticals was detected in all sampling sites. Higher concentrations were found in sites closer to wastewater discharges. Within the NSAIDs, ibuprofen was the most frequently found and at highest concentrations (0.6 – 9.7 µg/L), while diclofenac was frequently detected and at lower levels of concentration (N.D – 0.2 µg/L). The prevalence of antibiotics was in the following order: cephalexin (0.54 – 6.3 µg/L) > clarithromycin (0.05 – 0.79 µg/L) > levofloxacin (N.D – 0.49 µg/L) > ciprofloxacin (N.D – 0.39 µg/L) > chloranphenicol (N.D). Although at low levels, the ubiquous presence in surface water samples of a diverse group of antibiotics and NSAIDs alerts on the potential aquatic ecological risk of pharmaceuticals mixtures in urban streams of Argentina. Acknowledgements: INTA Estación Experimental Agropecuaria Balcarce, CONICET PIP0410 y ANPCyT PICT1598.

MP093 Optimization and Mechanism of Metoprolol Degradation during chlorine disinfection combined with UV photolysis

S. Nam, Seoul National University; K. Zoh, Seoul National University / Department of Environmental Health, School of Public Health

Metoprolol (MTP), which is known as a hypertension depressor, has increasingly appeared in conventional water treatment processes (WTPs). Disinfection is known to effectively oxidize the refractory contaminants in WTPs. In this study, the degradation of MTP was investigated using various treatment systems such as chlorination (Cl_2), UV photolysis (UV-C), and chlorination/UV-C reactions. The effects of changing UV light intensity (1.1~4.4 mW cm^{-2}), chlorine dose (1~5 mg L^{-1} as Cl_2), pH (2~9), and dissolved organic matter (DOM, 1~4 mg C L^{-1}) on MTP removal were examined using a two-level factorial design. Among three reactions, chlorination/UV-C was the most effective process for MTP removal. To optimize MTP removal during chlorination/UV-C reaction, total sixteen experiments using four factors were conducted. Among four factors, DOM inhibition of OH radical was the most important factor in the terms of MTP removal. The established model fitted well with the actual results in both surface water and tap water samples. Using the established model, the optimized conditions (UV-C = 4.4 mW cm^{-2}; $[Cl_2]$ = 5 mg L^{-1}, pH = 7, and [DOM] = 0.8~1.1 mg C L^{-1}) were found to achieve at least 78.9% of MTP removal. During chlorination/UV-C reaction, total five intermediates (m.w.: 171, 211, 309, 313, and 341) were identified with LC/tandem mass. Using these intermediates found, the degradation mechanism of MTP during chlorination/UV-C reaction was constructed.

MP094 Degradation Mechanism of Carbamazepine During Ozonation Reaction

B. Jo, S. Nam, Seoul National University; K. Zoh, Seoul National University / Department of Environmental Health, School of Public Health

Occurrence of human pharmaceuticals in aquatic environment has been demonstrated and widely evaluated in the last decade. The presence of organic contaminants might have negative impact on the quality of drinking water together with unknown toxicological effects through chronic exposure. The incidence of them in raw waters used for drinking water production and their elimination through treatment must be considered an issue in terms of health safety for water production. Carbamazepine (CBZ) is one of the well-known pharmaceuticals to treat mental impairment associated with epilepsy. Advanced oxidation process (AOP) has been recently applied to treat pharmaceuticals in water, however, the detailed degradation mechanism along with identified byproducts have not been thoroughly examined. In the study, kinetics of carbamazepine was evaluated in ozonation process, and the identification of intermediates was conducted to predict its degradation pathway using LC-MS/MS. During 60 min of ozonation reaction, CBZ removal (%) increased up to 90%, but TOC decrease was minimal. Formation and the decomposition of the detected intermediates from CBZ during ozonation were examined using LC-MSMS. Total five identified intermediates (1-2-benzaldehyde-4-hydro-1H,3H-quinazoline-2-one, 1-2-benzaldehyde-1H,3H-quinazoline-2,4-dione, 1-2-benzoic acid-1H,3H-quinazoline-2,4-dione, acridine and CBZ-10,11-epoxide) and three unidentified intermediates (mw of 163, 167, and 207) were observed during ozonation. These three intermdiates was never observed in the previous studies. Using detected intermediates, the degradation pathway of CBZ ozonation was constructed, and might be helpful to understand the fate and removal of CBZ during ozonation reaction.

MP095 The degradation of the antidepressant venlafaxine in aerobic municipal wastewater sludge

M.M. Schultz, College of Wooster / Department of Chemistry; C. Staebler, M.J. Murphy, College of Wooster / Chemistry; Z. Lapp, E. Shi, College of Wooster / Biochemistry & Molecular Biology; M.J. Snider, College of Wooster / Chemistry; S.S. Strand, College of Wooster / Biology

Public and regulatory interest regarding the presence of pharmaceuticals and personal care products (PPCPs) in the environment is increasing. Human pharmaceuticals may enter into the environment through wastewater treatment plants, and these untreated PPCPs may negatively impact the physiology and/or behavior of the ecosystem's organisms. Within wastewater treatment there are many routes by which chemicals are transformed; however, biodegradation due to the metabolic activities of microorganisms during the treatment process is the predominant route of PPCP breakdown. Consequently, it may be possible to decrease the amount of biologically active PPCPs entering the environment by augmenting WWTPs with microbes capable of more efficiently degrading these molecules. However, few microbes with these abilities have yet to be identified; therefore, this is an important area for discovery. To establish a model system for exploring the biodegradation of PPCPs, initial studies investigated the degradation of the widely prescribed antidepressant venlafaxine. To learn whether microbial communities in the wastewater sludge environment have the ability to degrade venlafaxine and, thus, consequently reduce their environmental loads, microcosms containing aerobic sludge were spiked with deuterated venlafaxine. Samples were analyzed for the depletion of the added pharmaceuticals by microscale solvent extraction followed by liquid chromatography tandem mass spectrometry. Municipal aerobic wastewater sludge was found to degrade venlafaxine completely by 5 days, whereas no change in the added venlafaxine concentrations were observed in abiotic control microcosms. Two venlafaxine primary metabolites, *o*-desmethylvenlafaxine and *n*-desmethylvenlafaxine, and two secondary metabolites, *n,n*-didesmethylvenlafaxine and *n,o*-didesmethylvenlafaxine were observed in the microcosms containing aerobic sludge, but not in the abiotic control microcosms. Further investigations are underway to identify the microorganisms responsible for the observed degradation of venlafaxine in the aerobic sludge.

MP096 Soil Sorption Isotherms for Atorvastatin

P.J. Pagnozzi, Civil and Environmental Engineering; M. Barnes, Rensselaer Polytechnic Institute; M. Nyman, Rensselaer Polytechnic Institute / Dept. of Civil and Environmental Engineering

Atorvastatin is a member of the statin class of cholesterol lowering drugs and has been one of the most-prescribed drugs worldwide over the past decade. To date it has been found in untreated wastewater but not in ground or drinking water in measureable concentrations. It is moderately hydrophobic (log K_{ow} of 3.7 – 6.3 depending on method). This study examines soil sorption isotherms at 1 and 2 week intervals for atorvastatin to different types of soil. Stock solution was generated by dissolving 200 mg of atorvastatin calcium tablets in 400 ml of DI water for a concentration of 500 mg/L. Dilutions of 5 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L, 50 mg/L, 75 mg/L, 100 mg/L, 125 mg/L and 150 mg/L. Two 5 ml samples of each

solution were mixed with 0.1 g of soil, placed on a mechanical rotator for 1 or 2 weeks, then removed and centrifuged. Aliquots (1.5 ml) were withdrawn and analyzed by HPLC utilizing 50:50 v/v methanol-water or 40:60 v/v water-acetonitrile mobile phase and a C-18 column stationary phase. Retention time was between 4 and 4.2 minutes. Results were modeled for a variety of isotherms: The data fit Langmuir and Freundlich isotherms equally well for silty-clay soil type. For the 1 week isotherm the Freundlich N value was 0.81 and K_F was 0.177 $mg^{1-N}L^Ng^{-1}$; the Langmuir Q value was 9.62 mg/g and the b value was 0.015. For the two week isotherm the N value was 0.92 and K_F was 0.160 $mg^{1-N}L^Ng^{-1}$; Q value was 21.14 mg/g and b value was 0.007. While the data fit both models the r^2 value for Hanes-Wolf linearization of the Langmuir isotherms were poor (0.348 and 0.707 for 1- and 2-week isotherms for silty-clay soil type, respectively) favoring the Freundlich isotherm. These findings indicate that atorvastatin sorption to silt-clay soils occurs in a monolayer way on heterogeneous binding sites; mechanisms of both physisorption and chemisorption are involved. These findings are consistent with previous work from our lab examining atorvastatin sorption in sediment soils from Lake Macatawa (Holland, MI). Further short term work includes isotherm studies of sand and loam soil types, and sorption under simulated environmental conditions such as variation of pH, presence of co-solvents and presence of co-sorbents.

Wildlife Ecotoxicology

MP097 Endocrine Disruption in the Northern Water Snake (Nerodia sipedon): Effects of Waste Water Treatment Plant Effluent

H. Pimental, V.K. Kremer, Middle Tennessee State University; F.C. Bailey, Middle Tennessee State University / Department of Biology

Waste water treatment plants are suspected to be a primary source of estrogenic chemicals in aquatic environments. These estrogens mainly come from the urine of premenopausal women and the urine of women who are taking birth control pills or who are on hormone-replacement therapy. The goal of the present study is to explore the effects of endocrine disruption from estrogenic chemicals downstream of selected wastewater treatment plants in Tennessee, USA on male Northern Water Snakes (*Nerodia sipedon*). "Feminization" in male fish has been shown to occur due to effluent downstream from treatment plants at locations around the US and the world, but very little work has been undertaken to see if similar things are happening in squamate reptiles. To accomplish our objectives, treatment plant effluents from around Tennessee will be initially screened for estrogenic activity using a yeast estrogenicity assay and sampling sites will be selected accordingly. Next, blood samples for vitellogenin and sex hormone analysis will be taken from the caudal vein of male *N. sipedon* upstream and downstream from selected treatment plants. Indicators of "maleness" including size of hemipene pocket, length of tail, and gonad weight and size as well as basic measurements (snout-vent length, tail length, mass, etc) will be taken from the same snakes. This study is important not only because endocrine disruption is understudied in reptiles but also because *N. sipedon* in particular is a link between aquatic and terrestrial environments and could potentially lead to effects of endocrine disruption in terrestrial predators.

MP098 Health Assessment of tree swallows (Tachycineta bicolor) nesting in the Great Lakes

E. Bigorgne, Baylor University / Department Environmental Science; C.M. Custer, T.W. Custer, US Geological Survey / Upper Midwest Environmental Sciences Center; S. Schultz, US Geological Survey / Patuxent Wildlife Research Center; N.K. Karouna-Renier, USGS Patuxent Wildlife Research Center / Beltsville Lab; C.W. Matson, Baylor University / Environmental Science

Assessment of ecosystem health is essential to evaluate the effectiveness of dredging and other recent remediation actions across the Great Lakes. The aim of this study was to assess chromosomal damage and oxidative stress in tree swallow (*Tachycineta bicolor*) nestings collected from contaminated areas across the Great Lakes region in 2010, 2011 and 2012. The flow cytometric method (FCM) was used to quantify cell-to-cell variation in DNA content within red blood cells (RBC). Two others biomarkers were also performed on the same samples: EROD activity and lipoperoxidation by measuring TBARs. The quantity of DNA damage and EROD activity were significantly increased in samples from the Detroit River (MI), Rouge River (MI), Erie Lake (MI) and Lincoln Park (WI) compared to a reference site. Only Detroit River and Erie Lake samples showed an increase of lipoperoxidation compared to a reference site in 2010. Metal analysis (2010) revealed that lead and cadmium were accumulated by tree swallows (liver content) at Detroit River, Rouge River, Erie Lake and Lincoln Park locations. Analysis of the correlations between the different biomarkers and metal contents are still in progress and will be presented at the congress. These biomarkers in tree swallows seem relevant for monitoring the ecosystem health of the Great Lakes.

MP099 Maximum Allowable Soil Concentrations of Metals and Hydrocarbons for Wildlife Ruminants

S. Bard, J. Armstrong, J. Nielsen, J. Trowell, Keystone Environmental Ltd.

To fulfill the site-specific protection goal of no adverse effects to wildlife (specifically deer and elk) through grazing exposure (specifically ingestion of grass and soil), maximum allowable soil concentrations of 19 metals, 17 polycyclic aromatic hydrocarbons and light/ heavy extractable petroleum hydrocarbons were derived. Dose-based, mammalian wildlife toxicity reference values were derived from no-observed adverse effect levels, with study selection based on a priority system that focused on literature regarding on the toxicity of the metal or hydrocarbon to ruminants first; where that was lacking, studies on herbivores and finally rodents filled in the gaps. Dose modelling using literature values was used to calculate ingestion rate and daily oral dose of metals and hydrocarbons from soil and grass. By determining the ratio of toxicity reference value over the daily oral dose, the maximum allowable soil concentration of the constituent was calculated. Since baseline literature values were used in the dose modelling, the model can be further refined by incorporating the biology of the receptor (seasonality of diet and exposure, range) into the dose modelling equation.

MP100 PCB bioaccumulation of in three species of penguin and insight to biomagnification processes in aquatic food webs of Antarctic Peninsula and Patagonia

G. Chiang, K.R. Munkittrick, Canadian Rivers Institute, Biology; R. Barra, I. Rudolph, S. Jara, EULA Chile Environmental Science Centre, University of Concepcion, Chile / Aquatic Systems Research Unit; M. Diaz-Jaramillo, Environmental Ecotoxicology and Chemistry Laboratory / IIMyC, UNMdP-CONICET

Persistent Organic Pollutants (POPs) are organic substances of natural or anthropogenic origin, resistant to photolytic, chemical and biological degradation, so they have a high persistence in the environment. In recent years, studies in remote areas of the Northern Hemisphere have found high concentrations and high deposition rates of POPs, despite the lack of widespread use in these areas. Although there is information that supports global fractionation, mainly in the northern hemisphere, extensive background information and history was lacking in the south, especially in Antarctica and the Southern Chilean Patagonia. The aim of this study was to determine levels and possible effects of PCBs in biota of the Antarctic Peninsula. Collection of fresh feces of three *Pygoscelis* penguin species was used as a noninvasive sampling approach and contaminant analysis was complemented by determination of stable isotopes and porphyrin levels. Of the three species studied, the highest levels of PCBs were found in the Gentoo penguin (*P. papua*) with an average of 35.3 ng g^{-1} (fresh weight) compared to the other species, Chinstrap (*P. antarctica*) and Adelia (*P. adeliae*) with 6.4 and 12.9 ng g^{-1} ww, respectively. PCB concentrations correlated well with the stable isotope analysis, in particular with the $\delta^{15}N$ levels, indicating a possible role of diet in the observed contaminant levels. Furthermore, relatively high levels of porphyrin metabolites were observed in the PCBs contaminated feces samples that can be correlated to the contaminant concentrations. Further research is ongoing to assess levels and possible effects of POPs and trace metals in the Antarctic and Chilean Patagonia food webs.

MP101 Quantifying mercury exposure for multiple shorebird species across the North American Arctic using blood and feather samples

M. Perkins, University of Michigan / Department of Environmental Health Sciences; L. Ferguson, Biodiversity Research Institute; R.B. Lanctot, US Fish and Wildlife Service; I.J. Stenhouse, D.C. Evers, Biodiversity Research Institute; N. Basu, McGill University / Department of Environmental Health Sciences; J. Bety, Université du Québec À Rimouski; S. Brown, Manomet; R. Gates, S. Kendall, US Fish and Wildlife Service; J. Lamarre, Université du Québec À Rimouski; J. Liebezeit, Wildlife Conservation Society; B. Sandercock, Kansas State University

Many species of shorebirds are experiencing population declines, and the causes for these declines are unknown. Exposure to environmental contaminants, such as mercury, may be a factor. Chronic mercury exposure

in birds can reduce reproductive success, which may ultimately result in population declines. Arctic shorebirds may be at particular risk to mercury contamination, as recent studies indicate mercury deposition is increasing in Arctic habitats due to increases in anthropogenically-produced atmospheric mercury and the unique features of the Arctic. We quantified mercury exposure in blood and feather samples from Arctic shorebirds to establish and compare baseline mercury concentrations across multiple species over a large spatial scale. We analyzed a total of 250 blood and feather samples collected in 2008 and 2009 from breeding and post-breeding shorebirds from western and northern Alaska, including sites near Barrow, the Arctic National Wildlife Refuge, and the Yukon Delta National Wildlife Refuge. In 2012, the study was expanded and a total of 1257 blood and feather samples were collected from breeding shorebirds at five sites across Alaska located near Nome, Cape Krusenstern, Barrow and the Ikpikpuk and Colville river deltas; and three sites across Canada located on the Mackenzie River Delta, Bylot Island, and East Bay (Southampton Island). In total, 14 species of shorebirds were sampled. Blood mercury concentrations in individual shorebirds ranged widely from 0.01 – 2.20 µg/g. Mean blood mercury concentrations among species sampled at the same site differed significantly; as did mean blood mercury concentrations of species sampled at different sites across the Arctic. Feather mercury concentrations were generally higher than blood concentrations ranging from 0.10 – 12.14 µg/g in individual shorebirds. Blood mercury concentrations of 0.7 µg/g have been associated with a 10% reduction in nest success for Carolina Wrens. Only breeding shorebirds sampled at Barrow had blood mercury greater than 0.7 µg/g, with approximately 19% of individuals exceeding this value. Most post-breeding shorebirds had relatively low blood mercury concentrations, with mean values below 0.27 µg/g, and did not differ among species sampled at the same location. Overall, this study indicates that mercury exposure in shorebirds varies by species and breeding location, and that some shorebirds breeding at Barrow may exceed levels that could reduce reproductive success.

MP102 Review of Marine Mammal Inhalation Toxicity Following Oil Spills

C.S. Mori, Industrial Economics Incorporated (IEc); A.B. Rosenstein, Consultant, Industrial Economics Incorporated (IEc); T.K. Collier, University Corporation for Atmospheric Research / Oceans and Human Health Program; E. Ruder, Industrial Economics Incorporated (IEc)

Limited publications exist on observed health effects in marine mammals following oil spills. In the available published studies, exposure levels are not usually quantified and exposure routes are often unspecified or consist of concurrent inhalation, ingestion, and dermal exposures. It has been hypothesized that, following oil spills, inhalation of petroleum-associated compounds may be of most concern for causing health impacts in marine mammals, due to the time spent at the air-water interface where the greatest concentrations of volatile hydrocarbons are found. Determination of inhalation toxicity is complicated because different sources of oil consist of different mixtures of volatile compounds that are emitted in varying concentrations to the air overlying a spill. In order to specifically assess marine mammal inhalation toxicity, published inhalation toxic effect levels for individual petroleum-related compounds in laboratory animal studies were compiled. The potential application of these toxic effect levels to the assessment of marine mammal risks following oil spills will be discussed.

MP103 Size Matters: Insecticide Exposure may Create a Selective Pressure that Favors Juvenile Blue Crabs (Callinectes sapidus)

K.J. Schroeder, D.L. Smee, Texas A&M Corpus Christi / Department of Life Sciences

Adult and juvenile blue crabs (*Callinectes sapidus*) are an important predator in many estuarine systems (e.g., oyster reefs, saltmarshes) and serve as a food resource for many aquatic species and people. In many regions, blue crab populations have been in decline and in some cases, smaller bodied crabs are becoming more frequent than larger bodied crabs. This trend is commonly attributed to fishing pressure, but exposure to pesticides from mosquito abatement programs and storm run-off may also play an important role. Adult and juvenile blue crabs were exposed to three insecticides (malathion, carbaryl, resmethrin) and a synergist (PBO), individually and in combination; lethal and sublethal effects (e.g., behavior, reflexes, coordination) were recorded every 12-hrs for 7 days. Effective lethal/sublethal exposure levels varied with pesticide, but were often below or at USEPA standards for drinking water. For both life stages, effected crabs experienced reduced coordination, decreased neurological functioning and were less able to respond to stimuli. Resmethrin +/- PBO treatments were more toxic than individual carbaryl and malathion treatments. Mixture treatments also increased lethal/sublethal effects compared to single-pesticide treatments in both life stages. Juvenile and adult blue crabs responded similarly to malathion and carbaryl, but adults were consistently more susceptible to resmethrin, PBO and mixture treatments than juveniles. These results suggest that toxicant exposure may create a selective pressure that favors juvenile stages and smaller bodied blue crabs.

MP104 Temporal and spatial trends in POPs in seabird eggs from the north Pacific ocean

J.E. Elliott, Environment Canada; K.H. Elliott, University of Winnipeg; M.F. Guigueno, University of Manitoba; L.K. Wilson, S. Lee, Environment Canada

Seabirds are effective sentinels for monitoring xenobiotic contamination of the marine environment. Eggs provide a relatively non-intrusive tool to measure substances, such as persistent organic pollutants (POPs) and mercury, which are maternally transferred to the egg via yolk lipids or proteins. Here we report and discuss data from long term monitoring of POPs and mercury in seabird eggs from the northeast Pacific over four decades (1970-2011). For this program, the marine system was divided, and representative species selected. The nearshore subsurface is monitored using two cormorant, species, (double-creasted, *Phalacrocorax auritus*, and pelagic, *P. pelagicus*); both feed on a variety of benthic and pelagic fish. The offshore subsurface is monitored using the rhinoceros auklet, *Cerorhinca monocerata*, which feeds mainly on small pelagic fishes. The offshore surface is monitored with the Leach's storm-petrel, *Oceanodroma leucorhoa*, which feeds mainly on surface plankton and larval fishes. At three breeding colonies each along the Pacific coast ofCanada and at four year intervals, 15 eggs are collected and analyzed as five pools of 3 eggs each. Data will be presented on long term temporal trends and spatial variation for representative organic contaminants. For example, a recent retrospective study using archived samples, showed that polybrominated diphenyl ether (PBDE) and hexabromocyclododecane (HBCD) flame retardants increased 10-fold from 1990 to 2011 in storm-petrel eggs from theGulf of Alaska. Stable carbon, nitrogen and sulphur isotopes reveal some of the role of dietary variation in relative contaminant burdens within seabirds, possibly related to marine regime shifts.

MP105 What Factors Influence Condition in Ringed Seals? An analysis of stress and condition over the past decade

R. Anderson, University of Manitoba / Department of Biological Sciences; G. Tomy, University of Manitoba / Department of Chemistry; S. Ferguson, Department of Fisheries and Oceans Canada; G. Anderson, University of Manitoba / Department of Biological Sciences

Arctic marine mammals are increasingly exposed to a variety of both environmental and anthropogenic stressors (ex. climate change, contaminant exposure, disease). An animal's response to acute disturbances will temporarily alter their baseline cortisol levels whereas chronic disturbances cause continued glucocorticoid production. These effects on individuals often manifest at the population and ultimately ecosystem levels. A prolonged stress response will have negative health impacts that could affect reproduction and survival. Ringed seals (*phoca hispida*) depend on their thick blubber layer for thermoregulation and energy storage. Although blubber thickness will change seasonally based on reproductive status and food availability, a healthy individual is consistently able to maintain a level of insulation that will minimize heat loss and allow the body to allocate energy resources to physiological processes other than thermoregulation. Ringed seal blubber samples and morphometric data were collected by Inuit hunters during the fall subsistence hunts in Arviat, Nunavut from 2003-2012. Blubber cortisol concentrations were measured using a new, methanol-based, extraction technique followed by LC/MS/MS analysis. Blubber thickness and cortisol levels were assessed along with covariates year, sex and age class (< 1, 1-5 and >5 years old) to determine how different population segments may be impacted. Ringed seal body condition decreased significantly over the study period and was accompanied by significant increase in blubber cortisol levels. Trends were similar between sexes but differed among age classes. Cortisol levels were significantly higher in pups suggesting that the population impacts of long term stressors may disproportionately affect younger individuals. Understanding how the physiological condition of this species

has changed over time in response to cumulative ecological stressors is essential for development of wildlife management strategies in the Canadian Arctic region.

MP106 When hot is not too hot: an earthworm dwelling under extreme volcanic conditions

L. Cunha, Universidade dos Açores / Biology; M. Novo, Cardiff University; H. Arruda, Universidade dos Açores; M. Hodson, York University; D. Spurgeon, Centre for Ecology & Hydrology; A.S. Rodrigues, University of Azores / Department of Biology, (CVARG) Center of Volcanology and Geological Risks Assessment, Ponta Delgada, Portugal; R. Camarinho, M. Viveiros, C. Silva, Universidade dos Açores; M. Bruford, A. Morgan, P. Kille, Cardiff University

Animals inhabiting extreme terrestrial environments provide a unique opportunity to better understand how organisms' cope with challenges posed by multiple geogenic stressors. The megascolecid Amynthas gracilis, a highly abundant invasive pantropical earthworm in the Furnas geothermal field (Sao Miguel Island, Azores) indicates its outstanding tolerance to high soil temperature, exceptionally high carbon dioxide and low oxygen levels, and elevated metal bioavailability, conditions that would be lethal for the majority of terrestrial metazoans. In the present work, we assessed how this metazoan tolerates such conspicuously stressful environment. Two sites in São Miguel, differing in their contemporary volcanic activity (thermal and degassing outputs), were selected for mesocosms exposures: Furnas, which displays the most conspicuous degassing and geothermal activity in the entire Azores archipelago and Macela, which does not presently display any thermal and degassing phenomena. A group of adult earthworms from Furnas and another group from Macela were assigned to a factorial-design (with earthworm source and exposure site as factors) and exposed for 28 days. Twelve mesocosms were used per site, with six mesocosm per treatment. Metal concentrations in actively volcanic (Furnas) and volcanically inactive (Macela) soils were found to be similar; however, Furnas soil was characterised by elevated temperature (~15 °C higher than Macela), relative hypoxia, extremely high CO_2 tension, and accompanying acidity. RNA-seq data generated from the exposed animals revealed an inherent mechanistic answer to the multi-stressor environment. Morphometric analyses of the epidermal thickness were assessed to confirm that the in situ mesocosms successfully produced a stressed phenotype. The epidermis of earthworms transplanted to the non-volcanic soil was approximately twice the thickness of the epidermis of conspecifics exposed to Furnas' soil, regardless the origin source of the earthworm. Clearly, the active volcanic environment at Furnas poses a multifactorial stress challenge to the epigeic A. gracilis colonizer.

Applying Adverse Outcome Pathways to Human and Ecological Studies

MP107 Narcosis – A Source to Outcome Pathway Approach to refine Environmental Risk Assessment

S. Gutsell, Unilever / SEAC; G. Hodges, Unilever Research; S. Marshall, Unilever; I. Malcomber, Unilever R&D Colworth; O. Price, Unilever / Colworth Science Park; C. Rendal, Unilever / Safety and Environmental Assurance Centre

The existing approach to assessing the adverse effects of chemicals in the environment is largely based on a battery of in-vivo study methods supported by a limited number of accepted in-silico approaches. This approach incorporates predictions to attempt to protect ecosystems/communities. This is typically done through the use of assessment factors that are used to derive a predicted no-effect concentration (PNEC), although AFs do not add to the understanding of chemical interactions with organisms. The conceptual framework of Source to Outcome Pathways provides a structured way to explain the confidence associated with a particular prediction method by clearly articulating where assumptions are made in the causal chain of events leading to an adverse outcome. Existing alternative approaches to the use of apical endpoint hazard studies, such as QSARs, skip several steps in the pathway. Without mechanistic justification for skipping these steps, the confidence placed in these approaches is somewhat limited. In addition, a barrier to the use of mode of action (MoA)-specific prediction tools is assignment of MoA. An error at this stage can result in predictions several orders of magnitude from a representative value. When limited or no information is available on the mechanism by which a chemical will cause toxicity, conservative threshold-based approaches may be used. By using the Source to Outcome Pathways framework to structure research efforts we aim to answer several long standing questions about the narcosis Mode of Action: Non-polar, polar, ester and amine narcosis have all been proposed as separate mechanisms of action within the narcosis mode of action. Do these mechanisms reflect different Molecular Initiating Events (MIE) or differences in other key events at higher levels? Can differences in species sensitivities to narcotic chemicals be explained by understanding the detailed pathways involved in response to narcotic chemicals? What can be learnt from research in the human health domain around the mechanisms involved in anaesthesia, which may be relevant to other species? As our understanding of the detailed mechanisms of toxicity covered by the narcotic chemicals increases, the sophistication of predictive methods will also increase with an associated decrease in uncertainty. We will present our research strategy and insights gained to date in this field.

MP108 EROD activity as a biomarker of exposure in field and laboratory birds exposed to environmentally relevant PCB mixtures

M.E. Barton, University of Maryland, College Park / Animal and Avian Sciences; K.D. Dean, University of Maryland / Neuroscience; M. Ottinger, University of Maryland / Department of Animal and Avian Sciences

Ethoxyresorufin-*O*-deethylase (EROD) activity has been used as a biomarker of dioxin exposure and for assessing exposure of wildlife to dioxin-like compounds such as polychlorinated biphenyls (PCBs). We studied EROD activity in experiments conducted in field birds (*Tachycineta bicolor* and *Sialia sialis*) and in laboratory Japanese quail (*Coturnix japonica*). Birds were treated during embryonic development with selected doses of one of the following: two PCB mixtures found in the upper Hudson River environs, PCB 126, or PCB 77. Tree swallows had a relatively flat response to PCB 77, while the two PCB mixtures and PCB 126 stimulated increasing levels in EROD. Quail showed similar responses, with a strong response to PCB126 and the two mixtures (GENMOD procedure; personal communication, Franca Barton) . The maximal fold-difference over control in the 126-treated birds was 5.5, however there was increased mortality at the higher doses. Maximal induction was observed (9.3 fold) with one of the mixtures, however again the increased mortality at the higher doses of PCB 126 may have masked the EROD response. The PCB mixture also induced an initial strong response followed by a plateau and a further increase with increasing dose for a final fold-difference of 14, and a significant cubic function for this mixture's dose-response curve ($p < 0.0001$). There were mild gender differences. PCB 77 elicited a slow flat EROD response. These data provide evidence that PCB 77 may not have strong dioxin-like biological activity in some avian species, which is in agreement with a previous WHO report that has proposed a recalculation of the PCB 77 TEF from 0.0005 to 0.0001. As such, indices of toxicity such as TEQs and TEFs should be utilized to estimate potential effects, with the recognition that these calculations may not fully inform mechanisms associated with endocrine disruption and other non-aryl hydrocarbon receptor mediated actions. Supported in part by the Hudson River Trustees (FWS; MAO). The conclusions and opinions presented here are those of the authors, they do not represent the official position of any of the funding agencies, the Hudson River Trustees, or the United States.

Mercury Characterization and Contaminated Site Remediation: Methods, Challenges, and Lessons Learned

MP110 How Low Can You Go? Pre-Anthropocene Mercury Levels in Fish Tissue

B.K. Hope, CH2M Hill; J. Louch, NCASI

Mercury (Hg) is a naturally occurring element that has been entering the environment from both natural and anthropogenic sources for millennia, and humans have been influencing its environmental fate from well before the industrial revolution. Humans and piscivorous wildlife are typically exposed to mercury (as neurotoxic methylmercury) primarily through consumption of finfish, shellfish, and marine mammals. Since the 1960s, regulatory limits for methylmercury have continued to decline as further information on its health impacts has become available. Because mercury is naturally-occurring, there may be a natural "floor" for methylmercury residues in fish tissue, below which it is not possible to descend, regardless of any regulatory imperatives to do so. The proximity of current regulatory criteria to previously estimated natural levels prompted us to reexamine the question of a natural (pre-Anthropocene) mercury level in fish tissue. Mercury tissue concentrations were estimated with an integrated mercury speciation, transport

and fate, and food web model (SERAFM), using mercury concentration estimates in soil and sediment, and in atmospheric deposition, prior to the onset of significant human impacts (i.e., < 100 CE). Model results suggest that methylmercury residues in predatory fish may have approached or exceeded the current human health tissue residue criterion (HHTRC) during the pre-Anthropocene, and would be non-compliant with current regulatory guidelines due solely to mercury naturally prevalent in soil and air during that era. These results are not meant to suggest that current efforts to reduce anthropogenic mercury emissions should be curtailed. They do suggest, however, that attempts to comply with a sub-natural HHTRC – should one be established – would likely be costly, ineffectual, and frustrating for risk managers and stakeholders alike.

MP111 Critical Review of Mercury Toxicity Reference Values for Protection of Fish

P.C. Fuchsman, ENVIRON International Corp; M.T. Sorensen, ENVIRON International Corp / Senior Science Advisor; L. Brown, ENVIRON International Corp; M.J. Bock, ENVIRON International Corp; A.C. Daniel, ENVIRON International Corp; C. Beals, Environ International; V.S. Magar, ENVIRON International Corp / Ecology & Sediment Management; M.H. Henning, ENVIRON International Corp

We provide a critical review of prominent toxicity reference values (TRVs) and dose-response analyses for protection of fish from adverse effects due to mercury. These TRVs are within the range of present-day background concentrations in fish tissue. While such low TRVs would seem to suggest widespread risks to fish populations, there is a high degree of uncertainty in the data underlying the TRV derivations. For example, although some experimental results suggest possible effects on fish productivity associated with environmentally relevant mercury concentrations in tissue, none of these results are statistically significant. Further studies are needed to support statistically robust characterization of dose-response relationships for mercury effects on reproduction in representative fish species. Studies of mercury effects on fish populations are also needed; none were identified in our review. As an additional line of evidence, we examined field studies linking fish community quality with fish tissue mercury concentrations. The field data indicate that fish communities are more resilient than would be predicted from published mercury TRVs.

MP112 Mercury Exposure Assessment for High-End Fish Consumers in the US

M. Li, University of Michigan / School of Natural Resources and Environment, Harvard School of Public Health / Environmental Health; T. von Stackelberg, Harvard School of Public Health / Environmental Health; J.P. Shine, Harvard School of Public Health / Department of Environmental Health; E.M. Sunderland, Harvard University

The National Health and Nutrition Examination Survey (NHANES) provides a statistically representative overview of fish consumption patterns by individuals in the United States. However, insufficient data on high-end fish consumers limits our understanding of groups that are especially susceptible to high levels of MeHg exposure from fish consumption. To better quantify the fish species selection preferences and consumption magnitudes of high-end fish consumers (three or more fish meals per week) in the US, we launched a new national wide survey for these people (n=~1750) online. The survey includes one-month and 3-month recalls for three separate periods to assess seasonality of fish consumption. Questions in the survey cover detailed information on specific fish and shellfish species consumed and its corresponding consumption frequency, meal size, sources (self-caught, restaurant, or grocery) and preparation methods. The results present new findings on the fish consumption pattern of these individuals and associated mercury exposures estimated using a probabilistic model that explicitly simulates variability in fish Hg concentrations consumed by individuals in our survey. Predicted hair-Hg concentrations derived from estimated mercury exposures are further compared to measured hair-Hg data of these individuals to cross-validate results of the our survey.

MP113 Critical Review of Mercury Toxicity Reference Values for Avian and Mammalian Wildlife

P.C. Fuchsman, ENVIRON International Corp; M.H. Henning, ENVIRON International Corp; E. Perruchon, ENVIRON International Corp; L. Brown, ENVIRON International Corp; V.S. Magar, ENVIRON International Corp / Ecology & Sediment Management

Widely used mercury toxicity reference values (TRVs) for birds and mammals are critically reviewed in the context of ecological risk assessment for contaminated sediment sites. Many assessments have relied on a 1970s study of methylmercury toxicity in mallards, but more recent studies using the same species have yielded very different results, indicating that mallards are relatively insensitive to mercury. Toxicity thresholds for other avian species are reviewed. Our review of mammalian TRVs focuses on their application to bats, mink, and river otters. Marine mammals are considered relatively insensitive to mercury exposure. The utility of various measures of mercury exposure is evaluated with regard to interspecies extrapolation of TRVs. In particular, we question the typical dose paradigm (mg mercury ingested per kilogram body weight per day), which does not account for differences in mercury toxicokinetics among species. This issue is of particular importance for bats and small birds, due to their high food ingestion rates relative to body weight and the limited availability of mercury toxicity data for these species.

MP114 An assessment of a diet-based mercury toxicity reference value for songbirds

H. Summers, Integral Consulting / Institute of Environmental Toxicology and Chemistry; J.L. Durda, Integral Consulting Inc.

The potential toxicity of mercury in songbirds has received increasing research focus over the last decade following an emerging understanding of the importance of mercury accumulation in invertebrate prey species that inhabit wetlands and riparian zones along contaminated water bodies. The developing literature has heightened awareness amongst environmental managers and risk assessors on the importance of the evaluation of the songbird exposure pathway when conducting risk assessments at mercury contaminated sites. Though longer term field studies of songbird populations can be used to evaluate potential effects, they are not always economical, feasible, or needed to support management decisions. Instead, predictive risk assessment which compares measures or estimates of exposure in the songbird diet to toxicity reference values (TRVs) is a commonly relied upon tool to support risk assessment and risk management at these sites. Many TRVs that are used to support avian risk assessments are from standard laboratory test species, some of which are substantially more sensitive to mercury toxicity than wild populations of songbirds. To support the development of a TRV that can be used in songbird risk assessment, we reviewed the published toxicological studies for mercury in songbirds to identify those studies that measured both dietary exposures and effects. Toxicological endpoints related to growth, reproduction, and survival were the focus of the review, consistent with common risk assessment guidance, but other physiological responses and behavioral endpoints also were considered. This paper presents the results of that review and recommends dietary TRVs for use in songbird risk assessments. Implications of these findings for future risk assessments as well as field studies are also discussed.

MP115 Characterizing the complex distribution, physico-chemical changes and hydrologic factors impacting fate and transport at a mercury spill site

D. Watson, Oak Ridge National Laboratory; C. Miller, L. Liang, M. Mueller, B. Lester, K. Lowe, E. Pierce, Oak Ridge National Laboratory

In Oak Ridge, TN, the DOE Y-12 National Security Complex (Y-12 NSC), used 11 million kg of Hg(0) between 1950 and 1963 for lithium isotope separation. Large quantities of mercury released during this period still remain in soils, sediment and in and under buildings at the facility, as shown in recently recovered subsurface cores. A system of subsurface drains provides avenues for rapid transport of Hg to surface water and offsite, however, the distribution and long-term fate of the onsite Hg(0) spills are not well understood. Site alterations during construction and operation of the industrial facility has created many ill-defined conduits and preferred pathways for contaminant migration. The Hg spatial distribution, physico-chemical changes, and hydrologic factors make predicting the mobility and reactivity of Hg in industrial settings difficult; nonetheless, a comprehensive understanding of these factors will enable the development, evaluation, and

optimization of remediation strategies that will succeed at reducing Hg flux and risk. Previous studies examining Hg in soils within the Y-12 facility have primarily quantified total Hg concentrations only, although it is known that Hg(0) is present based on visual observations. Through evaluation of historical data and new sampling and analysis efforts we have started to develop a better understanding of the migration pathways and forms of Hg present at the Y-12 NSC spill sites needed to conduct risk assessments, assess disposal options and evaluate the potential effectiveness of treatment options. A systems analysis approach is also being employed in which the bounds of known and suspected transport pathway features are documented using geographic information system (GIS) technology, and assigned a value associated with their risk of migration.

MP116 Storm dynamics of Hg and MeHg in an industrially contaminated creek: What can it tell us about source areas of Hg and MeHg within the catchment?

A. Riscassi, Oak Ridge National Lab / Environmental Sciences Division; S. Brooks, Oak Ridge National Laboratory; C. Miller, Oak Ridge National Laboratory; X. Yin, Oak Ridge National Lab

Sediments in East Fork Poplar Creek, Oak Ridge, TN as well as floodplain soils in the surrounding watershed, are contaminated with high levels of mercury (Hg) and methylmercury (MeHg). Concentrations of Hg and MeHg in floodplain soils (mean 25.3 and 0.020 ng/mg dw, respectively) are equal to or higher than creek sediment (mean 5.4 and 0.006 ng/mg dw, respectively). Floodplain soils are a potentially significant source of both Hg and MeHg to the stream, however their contributions have not been evaluated. We investigate the variability in stream Hg and MeHg with changes in discharge and corresponding hydrologic connectivity between the creek and surrounding floodplains. Understanding concentration dynamics during high-flow periods will help elucidate the relative importance of floodplains as source areas of in-stream Hg and MeHg. From March 2012 through March 2013 four high-flow events were sampled along rising, peak and falling limbs of each storm hydrograph in Spring, Summer, Fall and Winter seasons. Monthly baseflow samples were also taken. The range of discharge conditions sampled (n=124 to date) is approximately 20 to 4100 cfs. Samples were analyzed for filtered and unfiltered Hg and MeHg, dissolved organic carbon (DOC), UV-vis spectra, anions, metals and total suspended solids (TSS). During all storms particulate Hg and MeHg concentrations increased (up to 150X greater than baseflow) during the rising hydrograph limb and were closely correlated with TSS (r^2=0.92 and 0.78, respectively). The amount of Hg and MeHg per mg sediment in suspended particles (~ 12 and 0.013 ng, respectively) is between that for bed sediment and floodplain soils indicating a mixture of those sources. Similar to the particulate form, dissolved Hg (HgD) concentrations increased during high-flow events, though more moderately (up to 5X greater than baseflow) and offset in time (typically on the peak and falling hydrograph limb) and were associated with an increase in DOC (r^2=0.71). The amount of HgD per mg DOC varied between 3-8 ng for all flow conditions indicating the DOC transported from the watershed during high flow is similar in Hg content to that measured during baseflow conditions. Unlike HgD, dissolved MeHg (MeHgD) concentrations either decreased or remained stable during storm events indicating floodplain soil water mobilized during storms does not increase in-stream MeHgD concentrations.

MP117 Quantitative Basis for Design of Monitoring of Remedial Effectiveness in a River System

J.R. Flanders, PerkinElmer, URS; R.G. Stahl, E.I. DuPont de Nemours and Company / Corporate Remediation Department; N.R. Grosso, DuPont Company; C. Patmont, Anchor Environmental, LLC

The South River is a high-gradient stream in central, VA with elevated mercury (Hg) concentrations in soil, sediment, surface water and biological tissue as the result of historic industrial Hg use. In addition, benthic invertebrate communities have been negatively impacted in certain locations due to increased phosphorous (P) loading and suspended sediment deposition from degraded riparian zones. Remedial actions to address elevated Hg concentrations in environmental media (e.g., bank soils and sediment) and the impaired benthic invertebrate communities are being considered for implementation. A monitoring regime for the South River has been designed to measure potential short-term and long-term improvements. The short-term remedial action objectives (RAOs) are to reduce mercury transport and improve river bank habitat from a focused area of the South River. Short-term RAOs will be linked with specific projects (e.g., bank stabilization projects) and will be designed with a before-after type of monitoring design. Short-term monitoring will focus on river bank habitat and concentrations of Hg in near-bank media. Short-term monitoring results will be evaluated within an adaptive management framework. Long-term monitoring RAOs are to reduce methylmercury (MeHg) exposure and to improve habitat conditions throughout the South River. Reductions in MeHg concentration in fish tissue may take several years following Hg loading reductions. As a result, the long-term monitoring needs to quantify interannual variability and build on the substantial existing baseline data set. The long term monitoring regime has three components: annual young of the year (YOY) smallmouth bass tissue mercury concentrations, monthly surface water monitoring for mercury and water quality parameters, and biannual habitat quality and benthic invertebrate community monitoring. YOY smallmouth bass sample sizes were determined through a power analysis based on BASS model predictions of the range of MeHg in YOY fish (~75mm total length). Collection of approximately 10 YOY smallmouth bass should detect a 30% decline in MeHg concentration at 70% power. Monthly baseline water quality monitoring has observed significant variation in MeHg resulting from changes in water temperature. Biannual monitoring of the benthic invertebrate community will be compared to baseline data and incorporated into a multivariate statistical framework to identify stressors and long-term changes.

MP118 Development of a Mercury-Specific Hydrogel for the Assessment of Bioavailable Mercury and Methylmercury in Sediment

L. Brown, N. Steenhaut, ENVIRON International Corp; A. Amirbahman, D. Massey, University of Maine; G. Lotufo, USACE, ERDC; V. Magar, ENVIRON International Corp

The goal of our on-going project, funded by the Strategic Environmental Research and Development Program (SERDP), is to develop a cost-effective mercury-specific hydrogel as a biomonitoring tool for assessing mercury and methylmercury bioavailability in sediment. The hydrogels operate on the principle of diffusive gradients in thin films (DGT), and we investigated the relationship between lability (defined as uptake by hydrogels) and bioavailability (defined as uptake by benthic invertebrates) for mercury and methylmercury. During the development phase we optimized hydrogel parameters (i.e., gel material and thickness, deployment time, and extraction) for mercury and methylmercury uptake in sediment. Following optimization, we concurrently deployed hydrogels and benthic invertebrates in several laboratory time series exposure experiments to assess the relationship between lability and bioavailability. Relationships were assessed in three experiments for 1) multiple benthic invertebrate species (*Macoma nasuta, Nereis virens*, and *Leptocheirus plumulosus*) in estuarine sediment collected from a single location (Penobscot River Estuary, ME); 2) *M. nasuta* in estuarine sediment (Penobscot River Estuary, ME) with multiple organic carbon contents (8%, 4%, and 2%); and 3) *Lumbriculus variegatus* in freshwater sediment (Dodge Pond, CT) with multiple sediment mercury concentrations (10 ppm, 4 ppm, and 1 ppm). In Experiment 1, stronger mercury and methylmercury correlations were observed between hydrogels and tissue concentrations for *M. nasuta* and *L. plumulosus* compared to *N. virens*. Lower hydrogel-tissue correlations were observed in Experiment 2 compared to the Experiment 1 *M. nasuta* results, and correlations were higher for 8% and 4% OC than 2% OC for both mercury and methylmercury. In Experiment 3, there was good agreement between hydrogels and tissue for mercury; however, little-to-no methylmercury was measured in *L. variegatus* tissue while clear methylmercury uptake was observed in hydrogels.

MP119 Dendrochemistry and Phytoscreening of Hg in Trees

G.N. Bigham, Exponent; J. Balouet, Environment International; M. Chalot, Universite de Franche-Comte; L. Liang, CEBAM

A preliminary study of the uptake of Hg by trees was conducted to determine if dendrochemistry and phytoscreening techniques could be used to characterize the distribution of Hg in soil at contaminated sites. Objectives of the study were: 1) establish analytical techniques sensitive enough to quantify Hg, at background concentrations, in individual annual growth rings; 2) determine if phytoscreening (PS), analyses of the most recent rings, could be used to characterize the distribution of Hg in contaminated soils; and 3) determine if dendrochemistry (DC) techniques, analyses of dated tree cores, provide a reliable history of a tree's exposure to Hg via the roots. The study was conducted at a Hg-contaminated site on the South River, Virginia, where an estimated 500 to 1,000 kg of Hg was released

to the South River in the 1930s and 1940s from a synthetic fiber manufacturing plant located in Waynesboro, Virginia. Subsequent flood events deposited Hg-contaminated sediments on the floodplain for approximately 40 km downstream of the plant to the South Fork of the Shenandoah River. Fourteen trees were sampled in 2010 and 2012 on the South River floodplain at locations where vertical profiles of soil Hg had been previously characterized by the South River Science Team (www.southriverscienceteam.org). Soil total Hg concentrations at 0- to 12-in. ranged from 0.3 to 94 µg/g dw. Samples were analyzed for total Hg by a combustion/trap/CVAFS method developed by CEBAM in Seattle, Washington. Sample sizes were in the range of 0.2 to 0.01 g. The method detection limit was about 0.1 (ng/g), which was adequate to analyze tree rings as thin as 1 mm from a 5-mm diameter core. Additional analyses were performed at the Laboratoire Chrono-environnement at the Université de Franche-Comté using a Leco AMA 254 Hg Analyzer. Results of analyses of split samples by the two labs were comparable. Hg concentrations in PS samples at control sites ranged from 1.9 to 3.9 ng/g dw and from 5 to 145 ng/g dw at contaminated sites. PS sample results showed significant variability within individual trees. DC results for trees that were resampled were not consistent. Nevertheless, the PS technique was found to be a reliable indicator of the presence of Hg contamination. This study was part of the Pollution Investigation by Trees (PIT), an international research program funded by the French Environment and Energy Management Agency (ADEME) to test the use of trees to investigate polluted sites.

MP120 Removal of mercury and other trace metals through a constructed vertical flow wetland receiving flue gas desulfurization leachate wastewater

R.J. Reash, C. Svoboda, American Electric Power

To evaluate cost-effective removal technologies needed to reduce levels of mercury to regulatory targets, a vertical flow wetland (VFW) was constructed at the James M. Gavin coal-fired power plant (SE Ohio, USA) to treat flue gas desulfurization landfill leachate wastewater. The wetland, constructed during 2011, is composed of the the following media layers: 35 cm long-leaf sphagnum moss, 30 cm sand, 15 cm pea gravel, and 45 cm of #57 aggregate. VFW inlet and outlet water samples were collected (typically monthly) during 2012 – April 2013 and analyzed for trace elements (including THg and MeHg), dissolved ions, nutrients, and routine parameters. Inlet flows ranged between 0.01 – 0.22 MGD. Significant reductions (> 50%) in concentrations of three trace elements were observed in wetland outlet samples: THg (93%), Al (91%), and Se (62%). More modest removal efficiencies were observed for Co (28%), Cd (21%) and $NO_3 + NO_2$ (20%). Concentrations of both TOC and DOC were higher in wetland outlet samples (mean % increase 64 and 48%, respectively). Levels of MeHg and three dissolved ion parameters (B, SO_4, TDS) neither increased or decreased following passage through the VFW (% change < 20%). Of particular significance was the removal efficiency of THg: the mean inlet and VFW outlet concentrations were 90.4 and 6.3 ng/L, respectively. As the regulatory target goal for the leachate wastewater is 12 ng/L THg, VFW treatment appears to be a viable alternative to traditional chemical precipitation/coagulation processes, which are more costly and can result in higher net concentrations of TDS depending on the specific chemicals utilitized.

MP121 Mercury Pollution, Population Declines and Species Loss: A Review of the Evidence

M.S. Bank, University of Massachusetts / Department of Environmental Conservation

Contaminants such as mercury and its effects on species have been relatively well studied. Recent research investigations have identified methylmercury exposure as an overlooked environmental stressor that can cause negative effects on wild animal populations, even in remote areas. Species sensitive to environmental degradation hold great potential as eco-indicator species. However, eco-indicators are often merely monitored over time without recognizing the processes and mechanisms related to the factors that reduce the overall health and performance of local populations. Mercury contamination in the United States is a well-documented "contaminant biology" example and continues to be an environmental and public-health issue of great concern for certain sectors of the global human population. Documentation of the pervasiveness of this contaminant is a first step toward understanding the potential environmental health and ecological implications of mercury pollution, including species loss and potential population declines of biological organisms. Using weight of evidence approaches I synthesize and evaluate variation in mercury bioaccumulation and distribution across a broad gradient of physical, climatic, biotic, and ecosystem settings to identify the environmental conditions and ecosystem types and species that are most sensitive to mercury pollution. Implications of this work as it relates to the UNEP mercury convention will also be discussed.

MP122 Hg environmental quality standards in aquatic environments: Scientific underpinnings and regulatory implications

D.A. Vignati, CNRS, LIEC, CNRS UMR 7360; S. Polesello, CNR-IRSA, UOS Brugherio; M.S. Bank, University of Massachusetts / Department of Environmental Conservation

Environmental regulatory compliance for Hg pollution has long been assessed in terms of Hg concentrations in the water phase. In such frameworks, Environmental Quality Standards (EQS) are enforced as total Hg concentrations, although methylmercury (MeHg) is the form of highest concern. As a consequence, water quality criteria based on total Hg levels in the water phase may be underprotective against secondary poisoning unless they are adjusted for MeHg levels; a practice that so far is not widespread. Furthermore, monitoring procedures are not necessarily harmonised and even simple filtration before analysis can result in marked differences in Hg levels. To correct for these potential biases, the European Union has introduced, in 2008, a major shift in Hg monitoring and management by adopting an EQS based on total Hg concentrations in biota as an alternative to the water EQS, The use of a Hg-EQS for biota is scientifically sound because it automatically takes into account the bioaccumulation and biomagnification processes that are the main causes of concern from Hg pollution. On the other hand, the proposed EQS numerical value (20 µg kg^{-1} wet weight) risks to create a situation of widespread non compliance in EU water bodies if applied to fish. The Proposal for a Directive of the European Parliament and of the Council as regards priority substances in the field of water policy (published in 2012) states that Member States may classify Hg as an Ubiquitous, Persistent, Bioaccumulative and Toxic substance. Provided that a robust monitoring baseline exists, Member States may present separate classification maps showing that the possible 'not good' status is caused by an UPBT. Hg classification as an UPBT substance must not relax the pressure on Member States to continue targeting important local pollution issues that can cause large differences in neighbouring water bodies. Rather than unduly relaxing a scientifically sound EQS, more state-of-the-art science must be brought in to refine it. We will examine available data for selected European water bodies to show that a biota-based EQS for Hg is scientifically sound and to explore critical issues for consideration and inclusion in further regulatory updates.

MP123 Get Your Hands Out of Your Mouth! Implications from ingestion of Hg-contaminated particles in adobe brick homes in Huancavelica, Peru

N. Hagan, University of North Carolina at Chapel Hill / Environmental Sciences and Engineering; N. Robins, North Carolina State University / Department of History; H. Hsu-Kim, Duke University / Department of Civil & Environmental Engineering; J. Vandenberg, USEPA / National Center for Environmental Assessment

Huancavelica is the capital of the poorest department of Peru, where its 42,000 inhabitants live with the toxic legacy of over 400 years of mercury (Hg) contamination from historic cinnabar refining. Many of the residents are not aware of the high levels of Hg contamination throughout the city, and those who are informed often lack the resources to reduce exposure and alleviate the problem. Health effects from exposure to Hg contamination may be exacerbated, as more than 80% of homes in Huancavelica are constructed using adobe bricks fabricated on-site from contaminated soil. Interior walls and floors are generally unsealed, allowing Hg to emanate in vapor and particulate forms into a largely unventilated space. Concentrations of total Hg in adobe bricks and dirt floors from 60 residences ranged up to 1070 µg/g and 926 µg/g, respectively. Because Hg-contaminated particles can be re-entrained from the walls and floors and settle on surfaces, it is important to recognize potential health implications from the ingestion of particle-bound Hg, particularly for children who are especially vulnerable due to hand-to-mouth activity. Total Hg concentrations are often compared to health benchmarks for ingestion, such as the USEPA's Reference Dose or Superfund's Soil Screening Limit for Residential Soil. These benchmarks, however, are derived for highly soluble, bioavailable forms of Hg, such as

mercuric chloride. In this study, sequential selective extractions were used to characterize the species of Hg present in adobe bricks and dirt floors and simulated gastric fluid extractions were used to estimate bioaccessibility of Hg following ingestion. The results of the present study use this detailed speciation and bioaccessibility data to investigate the potential implications to health from ingestion, particularly for children, in the context of existing health benchmarks. Disclaimer: The information in this abstract has been funded in part by the US Environmental Protection Agency and in part by the EPA/UNC Toxicology Training Agreement CR-83515201-0, with the Curriculum in Toxicology, University of North Carolina at Chapel Hill. It has been subjected to review by the National Center for Environmental Assessment and approved for submission. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US Environmental Protection Agency.

Aquatic Toxicology and Ecology

MP124 A Brazilian port dredged sediment toxicity identification evaluation

T. Furley, APLYSIA Environmental Research and Solutions; K. Chagas, Aplysia technology for the environment / Ecotoxicology Laboratory; G. Lotufo, USGS; A. Guimaraes, V. Bof, Vale

This study evaluated the chronic toxicity of sediment associated with sediment dredging and disposal evaluation for a harbor in Brazil where iron ore and coal are unloaded into cargo ships. Sediment samples (two from the harbor area, four from the disposal area and one from the area between the two sites) causing a reduction of the reproductive output of the benthic copepod Nitokra sp. in standard laboratory bioassays and were submitted to a toxicity identification evaluation (TIE) following procedures developed by the US Environmental Protection Agency. To identify compounds likely causing the observed toxicity, sediments were amended with granular activated carbon (GAC) or one the resins SIR-300, SIR-600 (zeolite) or AGC-50-CS. Hydrophobic organic compounds were identified as the likely toxicity causative agents. This study pioneered the use of the test organism Nitokra sp. in sediment TIE studies in Brazil. This study also provided valuable information on the toxicity of GAC and various resin amendments to *Nitokra* sp.

MP125 An Approach to Examining Toxicity Mechanisms via Microscopy Using Fluorescently Labeled Olefins within Leptocheirus plumulosus

G.L. McMennamy, M-I SWACO / Analytical; K.D. Nguyen, M-I SWACO / Quality Assurance

Benthic amphipods are finding favor among researchers for assessing sediment toxicity because they are sensitive to a wide range of toxicants. *Leptocheirus plumulosus* is a significant ecological estuarine species because it is easily cultured, exhibits a high survival rate under varied environmental test conditions and displays sensitivity to reference toxicants similar to other amphipods test species. Numerous studies have been conducted by industry, resulting in an effective sediment toxicity test. Assorted studies have validated the legitimacy of the test which is currently employed by US Environmental Protection Agency's Gulf of Mexico compliance program for synthetic drilling fluids. Olefins are among some of the synthetic base fluids used in the drilling fluids industry. Olefins come in various molecular weights which can affect the hydrophobic/hydrophilic (HLB) rating thus affecting its properties. Toxicity mechanisms in *Leptocheirus plumulosus* have been researched over the years with various success. The criticality of the research is crucial to the current regulatory requirements in Gulf of Mexico's sediment toxicity testing. To further understand the mechanistic pathway of how drilling fluids in general and base fluids in particular affect the toxicity levels of the amphipods, research efforts have focused on how these chemicals enter the organisms. Multiple studies using different fluorescent tags and functional groups and tracking them inside the organisms showed promising results. These were done by tracking various base fluids inside the amphipods and focusing on particular regions where the chemicals may accumulate and thus confirm via sediment toxicity testing. This study focused on tracking different fluorescently tagged base fluids internally thru the Leptocheirus plumulosus. Olefin based chemicals with different hydrocarbon chains were utilized to study the effect of toxicity within the amphipod during sediment toxicity testing. These chains, with terminal functional groups, were reacted with fluorescent dyes and observed via fluorescent microscopy. It was demonstrated that the toxicity of the olefins were related to different hydrocarbon chain lengths and HLB rankings. Furthermore, tracking of different olefin based fluids were conducted by comparing images of different physiological regions within the amphipod using fluorescent imaging microscopy.

MP126 Assessing potential effects of low-level sediment metal contamination: conflicting results from community-level metrics and toxicity test assessments

A.Y. Oguma, University of Louisiana at Lafayette; P.L. Klerks, University of Louisiana at Lafayette / Department of Biology

Separating the ecological effects of low-level contamination from the confounding effects of natural variation is often a difficult challenge for risk-assessors and ecotoxicologists. Our objective was to determine whether low-level sediment metal contamination is affecting the benthic community of a shallow, cypress-forested lake. Due to prior activity of a now-retired ammunition factory, Caddo Lake currently has a gradient of minor sediment metal contamination (mainly lead) which fades to background levels as one moves away from this source. Sediment samples (n=20) were taken along this gradient for analyses of metals and a variety of other physical and chemical properties, as well for assessment with a standardized 42-d amphipod survival, growth, and reproduction toxicity test. The benthic community was also characterized, to the family level, at each site. Correlation analyses suggested that organic content had a significant positive effect on amphipod reproduction and growth, but that sediment metals were below threshold effect concentrations for the toxicity test. However, a significant negative correlation was found between the concentration of lead in sediment porewater and the total abundance of macrobenthos. Canonical correspondence analysis also suggested that porewater metal concentrations may be affecting community structure. These results suggest that further examination regarding the community-level effects of low-level sediment metal contamination are warranted even when sediment metal concentrations are below currently accepted threshold effect concentrations derived from standardized toxicity tests.

MP127 Cellular Biomarker Assays and Microbial Community Diversity Analyses for Assessing Oyster Health and Sustainability Projects

M. Lowder; B. Khan, S. Clinton, A. Ringwood, UNC-Charlotte

Oysters are important estuarine organisms, both environmentally and economically, and serve as habitat engineers. However, commercial fishing, anthropogenic effects and disease have caused extreme declines in the stocks and viability of oysters over the last hundred years. In recent years, a number of states have made efforts to restore the oyster populations by constructing artificial reefs as sanctuary sites. The intention of the reefs is to increase the overall stock of oysters, and rebuild reef systems that are important to many other species as well. For this study Eastern oysters were collected from various sites off the NC coast, both sanctuary and natural oyster reefs established by university and natural resource management agencies. Some of the sanctuaries, constructed at sites that were expected to be sustainable reefs, started out well but quickly decreased in viability. Data from 2010 samples suggested cellular-level stress in oysters from these sites. The goal of this study was to compare previous results of cellular biomarker assays with more recent studies to assess stress levels using lysosomal destabilization and lipid peroxidation assays, and to compare differences in microbial communities inhabiting oysters from multiple sites using ARISA (automated ribosomal intergenic spacer analysis). The results indicated continued cellular stress in sites that previously showed high levels of lipid peroxidation and lysosomal destabilization, especially at the Hatteras site, suggesting exposure to some type of environmental toxins or physiological stressor. This was particularly interesting because Hatteras is in a remote region that was expected to be a good location for augmenting oyster reef restoration. From the more recent data, there were still signs of sublethal stress that could lead to overall poor health of the population. Results also suggested that oysters identified as stressed using cellular biomarker indicators actually showed an increase in microbial diversity. Changes in microbial diversity may further affect oyster health as well as the safety of consumable seafood. These types of data are essential for assessing sublethal responses to environmental conditions that threaten benthic populations, and can also be used to inform the potential success of environmental management strategies in a timely manner.

MP128 Chironomus spp. test systems: one species fits all?

S. Kimmel, Harlan Laboratories Ltd. / Environmental Toxicology; J. Handley, Harlan Laboratories Ltd; S. Hoeger, Harlan Laboratories Ltd. / Environmental Toxicology

Towards ecotoxicology testing, a tiered approach starting at worst-case testing and ending with intense realistic lifespan testing strategies has proven to be a senseful and sophisticated approach. Further on, a few representative species have become stakeholders for the whole animal kingdom in terms of testing effects of chemicals, agrochemicals and pharmaceuticals. Species of the Genus *Chironomus* have proven to be a suitable insect representative and serve as indicator species from worst-case acute (OECD 235) over chronic (OECD 218/219) to full life-cycle testing (OECD 233). Further on, a combination between all mentioned setups and guidelines is possible, which makes *Chironomus spp.* an ideal testing species for covering all necessary aspects of a robust risk assessment for potentially hazardous substances and active compounds in terms of aquatic and soil & sediment ecotoxicology as well as higher-tier testing strategies. Several examples and setups as well as critical discussion of possible benefits/disadvantages of using one species extensively will be presented. Further on, modifications/adaptations according to individual study challenges (e.g., difficult test items, radiolabeled substances) and recent improved requirements according to regulation 1107/2009 will be displayed. A clear advantage of using organisms like *Chironomus riparius* is given by the different possible testing strategies and covering different mode of actions, entries into the environment as well as persisting behaviour of a substance, whereas focussing exclusively on one species might lead to a certain limitation of knowledge towards critical substances.

MP129 Community-Level Effects of Excess Total Dissolved Solids Doses Using Model Streams

C.T. Nietch, USEPA / Water Supply Water Resources Division; J.M. Lazorchak, USEPA / National Exposure Research Laboratory; B. Johnson, USEPA; B. Ramakrishnan, CBI, Inc.; P. Weaver, McConnel Group; D. Macke, K. Daniels, USEPA; S. Decelles, McConnell Group; D. Brown, USEPA; C.A. Impellitteri, USEPA / Water Supply and Water Resources Division

Model stream chronic dosing studies (42 days) were conducted with four different total dissolved solids (TDS) recipes. The recipes differed in their relative dominance of major ions. One was made from sodium and calcium chloride salts only. Another was similar to the first, but also included minor additions of salts of strontium, barium, magnesium, potassium, and bromide. A third was dominated by sulfate and bicarbonate while attempting not to skew sodium to calcium ratios beyond what is found in real systems. The last was comprised of sodium bicarbonate. The TDS composition and dosing gradients were derived from field data for streams where high potential for excess TDS impact exists. The recipes were dosed to a continuously renewed natural river water feed that represented a moderately hard water quality. All tests followed a dose response design and pair single-species exposures in both bench- scale whole effluent testing (WET) and ex-situ formats with whole community responses. In the model streams, measurements of periphytic biomass, chlorophyll content, and algal speciation were made. Macroinvertebrate counts, biomass, emergence and drift were assessed. Nutrient cycling, metabolism, and litter decomposition indicators were also tracked during each TDS dosing period. When the ions in excess came from the addition of sodium and calcium chloride only, the results indicated change in the stream periphyton occurring at TDS around 700 mg/L (EC20), macrobenthic structure was significantly altered after 1000 mg/L, and insect development (growth and emergence) was affected at TDS as low as 300 mg/L. These TDS sensitivity thresholds appeared to increase (i.e., communities are less sensitive) when the recipe included the minor cations of strontium, barium, magnesium, and potassium in relevant proportions. Communities appeared more sensitive to the TDS recipe dominated by sulfate and bicarbonate anions. The results provide community-level context for existing State-level TDS criteria for aquatic life, which generally ranges between 500 and 1500 mg/L pending designated use and natural background conditions, and is often qualified by chloride and sulfate content. The observed whole community responses suggest reconsideration may be warranted in some effluent dominated systems.

MP130 Dopamine and serotonin metabolisms in freshwater planarian, Dugesia japonica, and the effects caused by cadmium exposure

J. Wu, Department of Geography, National Taiwan University / Environmental Toxicology Laboratory, Department of Geography; M. Li, National Taiwan University / Environmental Toxicology Laboratory, Department of Geography; H. Lee, S. Zhong, Fu Jen Catholic University / Department of Chemistry

Although flatworms, or Platyhelminthes, are acoelomate and are considered lower taxonomically and evolutionally, they are the simplest bilateral animals that possess the nervous systems concentrated at the head end, which was considered as the most primitive form of brain. In flatworms, occurrences of neurotransmitters and their corresponding receptors and/or enzymes had also been reported and reviewed since 1970s. Hence, they were always regarded as important animal models for neurology related researches, particularly free-living flatworms, or planarians, of which their maintenance and experimental manipulation in laboratory were relatively easy. Dopamine (DA) and serotonin (5-HT) are two of major neurotransmitters found in freshwater planarians. DA modulates the locomotor activity of planarians, and 5-HT controls its regeneration and mediates the regulation of some neuromuscular functions. The synthetic pathways of DA and 5-HT were relatively clear in free-living planarians. In contrast, however, metabolisms of DA and 5-HT in planarians were not investigated. Hence, in the present study, we attempted to develop a high performance liquid chromatography (HPLC) coupled with carbon black nano (CBN)-modified electrochemical detector (ECD) method for determinations of DA, 5-HT and their metabolites in *Dugesia japonica*. Furthermore, monoamine oxidase (MAO) and catechol-O-methyla-transferase (COMT), two important enzymes involving in the metabolisms of DA and 5-HT in animals, were characterized and their activities were measured in planarian. Finally, effects of toxic metal, cadmium (Cd), on the metabolisms of DA and 5-HT, in terms of levels of DA and 5-HT, and activities of MAO and COMT in treated planarians, were studied.

MP131 Effect of elevated temperature and salinity on swimming endurance of the western mosquitofish (Gambusia affinis)

C. Funkhouser, Texas Tech University / Department of Natural Resources Management; D. Rogowski, Texas Tech University

Climate and anthropogenic changes are predicted to increase temperatures and salinities in aquatic systems. These changes may negatively affect a variety of aquatic species. By testing swimming performance one can assess the potential impacts of increased temperatures and salinity (specific conductance) on the physiology of exothermic organisms. We used the western mosquitofish, *Gambusia affinis,* as our study organism. Twenty fish were exposed to either, temperature (23, 26, 30°C) or specific conductance (900, 10,000, 17,000 $\mu S/cm^2$), and exercised individually in a flume at a water velocity of 0.2m/s. Time to exhaustion was recorded when the fish was no longer able to maintain its position in the water column and was ejected from the flume. Temperature had no significant effect on time to exhaustion, though specific conductance did ($F_{1, 59}$=4.4, p=0.04). At the higher and lower specific conductances (900 and 17,000$\mu S/cm^2$) time to exhaustion was significantly less than the control at 10,000 $\mu S/cm^2$. Sex had no effect on time to exhaustion for either the temperature or specific conductance trials. These results showed consistent swimming performance of *G. affinis* across a seven degree range of temperature, but decreasing performance with elevated specific conductance (salinity). These tolerances may potentially provide *G. affinis* an advantage over less tolerant native species as climate continues to change and habitats are altered.

MP132 Effects of elevated CO2 levels on accumulation and sub-cellular compartmentalization of trace metals (copper and cadmium) in marine bivalves

C.A. Hawkins, I.M. Sokolova, University of North Carolina at Charlotte / Department of Biology

Estuaries and coastal zones act as natural sinks for naturally and anthropogenically derived trace metals such as cadmium and copper that can strongly affect the survival and performance of resident organisms. Estuaries also experience short-term fluctuations in the partial pressure of carbon dioxide (P_{CO2}) levels as well as a long-term trend of increasing P_{CO2} due to the accumulation of CO_2 in the atmosphere. An increase of P_{CO2} leads to a decrease in pH and shifts in the carbonate chemistry of the ocean waters that can affect solubility and bioavailability of metals present in the estuarine waters and sediments. Currently, the interactive effects of elevated P_{CO2} and trace metals on estuarine organisms are not well understood. We determined

whether elevated P_{CO2} levels affect bioavailability, accumulation, and intracellular distribution of trace metals (Cd and Cu) in marine bivalves, the hard clams (*Mercenaria mercenaria*) and the eastern oysters (*Crassostrea virginica*) that are commonly exposed to metals and elevated CO_2 levels in their habitats. Clams and oysters were exposed for 28 days to clean artificial seawater (ASW) (controls) or to ASW with 50 µg L^{-1} Cd or Cu at three different P_{CO2} levels (~400, 800 and 2000 µatm) corresponding to the present day conditions and the projections of the Intergovernmental Panel for Climate Change (IPCC) for the years 2100 and 2250, respectively. Total content of Cd and Cu was measured in the gill tissues of clams and oysters acclimated at different P_{CO2}, and the distribution of these metals among subcellular fractions (insoluble metal-containing granules, mitochondrial, lysosomal, and microsomal fractions, as well as cytosolic heat labile and heat stable proteins) was determined using atomic absorption spectroscopy. This study determines the effects of elevated P_{CO2} in environmentally relevant range on accumulation and intracellular handling of two important metal pollutants and could shed important new light on the interactive effects of common environmental stressors in estuaries and coastal zones on metal physiology and homeostasis of marine mollusks. Supported by UNC Charlotte's Faculty Research Grant.

MP133 Effects of fate and transport of 17-β-trenbolone in sediment on endocrine function in female fish

S.Y. Skolness, DepartmentBiology; S. Slobotski, University of Nebraska Omaha; A.S. Kolok, University of Nebraska – Omaha / Nebraska Watershed Network

The fate and transport of steroidal compounds from agricultural runoff is not well understood; where the steroids may either remain in the sediment, desorb from the system, or breakdown to become bioavailable to aquatic organisms. The primary objective of the current study was to evaluate the fate and transport of 17-β-trenbolone in sediment from Elkhorn River, Nebraska. Preliminary data of spiked sediment was used to profile of the breakdown and release of 17-β-trenbolone to the metabolite trendione. From the initial spike, 17-β-trenbolone rapidly degrades to trendione within the 96 hours. From these data, we designed a time-course exposure to capture the breakdown profiles, complete breakdown profile (1-14 d), 17-β-trenbolone profile (1-4 d), and the metabolite trendione profile (4-18). Female fathead minnow (*Pimephales promelas*), were exposed to 17-β-trenbolone spiked sediment in 4 treatment groups: 1-4 d, 1-14 d, 4-18 d, and 1-4 d exposure with 10 d depuration. Upon completion of the exposures, liver and gonadal tissues were collection for gene expression analysis. Gene expression analysis of the target gene vitellogenin (vtg), important to normal biological function in female fish, was down-regulated in the treatment groups 1-4 d, 1-14 d, 4-18 d, and back to control levels in the 4 d exposed/10 d depurated fish. Tubercle production was noted in three of the treatment groups, 1-14 d, 4-18 d, and 4 d exposed/10 d depurated. Overall, female fish exposed to 17-β-trenbolone spiked sediment were defeminized in all treatment groups. The anti-estrogenic effect, reduced vtg expression, was remediated in the depurated fish; however, the androgenic effect, tubercle production, remained. Overall, this exposure analyzes the defeminization of female fathead minnows over the breakdown and release profile of 17-β-trenbolone in sediment.

MP134 Effects of hypoxia on embryonic development in Rana pipiens

J.D. Fernandez, J.R. Schroeder, Millikin University / Department of Biology

Low oxygen levels, or hypoxia, affect many aquatic organisms. While there is ample research on the effects of hypoxia on developed *Rana* species, there is considerably less literature focused on the effects of hypoxia on developing embryos and tadpoles. Thus, our study focused on examining the effect of hypoxia during early embryonic development. *Rana pipiens* eggs were separated into four different hypoxia treatment groups and one control group and were subjected to hypoxia at different stages of development. Our study shows that if embryos were exposed to hypoxia 3-8 days post-fertilization, during the specification of the central nervous system and organogenesis, the embryos were less likely to survive to the final stages of development.

MP135 Enhancing environmental education in middle school classrooms by graduate students in the Arkansas Delta through an NSF GK12 Program

J.L. Bouldin, Arkansas State University / Department of Biological Sciences, Environmental Sciences Graduate Program / Biological Sciences; K.L. Yanowitz, Arkansas State University / Psychology & Counseling; A.A. Grippo, Arkansas State University / Biological Sciences; C.A. Miller, Arkansas State University / Delta Institute for Mathematics & Science Education; J.T. Kennon, Arkansas State University / Dept of Chemistry & Physics

Interest in science careers begins during the early years of a child's education. Enhanced science curriculum by Graduate Fellows was incorporated into grades 6-8 science activities for five continual years. This National Science Foundation GK-12 grant facilitated Arkansas State University to target rural public school districts in the Arkansas Delta region which are represented by diverse ethnic student populations. Throughout the duration of the project 6,812 students in 10 school districts were visited weekly by the Graduate Fellow assigned as a resident scientist. Each Graduate Fellows team-taught with two Mentor Teachers to enhance the science environment and strengthen Mentor Teachers' content knowledge and experience. Hands-on learning for the students was directed by the Graduate Fellows based on their research experiences. Lesson plans written by the Fellows are aligned to the Arkansas Science Curriculum Frameworks, and sparked excitement and inquisitiveness in the students. Graduate Fellows found increased understanding of their research and improved communication skills while middle school students were introduced to cutting-edge science curriculum. Lesson plans are available to classroom teachers for further use and facilitate continued enhanced science curriculum to encourage student interest in STEM careers.

MP136 Immunomodulatory potencies of cyanobacterial toxic metabolites

O. Adamovsky, Masaryk University, Faculty of Science, RECETOX / Research Centre for Toxic Compounds in the Environment (RECETOX); L. Svihalkova, L. Kubala, Academy of sciences of Czech Republic / Institute of biophysics; L. Blaha, Masaryk University / Faculty of Science / Research Centre for Toxic Compounds in the Environment (RECETOX)

Massive growth of cyanobacterial water blooms became one of the most serious environmental and health problems worldwide. Cyanobacteria produce many biologically active metabolites including various pro-inflammatory and immunomodulatory agents. Up to now, immunotoxic/immunomodulatory potencies were evaluated only for a limited set of selected cyanobacterial strains or few known cyanobacterial toxins such as microcystins (MC) and lipopolysaccharides (LPS). In our study we focus on the characterization of the imunomodulatory potency of known cyanobacterial toxins to interfere with natural macrophage (RAW 264.7 cell line) function. Non-physiological modulation of macrophage natural functions plays critical roles in chronic inflammatory diseases, initiation of inflammatory and immune responses and other adverse health effects. Two experimental designs were studied, (i) direct effects of cyanobacterial toxins on macrophage viability and inflammatory response, (ii) and modulation of macrophage responses by cyanobacterial samples in co-exposures with selected model stimulatory molecules (e.g., LPS from heterotrophic bacteria). In addition to nonspecific effects (viability etc.), we focused on production of nitric oxide, which gives macrophages cytotoxic activity against viruses and bacteria and production of pro-inflammatory cytokines such as interleukin IL-6 and tumor necrosis factor TNFα. The study showed significant effects of toxic cyanobacterial blooms on immune responses, which has not been included in serious assessment of cyanobacteria risks. The research was supported by the grant from the Czech National Science Foundation (GAČR GP13-27695P), infrastructure is supported by the project CETOCOEN from the European Regional Development Fund.

MP137 Innate Immune defenses against disease in the Hawaiian coral Montipora capitata

M.S. Ansley, University of Mississippi – ETRP / Environmental Toxicology Research Program; D.J. Gochfeld, C. Williams, D.J. Lee, University of Mississippi / National Center for Natural Products Research; G.S. Aeby, Hawaii Institute of Marine Biology

Emerging infectious diseases are a critical stressor to marine communities, particularly coral reefs. These diseases can cause bleaching, tissue necrosis, and even death. Corals possess a diversity of innate immune defenses, such as physical barriers and antibacterial chemical defenses, which may protect them from disease. For example, the coral surface mucus layer serves as a first line of defense against invading pathogens, while the coral holobiont produces antibacterial chemical defenses. This study addressed variability in the antibacterial chemical defenses in the Hawaiian coral *Montipora capitata*, which is susceptible to *Montipora* White Syndrome. *M.capitata* occurs

in two color morphs, orange and red. These color morphs have different susceptibilities to this disease on the reef, which may be due to differences in immune response to pathogenesis. Mucus, as well as aqueous and organic extracts of the coral tissue, were tested for antibacterial activity in bioassays against a panel of known marine and human pathogens. In addition, chemical profiles of the two color morphs were generated by high performance liquid chromatography, and compared. Mucus and aqueous extracts showed selective activity against the tested pathogens; however, the organic extracts were relatively inactive. Extracts from the red color and orange color morphs showed differences in bacterial inhibition and the chemical profiles also exhibited differences between the color morphs. Differences in antibacterial chemical defenses may play a role as part of the innate immune system of *M. capitata* in protecting the coral against stressors in the marine environment.

MP138 Introducing Partition Coefficients to K-12 Students: A Fun Teaching Activity That Can Be Modified for Many Grade Levels

A.A. Grippo, Arkansas State University / Biological Sciences; J.T. Kennon, Arkansas State University / Dept of Chemistry & Physics; M. Hall, Arkansas State University / Mathematics & Statistics; K.L. Yanowitz, Arkansas State University / Dept of Psychology & Counseling; R. Engelken, Arkansas State University / Engineering; J.L. Bouldin, Arkansas State University / Department of Biological Sciences, Environmental Sciences Graduate Program

As part of the Arkansas State University/NSF Noyce Scholarship program, our Try out the Classroom (TOTC) workshop participants designed and tested a simple, hands-on experiment that mimics a real-life method for detection of an important physical/chemical parameter: partition coefficient. The seven Noyce TOTC participants, who registered for the workshop in order to decide whether they would like to become teachers in a STEM field, included undergraduates and graduate students majoring in biology, mathematics, and chemistry. After learning some basic chemistry on hydrophilicity/hydrophobicity and how that is important to environmental and life scientists, the workshop participants identified and tested different, easily obtainable vessels (such as empty water bottles); various types and amounts of oils; and common household products that could be analyzed for hydrophobicity, using color as an indicator. Guided by ASU faculty and area classroom teachers, participants researched background information on environmental, pharmaceutical, and other chemicals for which partition coefficient data is known, as well as AR frameworks for biology, chemistry and mathematics at all K-12 grade levels. Participants wrote pre- and post-tests and a 5E lesson plan, and tested their activity with high school Upward Bound chemistry students. This activity is fun, easy and instructive to students and teachers, and can be adapted to many grade levels. It introduces real-life physical science parameters and testing useful to environmental and life scientists, and can integrate science with mathematics. It also proved to be helpful to workshop participants in learning to design and trouble-shoot an experiment, as well as influencing their decision about teaching in a STEM field.

MP139 Polychlorinated biphenyl distribution and bioavailability in a Southern Illinois lake

M.C. Archer, A.D. Harwood, J.J. Hillis, Southern Illinois University Carbondale / Department of Zoology, Center for Fisheries, Aquaculture, and Aquatic Sciences; M. Mills, USEPA / National Risk Management Laboratory, Office of Research and Development; M.J. Lydy, Southern Illinois University Carbondale / Department of Zoology, Center for Fisheries, Aquaculture, and Aquatic Sciences

Persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) are a concern to environmental and human health. Previous studies have detected PCBs in both sediment and bluegill from Campus Lake of Southern Illinois. The primary objective of this study was to build on preliminary data to evaluate the distribution and bioavailability of PCBs in Campus Lake. After examination of whole sediment concentrations throughout the lake, one PCB hotspot was identified. This hotspot was then further investigated through sediment extractions, laboratory-based bioaccumulation bioassays using *Lumbriculus variegatus* and *Chironomus dilutus*, and Tenax extractable concentrations. These studies revealed that PCBs from Campus Lake are bioavailable to organisms inhabiting the lake. While this data provides information on the magnitude of the contamination, the ultimate goal of this project will further evaluate the PCB concentrations of several species of different trophic levels including: tetragnathid spiders, emerging chironomids, and various different fish tissues and relate them to Tenax extractable concentrations. This will provide further insight on the potential of Tenax extractable concentrations to evaluate risk in sediments.

MP140 Sorption and Anaerobic Degradation of Explosives in Coastal Sediments Using Isotopically Labeled Compounds

T.S. Ariyarathna, University of Connecticut / Dept. of Marine Sciences; P. Vlahos, University of Connecticut / Departments of Marine Sciences and Chemistry; C. Tobias, University of Connecticut; M. Ballentine, University of Connecticut / Department of Marine Sciences; R. Smith, University of Connecticut

The explosive compounds, 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) are synthesized globally, mainly as military munitions, and have become priority pollutants of concern. As coastal zones and estuaries are commonly impacted zones it is important to understand the fate and transport of these compounds in these environments. Here we present studies focused on the sorption kinetics and degradation of TNT and RDX in intertidal silt, fine, high carbon containing sediment at 23^0C under anaerobic conditions. Experiments were carried out by spiking ^{15}N labeled TNT and RDX solutions into sediment slurries in two separate experiments. Water and sediment samples were analyzed for parent compounds and degradation products over a two week period of experiments. Sediment uptake rates for TNT and RDX are 5.68 μmol L^{-1} hr^{-1} and 1.57 μmol L^{-1} hr^{-1}respectively. Equilibrium partition constant for TNT was 0.076 L/g whereas it was 0.0009 L/g for RDX. Isotope analysis of the bulk sediments revealed an initial rising inventory of ^{15}N illustrating the role of sediments on sorption and degradation of both compounds. Water samples from the slurries will be analyzed for ^{15}N inventories in different pools of nitrogen including ammonium, nitrate, nitrite and nitrogen gas. The results will be used to identify the mineralization pathways of parent compounds into both organic and inorganic nitrogen pools entering the marine nitrogen cycle. Quantification of degradation and transformation rates of TNT and RDX will lead to mass balances in the systems and help identify gaps. These experiments will be extended into ecocosms with biota to address the importance of sediments on mineralization of explosives in large scale in near future and ecosystem coupling. Chemical parameters evaluated include pH, salinity, dissolved oxygen, dissolved organic matter, iron and sulfide in sediment profiles that are correlated to explosive metabolism.

MP141 Taxonomic and Spatial Variation in Trace Element Bioaccumulation in Odonate Nymphs in a Stream Receiving Coal Combustion Waste

A.H. Lindell, University of Georgia – Savannah River Ecology Laboratory; G.L. Mills, J.V. McArthur, University of Georgia / Savannah River Ecology Laboratory; D.E. Fletcher, Savannah River Ecology Laboratory

Dragonfly nymphs provide an exceptional opportunity to compare macroinvertebrates that are all predators within the same order (Odonata) and suborder (Anisoptera), but differ vastly in their habitat use and body form. Ecological habits range from those that bury in the stream sediment through those that sprawl across the surface of it, to those that cling to suspended wood debris or root masses. Body form ranges from long and slender to those with broad, palmate abdomens. Their predaceous behavior and relatively long nymphal stage renders them potentially excellent integrators of contaminants in both space and time. Moreover, odonate nymphs have relatively ubiquitous distributions in diverse habitats ranging from swift streams to isolated wetlands. Their relatively large size for a macroinvertebrate also makes them suitable for contaminant analyses. We incorporated four families and a total of eight genera of dragonflies into our current analyses. Dragonflies were collected from two sites on Beaver Dam Creek, a Savannah River tributary located on the Savannah River Site, South Carolina, USA. Beaver Dam Creek received effluents containing coal combustion waste from a coal fired power plant in its headwaters for over 50 years. Trace element (Be, V, Cr, Ni, Cu, Zn, As, Se, Sr, Cd, Sb, Cs, Ba, Hg, Tl, and Pb) and stable isotope (C and N) analyses were conducted on over 100 composite dragonfly samples. Dragonflies from the downstream site were more enriched with ^{15}N, but the N isotopic signature indicated broad overlap in trophic position. Patterns of carbon sources utilized among taxa differed between sites with the climber-sprawlers and climbers diverging. Both taxonomic and spatial differences in trace element bioaccumulation were observed, including differences between genera within a family. Interestingly, nymphs of different families but of similar body form had more similar patterns of bioaccumlation to each other than to members of their own family with different body forms. For some elements such as Ni, Ba, Sr, V and Be, concentrations differed in consistent patterns based on habitat use. Sprawlers

and climber-sprawlers that are often associated with finer sediments in depositional zones accumulated the highest concentrations.

MP142 The effect of cadmium on ubiquitin-proteasome pathway involved in degradation of hemoglobin proteins in larvae of 4th instar Chironomus riparius

J. Oh, Department of Biological Sciences; C.S. Bentivegna, Seton Hall University / Department of Biological Sciences

Larvae of 4th instar Chironomidae or chironomids are a convenient animal model for studying environmental quality. In order to generate a useful biomarker, we have been attempting to characterize various molecular responses exhibited by chironomid upon a change in their environment – especially upon exposure to heavy metals, which are frequent toxic pollutants found in urban environments. Our findings indicated that hemoglobin (Hb) proteins of chironomid – major proteins found in their hemolymph – are an appropriate biomarker for evaluating environmental quality because they are modulated by heavy metals like cadmium (Cd). Effects on Hb were detected by observing protein profiles in the range of 4-17 kDa as detected using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Cd affected Hb protein profiles by causing either the disappearance or decreased intensity of bands ranging from 4-17 kDa. Liquid chromatography-mass spectrometry (LCMS) data in control chironomid indicated that the affected bands were either whole or fragmented products of Hb VII gene, suggesting that Cd might exacerbate the normal degradation process of Hb. In this study, we investigated the Ubiquitin (Ub)-proteasome pathway as a mechanism for Hb degradation in the presence of Cd using larvae of *Chironomus riparius*. More specifically we studied: 1) the presence and expression of Ub gene and 2) the effect of native proteases – chymotrypsin, pepsin, and trypsin – on Hb profiles of control and Cd exposed chironomids.

MP143 Tissue alterations in catfish gill from Tecolutla, Veracruz, Mexico

C. Garcia Gonzalez, X. Guzman-Garcia, I. Hernandez Calderas, P. Ramirez Romero, U.A.M. Iztapalapa / Hidrobiologia

Fish is the most abundant and threaten vertebrate group. There is a lack of information on the natural distribution and environmental requirements of most Mexican fish species. Catfishes have been used as water quality bioindicators, however in Mexico there are very few studies of fish communities in degraded sites. Tecolutla is located on the Gulf of Mexico Coast, and there have been reports of toxicity in its sediments, in contrast water quality has been heterogeneous. Fish gills, kidneys, liver and skin have been considered pollutant target organs, useful to evaluate pollution effects and therefore important to be used in the evaluation of biological responses through histopatological biomarkers. In the present study 46 *Arius felis* gill samples, from an annual cycle, were stained with Hematoxilin-Eosin and analyzed. Tissue lesions found include: circulatory alterations, reversible changes and progressive changes of different degrees. The most frequent circulatory alteration registered was congestion, in different progession levels. Mucus cell proliferation was observed associated to hyperplasia and secondary lamella fusion. Hyperplasia in different degrees was the most frequent progressive damage observed. The prevalence of lesions in catfish gills suggests that these organisms' health is compromised and therefore the environmental health of this ecosystem is too.

MP144 Tissue evaluation of the oyster Crassostrea virginica in the Gulf of Mexico

I. Hernandez Calderas, C. Garcia Gonzalez, X. Guzman-Garcia, P. Ramirez Romero, U.A.M. Iztapalapa / Hidrobiologia

Histopathology is a tool that allows the evaluation of tissue changes and has been proposed as a biomarker. Bivalve mollusks have been used internationally in a variety of monitoring programs. The objective of the present work was to evaluate the tissues of the oyster *Crassostrea virginica* in the Gulf of Mexico. 158 samples were collected in five sites, these were fixed, cut (three serial cuts of 5 um) and stained with H-E, PAS and Masson. Analyses included observations with optic microscope and the elaboration of a diagnostic table with selection criteria for special hystochemical staining. The tissue description emphasized the alimentary channel (esophagus, stomach, stile sac, intestine and digestive gland). The application of special staining techniques, like PAS, demonstrated the type of mucopolysaccharides of the basal membrane in healthy individuals. Collagen presence was recognized and was related to tissue reparation processes. Tissue analysis tables showed lesions prevalence in oysters collected in four out of the five collection sites. Tissue responses included the presence of brown cells, inflammations, parasites, eosinophil secretions, epithelial atrophy of the digestive tubules and the connective tissue, fibrosis and granulomas.

MP145 Transcriptome sequencing and De novo Analysis for the Asian clam (Corbicula fluminea) using the Illuminea GAIIx method

H. Chen, State Key Laboratory of Environmental Aquatic Chemistry; J. Zha, Z. Wang, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences / State Key Laboratory of Environmental Aquatic Chemistry

We used a next-generation high-throughput DNA sequencing technique, the solexa/Illuminea GAIIx method to analyze the transcriptome from the whole bodies of *C.fluminea*. More than 6.7 Gb high quality date were generated based on the raw date, and 134,684 unigenes with a mean length of 791 bp were assembled using Velvet and Oases *de novo* software. All the assembly unigenes were obtained by running BLASTx and BLASTn similarity searches on the Nt, Nr, SWISS-PROT, COG and KEGG databases. In addition, the Clusters of Orthologous Groups (COGs), Gene Ontology (Go) terms and Kyoto Encyclopedia of Gene and Genome (KEGG) annotations were also assigned to each transcript unigenes. More than 2,151 simple sequence repeats (SSRs) and 268,126 single nucleotide polymorphisms (SNPs) were also detected. To primarily verify part of the results of assembly and annotations and search for some environment pollution biomarkers, two cytochrome P450 genes, three Hsps (Hsp60, Hsp70 and Hsp90) genes and three GABA-related genes were cloned based on related transcripts and their expression in tissues were measured by real-time quantitative PCR.

MP146 Trophic Characterization and Differential Trace Element Accumulation in Congeneric Catfishes (Ictaluridae: Ameiurus)

D.E. Fletcher, Savannah River Ecology Laboratory; A.H. Lindell, University of Georgia – Savannah River Ecology Laboratory; G.K. Stillings, G.L. Mills, J.V. McArthur, University of Georgia / Savannah River Ecology Laboratory

Differences in morphology of congeneric species may indicate differential habitat use, feeding habits, and consequently disparity in contaminant bioaccumulation. We compared trophic position, carbon sources, and contaminant bioaccumulation among and within three species of *Ameiurus* (bullhead catfish) that differ in varying degrees in coloration, body form, caudal fin shape, and mouth position. Fish were stratified across the broadest size ranges available from two sites on Beaver Dam Creek, a Savannah River tributary located on the Savannah River Site, South Carolina, USA. This stream received effluents containing coal combustion waste from a coal fired power plant in its headwaters for over 50 years. Trace element (Be, V, Cr, Ni, Cu, Zn, As, Se, Sr, Cd, Sb, Cs, Ba, Hg, Tl, and Pb) and stable isotope (C and N) analyses were conducted on muscle samples of 97 fish. Bioaccumulation in the liver was explored by analyzing trace elements on a subsample of 22 fish. Trophic level ($\delta^{15}N$) and carbon sources ($\delta^{13}C$) did not differ among species within size classes, but greater variability in these measures occurred at the more disturbed upstream site. This variability was not due to body size or sex. Based on the restricted subsample of fish, bullhead livers effectively sequestered many elements to higher concentrations than muscle. Muscle element concentrations were often highest in the smallest individuals. This pattern may ameliorate human risk, but put predators of the smaller fish at greater risk. When accumulation varied spatially, concentrations were higher at the upstream site that was closer to the effluent source. Some elements (Be, V, Cd, Sb, Tl, Pb, and As) rarely accumulated in muscle while others (Zn, Cu) accumulated, but did not differ between species or sites. Bioaccumulation of Se, Hg, Ba, and Sr, differed both within and among species. Patterns of elemental accumulation were species specific. However, multivariate analyses suggest more similar patterns overall in the two more morphologically similar species supporting our initial hypothesis.

MP147 Effects of Wastewater Treatment Plant Effluent on Survival, Growth, and Vitellogenin Concentrations of Fathead Minnows (Pimephales promelas)

L. Griffin, University of WI-Whitewater; E. Harrahy, University of Wisconsin-Whitewater / Department of Biological Sciences

One source of pharmaceuticals and personal care products (PPCPs) pollution is wastewater treatment plants (WWTPs), where some PPCPs are being delivered and then discharged to receiving waters. Estrogens, the primary female sex hormones, have been found in natural and synthetic forms in effluents and in receiving waters. Studies conducted by others have shown that male fathead minnows (*Pimephales promelas*) exposed to estrogens or

estrogen mimics can produce vitellogenin, an egg precursor protein normally found only in female fish. Field and laboratory studies were conducted to determine if effluent from the Whitewater WWTP affects survival, egg production, and/or vitellogenin concentrations in fathead minnows. Results from a 24-day field reproduction assay conducted using caged fish showed no significant difference in survival, egg production, or vitellogenin concentrations between sites located upstream and downstream from the WWTP effluent pipe. Vitellogenin was detected (using ELISA) in males at both sites, though in higher concentrations downstream. To eliminate other sources of estrogenic contaminants, whole effluent toxicity (WET) tests were performed in the laboratory to determine if whole effluent from the Whitewater WWTP impacts survival or growth of fathead minnow larvae. Results of WET tests showed no significant differences in survival (acute and chronic) or growth (chronic). A 21-day reproduction assay conducted in the laboratory showed no significant differences in survival or egg production in adult fathead minnows. However, vitellogenin was detected in all male fish analyzed, including controls. There could be other sources of estrogenic compounds in the laboratory that interfere with test results. Future studies will examine potential sources of estrogenic compounds in the laboratory. Analysis of effluent samples may help identify estrogenic compounds of importance. Combining field and laboratory studies will give us a better understanding of the effects effluent can have on aquatic organisms.

MP148 Investigating distributions and baseline biology of Crangon septemspinosa at contaminated and reference sites in the Saint John Harbour, Canada

R.J. Power, Canadian Rivers Institute, Biology / Biology; S. Courtenay, D. Methven, University of New Brunswick; K.R. Munkittrick, Canadian Rivers Institute, Biology

The Saint John Harbour (SJH) estuary is one of the regional nodes in a national project looking at developing a cumulative effects assessment monitoring program. The focus of this study is to examine whether there are responses in sentinel species exposed to the sewage effluent from a new municipal wastewater treatment facility in the area of discharge. Nearshore sampling was carried out at low tide, during the day, with a 9 X 1.5 metre beach seine with 9mm stretch mesh. The most abundant epibenthic animal caught was *Crangon septemspinosa* (sand shrimp). The potential of sand shrimp as a sentinel species for contamination will be investigated. A good sentinel species is abundant, resident, demersal, and has easy to measure health endpoints. Sand shrimp cohorts will be followed throughout an annual cycle to detect spatial and temporal changes in abundance, size, condition factor (length-at-weight), sex ratios, and fecundity. Some preliminary results show differences in condition factor (a metric of energy storage), size-at-maturity, and abundances between sexes. Females are more abundant than males (> 70% of total catches), have a higher condition factor and a smaller size-at-maturity at all sites. Differences were detected when compared to reference sites, including more females bearing eggs, and larger males and females present at the wastewater discharge site and one other reference site. If sand shrimp are determined to be a good indicator of contamination, we can give this tool to industrial watershed end-users by determining optimal sampling times and what is normal at multiple reference sites so cyclical monitoring can be carried out.

Ecological Risk Assessment

MP149 A modified bioaccumulation model to evaluate trophic magnification of select perfluoroalkyl acids (PFAAs) in a terrestrial food web

M.R. McDougall, School of Resource and Environmental Management; F.A. Gobas, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment)

Predictive bioaccumulation models are tools developed to assist with chemical categorization and risk assessment for substances of bioaccumulative concern. These models, however, have been largely developed for neutral (i.e., non-ionic) substances, but not for ionizable substances, including perfluoroalkyl acids (PFAAs), a class of environmentally ubiquitous commercial chemicals used predominantly as surfactants. The octanol-water partition coefficient (K_{OW}) is often used within bioaccumulation models to describe chemical partitioning of neutral persistent organic pollutants (POPs) into lipids. However, protein partitioning of PFAAs is believed to be critical for understanding the bioaccumulative behaviour of these compounds, particularly in protein-rich tissues of terrestrial organisms, such as the liver. In the current study, an existing terrestrial bioaccumulation model for a lichen-caribou-wolf food web in the Canadian arctic is modified to account for the proteinophilic nature of PFAAs. Trophic magnification factors (TMFs) will be calculated from previous and modified model outputs and compared to observed biomagnification within this food web to test the hypothesis that existing bioaccumulation models accounting for lipid partitioning alone do not adequately evaluate the bioaccumulative behaviour of PFAAs. Ultimately, this modified model can be used to reassess bioaccumulation risks for PFAAs and contribute to modification of regulations surrounding such chemicals as necessary.

MP150 A Reduction/Oxidation Model for Abiotic Degradation of Munition Constituents in Soils

A. Miglino, Dept. of Civil & Environmental Engineering; R. Gonzalez, D.M. Di Toro, University of Delaware / Dept. of Civil & Environmental Engineering; H.E. Allen, University of Delaware / Dept. Civil & Environmental Engineering

Munition constituents (MC) are compounds of environmental concern on military testing ranges due to the potential for off-site transport. The behavior and fate of six of these compounds (nitroglycerin, RDX, HMX, nitroguanidine, 2,4,6-trinitrotoluene and 2,4-dinitrotoluene) in soil is explored. Laboratory work generated a set of sequential desorption and extraction data for the MCs on 30 soils for several adsorption times. From mass balance calculations it has been observed that MCs undergo various degrees of abiotic degradation in soil. It is thought that this degradation occurs via RedOx reactions. The extent of the reaction is controlled by the amount of available reductant in the system; when reductant is depleted, degradation ceases. Abiotic degradation for these chemicals is modeled as a function of soil and MC properties. A partitioning model is used to describe sorption to the reaction site and a bulk reductant is used to control the total amount of degradation in the system. Predictions of mass loss from this model and comparisons to observed mass losses will be presented for the soils under study.

MP151 Assessing the environmental fate, exposures, toxicity and risks of octamethyltrisiloxane L3 (also MDM)

C.A. Staples, Assessment Technologies, Inc.; G. Kozerski, Dow Corning Corporation; E. Mihaich, Environmental and Regulatory Resources; K. Woodburn, Dow Corning Corporation

Octamethyltrisiloxane (referred to as either L3 or MDM) is used in a variety of commercial and consumer applications. This presentation examines the nature and extent of the hazard posed by L3 to the environment. The available evidence suggests that L3 exceeds internationally recognized regulatory thresholds for persistence in air (half-life >2 days) and sediment (half-life >180 days); however, L3 should not biomagnify within food webs, as indicated by a BMF of 0.86 kg-food/kg-fish (lipid normalized) measured in a laboratory dietary feeding study with rainbow trout. The solubility of L3 in water is 0.034 mg/L, and in organic carbon it is 740 mg/kg-OC assuming $S_{OC}=K_{OC} \times S_W$. It is theoretically impossible for L3 to exceed its solubility limits in water or the organic matter in sediments or soils. With this in mind, the acute and chronic toxicity of L3 was measured in rainbow trout, *Daphnia magna*, and green algae. Further, the toxicity of L3 to a sediment dwelling oligochaete and midge have been measured in studies with artificial sediment. The results of the studies show that L3 is not toxic to any organism tested up to the limit of solubility in any environmental matrix (i.e., water or organic matter in sediment), with the exception of one study that used artificial sediment. To address bioavailability concerns associated with the use of artificial sediment, an additional toxicity test was conducted using the same organism (*Lumbriculus variegatus*), but exposing the organism to L3 incorporated into natural sediment. No toxicity was found in the test using natural sediment. L3 has been analyzed mainly in sediments as part of environmental monitoring programs in various locations in North America and Europe. L3 was not detected in any of more than 70 field sediment samples from Scandinavian locations. Taken together, the physical properties, fate characteristics, ecotoxicity data, field bioaccumulation studies, and environmental monitoring data suggest that L3 should not accumulate to concentrations sufficient to cause adverse effects in organisms in surface waters or sediments. Taking into account the intrinsic properties of L3 and all of the available scientific information, it should be concluded that L3 does not pose a hazard to the environment.

MP152 Assessment of Polychlorinated biphenyls (PCBs) Levels in Surface Water and Fish (Clarias gariepinus) from Ovia River, Nigeria

I. Tongo, L.I. Ezemonye, T. Adesina, University of Benin / Animal and Environmental Biology

The determination of polychlorinated biphenyls (PCBs) in surface water and fish (*Clarias gariepinus*) samples from Ovia River, Nigeria was carried out using gas chromatography with electron capture detector. PCBs concentrations were evaluated to indicate concentrations in the matrices, seasonal and spatial variations. Total PCBs concentrations (£PCBs) ranged from 0.001 – 0.014 µg/l, surface water and 0.001 – 0.027 µg/kg-dry weight (dw), fish. PCBs concentrations in water and fish samples were rather low, however, mean concentrations of PCBs in fish samples were higher than water indicating bioaccumulation. Seasonal variation showed higher concentrations in the wet season for all the matrices which may be attributed to increase erosion, precipitation and transportation of PCBs associated with municipal runoff. The pattern of distribution of individual congeners varied between the stations and matrices with Ekenhuan, the downstream station having the highest concentration of PCBs in all the matrices. The distribution of PCB congeners was dominated by tetra-PCBs (32%, water and 38%, fish) followed by tri-PCBs (20%, water and 25%, fish) and penta-PCBs (22%, water and fish). The mean concentrations observed were lower than the USEPA PCB limit for drinking water ($0.0005 mgl^{-1}$); however levels at some stations were higher. Further studies are therefore proposed to determine PCBs bioaccumulation through tissues of aquatic biota in order to assess the risk potentials on aquatic ecosystems and human health.

MP153 BACI Analysis for Large-Scale Environmental Studies: Effectiveness, Pitfalls and Implementation

T. Bohrmann, Cardno Entrix; W.J. Warren-Hicks, Cardno Entrix / CEO

Before/After, Control/Impact (BACI) analysis may be useful for inferring the impacts of a large-scale environmental event. A BACI analysis is meant to account for temporal effects (the before/after component) as well as spatial or location effects (the control/impact component) in order to elucidate the importance of the environmental event itself. In this talk, we present examples of effective BACI analyses and describe what makes them effective. We additionally describe complications likely to inhibit the effectiveness of a BACI analysis. Finally, we describe a novel, modern implementation of a BACI analysis in which the principles of a BACI model are embedded in a larger Bayesian model. Components of the larger Bayesian model account for additional environmental variation yielding improved power to detect the impact of the environmental event.

MP154 Beyond Screening Level Assessments for Terrestrial Vertebrate Risk Assessments

C. Habig, Compliance Services International, Compliance Services, Inc.

Currently, the USEPA uses two nomogram-based models, T-REX and T-HERPS, for evaluating potential exposures and risks to birds, small mammals, reptiles, and amphibians. Results of these models are applied to both endangered and non-endangered species, with an additional safety factor added into endangered species assessments. EPA currently conducts these screening-level assessments as national-level assessments. Both of these models are Tier I, screening level models that inherently and intentionally include conservative assumptions, including upper-end estimates of residues on feed items and upper-end assumptions concerning use scenarios. Both models rely on Day 0 and Day 1 nomogram estimates of exposure for evaluating both acute and chronic risks to terrestrial vertebrates. Currently, there are no clear methodologies for refining screening level assessments that suggest either acute or chronic risks to terrestrial vertebrates. There are, however, multiple options for refining these assessments. Two options that are particularly useful for endangered species include refinements based on habitat and diet. The diet information can be further refined to incorporate factors such as foraging behavior, as well as a probabilistic assessment of exposure. Additional options include incorporating more detailed information on use patterns of products, particularly at a local or regional level. Temporal considerations, particularly with respect to product use, also represent key considerations that currently are not included in risk assessments for terrestrial vertebrates. Examples of risk assessment refinements for several different species of terrestrial vertebrates are provided. These include an example of exclusion by habitat for an endangered small mammal for corn and soybean products, and several exclusions based on dietary refinements. These dietary refinements include estimating exposures for more representative diets for small birds and mammals rather than the single category of feed item that is used in the screening models. An example of a probabilistic exposure will be provided for these species based on analysis of a residue database. An example of temporal refinement for a mammalian chronic risk assessment will also be provided.

MP155 Bioavailability and risk of polycyclic aromatic hydrocarbons (PAHs) in the Big Blue River, Nebraska

J.A. Kountzman, Black & Veatch / Federal Services Division

The bioavailability of organics in the environment is of great concern particularly near industrial sites discharging wastes to recreational waters. Surface water and sediment in these areas may contain higher than average concentrations of Polycyclic Aromatic Hydrocarbons (PAHs). In order to evaluate ecological risks associated with elevated concentrations of these constituents, the fraction of the total concentration in environmental media that is bioavailable must be identified. Often, very high percentages (near 100%) of total PAHs are assumed to be bioavailable. While this assumption is conservative, it may not be a reasonable estimate of site conditions because the actual bioavailability of PAHs is often not assessed. The resulting risk may overestimate the true risk of exposure to site media. Overestimation of risk may result in lengthy and costly site remediation that may not be warranted. In order to understand PAH bioavailability in freshwater habitats, chemical and/or biological techniques must be used to measure the portion of PAHs that are bioavailable. For this study, direct measures of PAH concentrations were made from samples of surface water, sediment, and pore-water collected from the Big Blue River at Beatrice, Nebraska. Chronic toxicity tests were also performed on surface water (*Ceriodaphnia dubia* and *Pimephales promelas*) and sediment (*Hyalella azteca*) samples, and a benthic macroinvertebrate community survey was also conducted. In addition to chemical analysis, all sediment samples were also analyzed for grain size, and total organic carbon. PAHs were detected in surface water, sediment, and pore water from multiple sample locations, while toxicity was only observed in sediments at two locations. Regression analysis was used to evaluate the paired analytical chemistry and toxicity test and benthic community survey results to estimate bioavailability. PAHs in some sediment samples were found to be bioavailable in the laboratory tests; however, benthic macroinvertebrate samples suggested no bioavailability/toxicity to native species. This paper presents the results of these tests and how they were used to estimate bioavailability and ecological risk.

MP156 Case study for domestic application of EU SimpleBox

H. Kim; Y. Lee, L. Chang, J. Jung, J. Song, J. Lee, D. Lee, GSES, Seoul National University

As a new chemical registration and evaluation system was legislated in Korea, various exposure assessment tools should be developed to support the system. Particularly for the environmental exposure assessment at preliminary level, use of SimpleBox (ver. 3.24a)). Further modification is in progress to reduce the prediction uncertainty.

MP157 Chlorine: Impact Distances for Environmental Emergency Plans

K. Ketcheson, Environment Canada; A. Guitor, University Student at Waterloo

This poster presents graphs that estimate the impact distances of toxic plumes of chlorine with reference to Acute Exposure Guideline Levels (AEGL) and Emergency Response Planning Guidelines (ERPG), both set for a time duration of 1 hour. Also, when a tank of chlorine collapses, a pool of liquid chlorine can sometimes form. The poster provides a graph of the maximum pool radius based on the quantity (tonnes) of a chlorine spill.

MP158 Chronic exposure to volcanogenic and anthropogenic air pollution: lung injury assessment

R. Camarinho, Universidade dos Açores; P. Garcia, CITA-A – Azorean Biodiversity Group, Departamento de Ciências Agrárias, University of the Azores; A.S. Rodrigues, University of Azores / Department of Biology, (CVARG) Center of Volcanology and Geological Risks Assessment

Despite the fact that several pathologies of the respiratory tract have been associated with exposure to air pollutants of anthropogenic origin, few studies have been made regarding the exposure to volcanogenic air pollution, representing an unrecognized health risk for humans inhabiting non-eruptive volcanically active areas. In this study we tested the hypothesis whether air pollution of volcanogenic origin causes lung injury. Lung injury was assessed in wild mice (*Mus musculus*) exposed to volcanogenic or

anthropogenic air pollution. The extent of lung injury was determined using histological morphometric parameters, the inflammatory status and amount of black silver deposits. We found that mice exposed to air pollution have decreased percentage of alveolar space, alveolar perimeter and lung structural functionality ratio (LSF) and, increased alveolar septal thickness, amount of black silver deposits and inflammatory status. For the first time, we present evidence of these effects in individuals living in a volcanically active environment. Globally, our results clearly show that non-eruptive active volcanism has a high potential to cause lung injury in individuals chronically exposed. We also highlight the usefulness of *M. musculus* as bioindicator species, and of the developed biomarker of effect LSF ratio, for future animal and/or human biomonitoring programs.

MP159 Ecological models in chemical risk assessment –Recommendations of the SETAC workshop MODELINK

V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; A. Alix, Dow Agrosciences / DGAl – SDQPV – BRMMI; D. Auteri, EFSA; P. Carpentier, ANSES / Direction du Vegetal et de l'Environnement; P. Dohmen, BASF SE / Landw. Versuchsstation, APD/RO; V. Ducrot, INRA / Ecotoxicology and Quality of Aquatic Ecosystems; V. Grimm, Helmholtz centre for environmental research – UFZ / Department of Ecological Modeling; T.G. Preuss, RWTH Aachen University / Institute for Environmental Research; M. Reed, HSE – Health and Safety Executive; W. Schmitt, Bayer CropScience AG / Environmental Modelling; P. Thorbek, Syngenta / Environmetal Safety; L. Wendt-Rasch, Swedish Chemical Agency-Kemi; U. Hommen, Fraunhofer IME

Since the implementation of the first guidance document on the evaluation of risks of chemicals in a regulatory context, there has been a strong drive in risk assessment science to develop methods to determine which ecotoxicological effects are of ecological relevance. More recently, the European Food Safety Authority (EFSA) has in this context developed a framework, based on the ecosystem services approach, for deriving specific protection goals for environmental risk assessment of pesticides. Within this framework ecological modelling has been identified as a promising tool to link the results of ecotoxicological studies to specific protection goals because it can facilitate extrapolation from standard test endpoints to higher levels of biological organization and can explore the influence of various kinds of ecological complexity on the degree of risk. MODELINK was a two-part SETAC Europe technical workshop designed to provide guidance for when and how to apply ecological models for regulatory risk assessment, with a focus on the risk assessment of plant protection products and the main groups of organisms covered in EC Reg 1107/2009. MODELINK brought together approximately 60 experts from diverse backgrounds (ecotoxicologists, modellers, regulatory scientists and policy makers) and representing the tripartite structure of SETAC (Academia, Business, Government) and produced six case studies covering macrophytes, aquatic invertebrates, fish, soil invertebrates, non-target arthropods, and birds and mammals. The kinds of models considered included toxicokinetic/toxicodynamic (TKTD) models and population models of different complexity. The case studies demonstrated that ecological models can have an important role to play at all tiers of the risk assessment, with the exception of Tier 1 (screening level). Important conclusions from MODELINK are that well-tested and accepted ecological models can add value to risk assessment by adding ecological realism; by allowing the consideration of spatially explicit exposure scenarios; by facilitating extrapolation to different conditions and regions; by making use of more information from toxicity tests; by explicitly linking exposure and effects; by providing outputs that can be linked to ecosystem services and expressed in terms of relevant and measureable specific protection goals.

MP160 Effect of insecticide resistance status on environment in vineyards using by Environmental Impact Quotient

S. Mermer, Fac. of Agriculture Lab Env Tox, Adnan Menderes University / Faculty of Agriculture Department of Plant Protection Lab Environmental Toxicology; M. Usluy, C. Turgut, Adnan Menderes University

European Grapevine Moth (EGM) is the most common pest, which reduces yields and quality in the vineyards in all European andMediterranean-countries. Insecticides are used in season to control the moth in vineyards up to 9 times from mid-season to end of the season and affect non-target organism and pose a risk in the environment. Insecticide resistance development is the most important danger of EGM due to higher application rates and use frequency of insecticides. The aim of this study is to estimate the risk factors of the pesticides in the environment, beneficials, bees etc. by using Environmental Impact Quotient (EIQ). The EIQ is a mathematical model that calculates a risk factor of pesticide in terms of environment and health. Due to the occurrence of resistance, how often pesticide applications are required and the effect of this situation on non target organisms (consumer,farm worker, non-human biota) will be estimated using EIQ.

MP161 Effects of chronic exposure to volcanogenic pollutants on mice testis

A.F. Ferreira, University of Azores / Biology; P.V. Garcia, University of Azores / Biology; CITA_A; R. Camarinho, University of Azores / Department of Biology; CVARG; A.S. Rodrigues, University of Azores / Department of Biology, (CVARG) Center of Volcanology and Geological Risks Assessment, Ponta Delgada, Portugal

There is a global concern that human exposure to environmental contaminants can cause infertility problems or adverse reproductive outcomes. Volcanism is a source of pollutants impacting on health and environmental quality, being yearly responsible for the release of almost 150 million tons of carbon dioxide (CO_2), other gases and metals into the atmosphere. The island of S. Miguel (Azores – Portugal) is volcanically active, having a human population living inside an active volcanic crater. Recent studies have demonstrated that organisms, including humans, who live in these areas have a higher risk of harmful health effects. The present study tested the effects of chronic exposure to volcanogenic pollutants on mice testicular damage. Two groups of mice, one exposed and the other unexposed to volcanic activity were compared. Testicular damage was assessed by a double blind histological evaluation of the extent of damage in the seminiferous tubules (lumen with >50% of spermatozoa, lumen with < 50% of spermatozoa, without spermatozoa or germinal epithelium disorganized) and by histological morphometric parameter, namely the mean diameter of the seminiferous tubules. Mice exposed to volcanogenic pollutants have lower mean diameter of seminiferous tubules (F=6.147, p=0.019) compared with the control group. The extent of damage in the seminiferous tubules of mice exposed to volcanogenic pollutants was significantly higher (p< 0.001) than in the control group. A small and negative association (r=-0.084, p< 0.05) was observed between mean diameter of seminiferous tubules and the extent of testicular damage. Results show that chronic exposure to volcanogenic pollutants increases the extent of testicular damage in mice, highlighting the risks of male reproductive impairment in organisms, including humans, that live in volcanically active environments.

MP162 Effects of deltamethrin, dimethoate and chlorpyrifos on survival and reproduction of the collembolan Folsomia candida in African and European soils

O.J. Owojori, University of Saskatchewan / Department of Soil Science, Obafemi Awolowo University / Zoology; K. Jegede, Obafemi Awolowo University / Zoology; I. Jaabiri, National School of Engineering of Sfax, Tunisia / Laboratory of Water, Energy and Environment; J. Roembke, ECT Oekotoxikologie GmbH

Indiscriminate use of pesticides is rampant in most parts of Africa but only scanty data exist for protection of soil organisms. In order to assess the site-specific effects of three commonly used pesticides on soil fauna in Africa, non-contaminated surface soils were collected from Nigeria and Tunisia, and used in the collembolan (*Folsomia candida*) reproduction test. Results were compared with data gathered in standard OECD and European LUFA 2.3 soils. For deltamethrin, higher toxic effect on survival (LC50 = 1.32 mg/kg) was found in the Nigerian soil, than for the other soils which ranged between LC50 = 6.84 – 11.37 mg/kg. Significant effects on reproduction were similar in all the four soils (2.5-5.9 mg/kg). For dimethoate, toxic effect on survival was highest in the Tunisian LC50 = 0.5mg/kg, while Nigerian, LUFA, and OECD soils had comparable toxic values (LC50 > 0.8 mg/kg). Toxic effect on reproduction was highest in Tunisian soil (EC50 = O.36 mg/kg) followed by LUFA soil (EC50 = 0.5 mg/kg) while OECD and Nigerian soils had EC50s > 0.8 mg/kg. For chlorpyrifos, similar LC50 values were obtained for LUFA, Tunisian and Nigerian soils which ranged from 0.019 – 0.038 mg/kg while LC50 > 0.1 in OECD soil. The reproduction EC50 values of 0.019 and 0.037 mg/kg obtained in LUFA and Tunisian soils, respectively, were lower than those for Nigerian (EC50 = 0.058 mg/kg) and OECD (EC50 = 0.094 mg/kg) soils. Irrespective of the soils used, the toxicity of the chemicals was in this order: deltamethrin < dimethoate < chlorpyrifos. Soil and pesticide specific patterns of toxicity to *F. candida* were therefore evident and might be related to the physicochemical properties of the soils and the pesticides. Since the two African soils did not

cluster together in their toxicity pattern in comparison with the OECD and European soils, it is suggested that ecological risk assessment of pesticides in Africa should be done on a case by case basis using tests simulating the local environmental conditions.

MP163 Environmental Integrity and Human Health Issues in a Groundwater-Supplied Borrow-Pit Lake Ecosystem

K.J. Maier, East Tennessee State University / Department of Environmental Health

Borrow-pit lake ecosystems are formed after road construction activities and are designed to support recreational beneficial uses. Resource agency concerns about the lack of fish reproduction and potential human health issues associated with the consumption of fish from a groundwater-supplied borrow-pit lake ecosystem were evaluated. Water and whole-body fish (catfish, trout, carp, bass, and bluegill) samples were collected and analyzed (using standard methods) for selected ion, metal, metalloid, and pesticide concentrations. In a preliminary risk assessment the concentrations were compared to existing water quality criteria (WQC, to protect aquatic life) and human consumption thresholds (IRIS database). Concentrations of concern in the water included total Se at 5.75 ppb (WQC 5 ppb), and chloride at 376 ppm (WQC 230 ppm). Elements of concern, based on human health issues associated with the consumption of fish included Zn, As, Se, and Hg. Average whole fish tissue concentrations (std. dev.) for Zn, As, Se, and Hg were 128 (38), 0.32 (0.30), 0.84 (0.12), and 0.26 (0.12) mg/kg wet weight, respectively. The IRIS human consumption thresholds for Zn, As, Se, and Hg are 21, 0.021, 0.35, and 0.007 mg/day for a 70 kg adult, respectively. All organics were below detection limits. The results and any conclusions were confounded by the lack of quality assurance and quality control data, and lack of appropriate replication and experimental design. The elevated water and tissue Se concentrations could explain the lack of fish reproduction in this system. This preliminary data suggest potential human health issues associated with the consumption of fish. A more through and appropriate study is recommended to validate the preliminary conclusions.

MP164 Environmental Risk Assessment of Prasugrel

A.N. Perkins, Eli Lilly and Company; R.D. Meyerhoff, Eli Lilly and Company / Lrl- Health, Safety And Environmental

A cardiovascular human pharmaceutical, prasugrel is a pro-drug that is extensively metabolized by humans, eventually to inactive metabolites. Environmental fate and ecotoxicity studies were conducted with prasugrel and the predominant metabolite excreted by humans, M1. The data has been integrated into an environmental risk assessment for the therapeutic use of prasugrel. Prasugrel is extensively transformed in activated sludge and water sediment systems to degradation products that overlap human metabolites and are further degraded. Considerable mineralization is observed in these systems. M1 is hydrophilic (log Pow of minus 1.70). Therefore, prasugrel and its metabolic residues are not expected to persist in surface waters, wastewater sludge, aquatic sediments, or biological tissues. The maximum total predicted environmental concentration (PECs) of prasugrel residues for sewage treatment facilities, ground water, and surface water are 0.5 µg/L, 0.05 µg/L, and 0.0125 µg/L, respectively. The maximum PECs in those compartments for the M1 metabolite are 0.125 µg/L, 0.0125 µg/L, and 0.003125 µg/L, respectively. In ecotoxicity testing with prasugrel, fish were the most sensitive species tested compared to daphnia, algae, and sewage microorganisms. M1 was less toxic than the parent molecule. The predicted no-effect concentrations for prasugrel and M1 are at least 380 times higher than the predicted environmental concentrations. Therefore, excretion by humans of prasugrel residues, including the primary excreted metabolite, is not expected to result in any significant environmental risk.

MP165 EROD induction in brown trout caused by urban run-off after a longer period without rain

N. Hanson, EnviroPlanning / Department of Plant and Environmental Sciences, University of Gothenburg / EnviroPlanning; C. Aberg, EnviroPlanning; J. Parkkonen, L. Forlin, University of Gothenburg

Many pollutants are brought to the aquatic environment by urban run-off from hard surfaces such as roads and parking lots. Pollutants end up on these surfaces through emissions from car engines and rubber praticles from tires, etc. There is also a constant deposition from the atmosphere. When it rains, the pollutants are washed away from the hard surface. To avoid problems with high water levels, the water is led away through stormwater pipes. Most often, these pipes end up in a natural surface water that becomes the recipient for stormwater. During long periods without rain, it can be expected that higher levels of pollutants are accumulated on the hard surfaces. This means that high concentrations of pollutants can be expected in urban surface waters at the end of longer periods without rain. This is commonly known as the first flush effect. From January to April, 2013, western Sweden had a period of almost three months without rain. The average would be about 150 mm rain during this period, spread out over about 45 days. To examine if this extreme weather would result in an unusual first flush effect, brown trout were captured downstream and upstream an urban area with a motorway. The fish were captured twice, at the end of the dry period and two weeks later, when it had rained for several days. The fish were analyzed for EROD-activity, which is a measure of detoxification activity in the liver. The analyzes will be finished during June, 2013. The results may be important for how stormwater is considered in urban planning and to determine if it can be safely discharged into natural streams or if it should be lead to treatment plants.

MP166 Estimation of industrial acceptable daily usage of chemicals to protect human health and soil organisms from soil contamination

S. Ikeda, Meiji University / Department Applied Chemistry; E. Hase, Sumika Chemical Analysis Service, Ltd / Chemicals Compliance Division; M. Kitano, Shukutoku University / College of Integrated Human Health and Social Welfare; T. Nakamura, Meiji University / Department of Applied Chemistry

In Japan, risk assessment of chemicals in water and air has been well developed and extensively conducted. On the other hand, risk assessment of chemicals in soil has not yet been fully developed. Thus it has not been considered for the regulation of chemicals. In this study, "Industrial Acceptable Daily Usage (IADU)" of Designated hazardous substances under Soil Contamination Countermeasures Law (SCCL) and Priority assessment chemicals in 2012 under Chemical Substances Control Law (CSCL) of Japan were estimated in order to protect human health and soil organisms from soil contamination. Mathematical model was applied to calculate soil and pore water concentration. Especially in this study, the equations concerning atmospheric degradation process were introduced into the existing mathematical model in order to calculate more precise concentration in soil and soil pore water. This approach is quite important for chemicals which are mainly distributed into atmospheric compartment. By comparing these calculated concentrations and the existing environmental standards, the IADUs of these chemicals were estimated. Furthermore, this study developed a new procedure to propose the soil standards of substances which have not yet been established. In addition to IADUs for human health, IADUs for soil organisms were estimated from "Predicted Non-Effects Concentration (PNEC)". For some chemicals, IADUs for soil organisms were found much smaller than IADUsfor human health. This indicates that these chemicals must be handled more strictly and carefully taking into account their possible adverse effects on soil organisms.

MP167 Evaluating Inhalation Risk to Burrowing Animals

J. Holder, ERM; R. Ettinger, Geosyntec Consultants

Human health risk assessments regularly evaluate the vapor intrusion pathway which addresses potential exposure of people to volatile organic compounds (VOCs) in indoor air that have migrated from contaminant sources in groundwater or the vadose zone. Exposure estimates for building occupants (i.e., the exposure point concentration) can be modeled and/or measured. An analogous exposure occurs in wildlife that spend a portion of their day in burrows. VOCs from subsurface sources can migrate through the soil and enter the burrow where they can be inhaled. Many species can be exposed via this route including mammals (ground squirrel), birds (burrowing owl), reptiles (desert tortoise) and amphibians (spadefoot toad). Concentrations of VOCs in burrow air are dependent on many factors including: (1) the distance from the source, (2) the architecture of the burrows, and (3) air exchange with the outside atmosphere. This presentation will explore different methods that have been used to evaluate exposure of burrowing wildlife to VOCs in soil vapor, the uncertainties associated with these methods, and possible models and empirical data that could be collected to address these uncertainties.

MP168 Evaluating Non-Indigenous Species Eradication Options in a Bayesian Network Derived Adaptive Management Framework

C. Herring, Western Washington University; W.G. Landis, Western Washington University / Institute of Environmental Toxicology

Many coastal regions are facing problems with the introduction and spread of non-indigenous species (NIS). Common efforts addressing this issue include eradicating these species. Eradication methods removing non-indigenous species range from chemical eradication, mechanical eradication, or a combination of the two actions depending on the species being eradicated. These management options are often completed with little information as to the consequences of these toxicological impacts to the surrounding habitat. A regional risk assessment is being conducted at a National Estuarine Research Reserve in Padilla Bay, Washington to develop methods of analyzing these consequences. The method is derived from the current relative risk model used to estimate risk in other NIS scenarios. The current relative risk model uses Bayesian networks to calculate risk and evaluate uncertainty. The risk assessment is analyzing various vectors of NIS introduction and possible adaptive management strategies to reduce risk to the Padilla Bay region. The model specifically analyzes various adaptive management options for controlling types of NIS, comparing and contrasting methods such as chemical and mechanical eradication, as well as treating ballast water before releasing it into coastal waters. The study evaluates the likelihood of outcomes from each management option in respect to the surrounding habitats, including organism and sediment composition. The framework from the risk assessment and adaptive management will be adaptable for other regions interested in the eradication of NIS organisms.

MP169 Expression of antioxidant and endocrine associated genes on Chironomus riparius developmental stages following temperature changes

K. Park, Chonnam National University / Fisheries and Ocean Science; I. Kwak, Chonnam National University

Climate change is expected to lead to latitudinal and altitudinal temperature increases. Global warming is already posing severe risks for aquatic species. To investigate the potential effects of temperature on aquatic invertebrate, biological and molecular gene responses were analyzed during *Chironomus riparius* development, including the five stages from embryo to adult. Temperature change induced reduction of survival rate, changes of biological development including the sex ratio of emerged adults, success rates of pupation and emergence, and the developmental timing of pupation and emergence. Increased temperature induced gene expressions of ecdysone receptor, ultraspiracle, estrogen-related receptor in *C. riparius* fourth-instar larvae and pupae of developmental stages, and affected the activity of antioxidant genes, such as catalase, peroxidase, glutathione peroxidase, and superoxide dismutase during developmental stages from *C. riparius* fourth-instar larvae to adults. Increase of temperature on fourth-instar larvae increased oxidative stress in pupae and adults. Responses of antioxidant genes to increased temperature occurred in a developmental stage-dependent manner. However, decreased temperature did not induce expression of antioxidant genes in a developmental stage-dependent manner, although it induced oxidative stress during *C. riparius* developmental stages. These results suggest that climate change associated temperature alterations may disturb the endocrine system and several developmental and biological effects by induction of oxidative stress in aquatic environments.

MP170 Habitat considerations for ecological risk assessments of wildlife exposed to persistent organic pollutants

D.L. Tazelaar, Cardno Entrix / Animal Science; A.M. Neigh, , Michigan State University / Department of Zoology; A. Sookhai, Cardno Entrix; J.P. Giesy, University of Saskatchewan / Toxicology Centre and Department of Veterinary Biomedical Sciences; M.J. Zwiernik, Michigan State University / Wildlife Toxicology Laboratory, Department of Animal Science, College of Veterinary Medicine

Habitat characteristics may influence wildlife population level metrics; however, the influence of habitat is not always considered during ecological risk assessment hypothesis generation. In some instances, potential habitat effects are only implicated upon the completion of a risk assessment if no dose-response relationship is observed between persistent organic pollutant exposure and population level metrics. In order to improve upon what is typically a qualitative expert assessment of habitat quality, Habitat Suitability Index (HSI) models are useful tools for species-to-habitat relationship comparisons between locations. Unfortunately, HSI models have not been developed for all species that may be investigated during an ecological risk assessment. This is the case for the American robin (*Turdus migratorius*). A multiple lines of evidence risk assessment of American robins exposed to polychlorinated dibenzofurans and polychlorinated dibenzo-*p*-dioxins in the Tittabawassee River floodplain, Midland, MI, USA was conducted from 2005 to 2008. The assessment of reproductive endpoints revealed significant differences among the ranges of reference and study area hatching success in American robin populations; however, the effect was not dose-related. The ranges of percent hatching success were not significantly different between reference (n = 24) and study (n = 42) locations where exposure was greatest, while the range of percent hatching success was least at study (n = 10) locations where exposure was intermediate. A comprehensive habitat assessment was beyond the scope of the study; however, habitat characteristics, such as height of nest from the ground, nest tree circumference at breast height, and canopy cover estimates, were measured for a subset of nests. The objective of the present study is to determine if habitat quality differences explain significant differences for ecologically relevant endpoints from the American robin study. Further, data from related ecological risk assessments, where habitat data are available, will be assessed for the feasibility of generating HSI models.

MP171 Hotspot Considerations in Spatial and Non-spatial Exposure Point Concentration Analysis

W. Stiteler, T. Negley, Arcadis US, Inc.; M. Butcher, ARCADIS; M. Pattanayek, Arcadis US, Inc.

The exposure point concentration (EPC) is an estimate of the arithmetic mean concentration of a constituent that may be potentially contacted by a receptor over a period of time that can be associated with an adverse health effect. Typically, an EPC is calculated from a set of samples from an area that represent the potential habitat or home range of a receptor population. To reduce the chance of underestimating the true mean, a 95 percent upper confidence limit for the mean (95UCL) is used for the EPC. The assumption when calculating EPCs is that an unbiased sampling design is used and each observation is independent and identically distributed. In practice, these assumptions are often violated due to targeted sampling efforts in and around hotspots, and because many environmental contaminants exhibit positive spatial autocorrelation. Therefore, simply pooling data without accounting for spatial information can lead to an estimate of the mean that is biased towards subareas that are oversampled or disproportionately represented. Identification of hotspots and spatial weighting of a sample can yield estimates of an EPC that is more representative of potential exposure. While the risk assessment literature provides many examples of the application of spatial weighting methods, their performance has not been systematically studied for a range of conditions. In this poster, we illustrate methods to identify hotspots and the influence of hotspots on exposure concentrations with and without spatial weighting. Results suggest that relatively simple declustering with Thiessen polygons can be expected to yield more reliable EPCs under a wide range of contamination scenarios and sampling designs.

MP172 Identification of Atrazine Contamination in Macon County, Illinois

S.J. Wassenhove, T.B. Mansur, J.R. Schroeder, Millikin University / Department of Biology

Pesticide exposure has previously been indicated as a possible cause of reproductive and developmental abnormalities, as well as increasing the risk of cancer. Illinois is one of the top three states nationwide in amounts of atrazine applied annually. Currently the USEPA allows for contamination levels of up to 3 ppb daily for an annual average. However, the EPA does not sample in the Lake Decatur watershed. To determine whether local atrazine application results in water contamination, we have begun a multi-year study of several water sources within Macon County, Illinois. Water was collected weekly at ten different sites. An ELISA was performed to quantify any atrazine contamination within our samples. Our preliminary results demonstrate that there are detectable levels of atrazine in surface and drinking waters around Macon County.

MP173 Inconsistencies in the Estimation and Selection of Terrestrial Plant Endpoints used in Pesticide Risk Assessments

S.I. Rodney, Intrinsik Environmental Sciences; M. Whitfield Aslund, R.S. Teed, R.L. Breton, Intrinsik Environmental Sciences, Inc.

Registration of pesticides in the US requires the submission of seedling emergence and vegetative vigor toxicity studies (Guidelines 850.4100 and 850.4150). Data from these studies are used to derive effect metrics (e.g., NOERs and ER25s) for terrestrial plants. Such effects metrics may be derived for a variety of endpoints including percent germination, growth rate, plant biomass, root and shoot length and many others. There are, however, substantial inconsistencies in the selection of endpoints for use in risk assessment and the data analysis methods used to derive the quantitative effect metrics (particularly the ER25s). This point applies to both the original study authors and the re-analyses of data often conducted by EPA. The inconsistencies raise questions about the validity of the selected effect metrics and endpoints and ultimately the estimates of pesticide risk to terrestrial plants. Herein we highlight the issues arising from the 'mixed bag' approach of endpoint selection and ER25 derivation. We also provide suggestions for standardizing endpoint selection and data analysis in future toxicity studies for terrestrial plants.

MP174 Influence of Silica Nanoparticles on the Genotoxicity and Phytotoxicity on Allium cepa

G.H. Silva, University of São Paulo / Centro de Energia Nuclear na Agricultura; F. Antunes, University of Coimbra; A.M. Soares, Universidade de Aveiro / Department of Biology and CESAM – Centre for Environmental and Marine Studies; S. Mendo, University of Aveiro / department of Biology & CESAM; I. Lopes, University of Aveiro / CESAM; R.T. Monteiro, University of São Paulo / Centro de Energia Nuclear na Agricultura

Nanotechnology is rapidly expanding, and several new products (nanoparticles-NP) with improve performances have been developed. The NP environmental fate and toxicity is poorly known and needs to be studied for a safe use of this technology. This study aims to investigate phytotoxic, cytotoxic and genotoxic impacts of silica nanoparticles, with three different sizes, using root tip cells of *Allium cepa* as an indicator organism. *A. cepa* root tip cells were treated with four different concentrations (TM40: 24.3, 16.2, 10.8 and 7.2 gL^{-1}; HS30 and SM30: 18.225, 12.05, 8.1 and 5.4 gL^{-1}) of engineered silica nanoparticles (TM40 – 22nm; HS30 – 12nm; SM30 – 7nm) dispersion, to study endpoints like mitotic index, different types of chromosomal aberrations, disturbed metaphase, sticky chromosome, breaks, micronucleos, germination and growth rate. For each concentration ten sets of microscopic observation were carried out for the genotoxicity and citotoxicity test and for the phytotoxic test 20 seeds with 3 replicate were exposed for 7 days, then size and germination of plantlets was observed. In addition, the characterization of NP suspensions was carried out through dynamic light scatering, zeta potential and rheology. No chromosomal aberration was observed in the negative control and the mitotic index (MI) value was 18,84%. With increasing concentration of the nanoparticles decrease in the mitotic index was noticed, we could see significant decrease of the MI for all the particles and for all concentrations, except on the 7.2g/L for TM40 and 5.4 g/L for HS30. For all nanoparticles and on all concentrations (except for TM40 and 5.4 g/L for HS30) cytological effects including the chromosomal aberration were observed in treated cells. Increase of germination and growth was also observed on all treatments. We infer from this study that silica nanoparticles could penetrate plant system causing effects on growth, germinations and on cell division. Plants are an important component of the ecosystems so those findings suggest that they need to be included when evaluating the toxicological impact of the nanoparticles on the environment.

MP175 Not Your Average Average: Methods for Interpolating Contamination Data for Assessment Analyses

K. Doiron, J. Fydenkevez, Industrial Economics, Incorporated; S. Friedman, Industrial Economics, Inc.; R. DelVecchio, Industrial Economics, Incorporated

In the context of risk assessment, natural resource damage assessment, and other environmental assessments, analyses of environmental data routinely compare sediment concentrations to regulatory criteria and/or thresholds from the literature. How those data are analyzed can influence conclusions regarding risk, injury, and environmental hazards. The use of an arithmetic or geometric mean to represent data across a geographic area does not account for data unevenly distributed on a spatial scale throughout an assessment area. Neither existing data nor data collected through new studies are likely to be evenly distributed throughout an assessment area. The increasing availability of Geographic Information Systems (GIS) software now allows for utilization of interpolation analysis methods that provide a more representative result, including, but not limited to, Thiessen polygon area-weighted average and inverse-distance weighted average (IDW). Thiessen polygons define separate areas of influence (polygons) around a set of points. Polygon boundaries define the area that is closest to each point in relation to all other points. IDW estimates grid cell values by averaging the values of data in the vicinity of each processing cell. The closer a point is to the center of the cell being estimated, the more weight it has on the averaging process. In this paper we show how application of different interpolation methods to the same contaminant concentration data results in different averages, which can affect whether or not an average exceeds a criteria or threshold. In this study, we use the four data analysis methods listed above (arithmetic mean, geometric mean, Thiessen polygon, and IDW) to calculate average contaminant concentrations for multiple contaminants within an example assessment area, then compare each average to literature-based sediment quality guidelines (SQGs). The results of our study will allow decision makers to better understand how the chosen interpolation method may impact their results and decision-making process.

MP176 Pb2+ exposure as a risk factor for schizophrenia

S. Jeong; K. Stansfield, T. Guilarte, Columbia University Mailman School of Public Health

Lead (Pb^{2+}) is a ubiquitous developmental and environmental neurotoxin as well as a potent and selective N-methyl-D-aspartate receptor (NMDAR) antagonist that causes NMDAR hypofunction. Hypofunction of the NMDAR is thought to be involved in the pathophysiology of schizophrenia. The GABAergic hypothesis of schizophrenia suggests that NMDAR hypofunction may produce deficits of parvalbumin (PV) expressing GABAergic interneurons, leading to cognitive dysfunction in memory, attention, motor, verbal, and executive function in schizophrenia. Decreased PV expression was found in GABAergic interneurons in postmortem brain samples of schizophrenic patients and in experimental animal studies using antagonists that impair NMDAR function, suggesting that NMDAR has an important role in PV maintenance and integrity. Furthermore, recent epidemiological findings by Opler et al. have shown that there is a possible association between prenatal Pb^{2+} exposure and schizophrenia. Thus we hypothesize that developmental Pb^{2+} exposure is a risk factor for developing schizophrenia and leads to the impairment of PV expressing GABAergic interneurons and decreased levels of PV in the brain. We aim to examine the role of early life Pb^{2+} exposure in schizophrenia development by characterizing the trajectory of parvalbumin expression in GABAergic neurons in Pb^{2+} exposed animals and whether these changes occur in a dose-dependent manner. Specifically, this study will measure the cell density, soma size, and parvalbumin expression in the striatum, frontal cortex, and hippocampus of female and male rats at 14 and 35 days after birth. This study is one of the first to examine the effects of early life Pb^{2+} exposure as a possible risk factor for schizophrenia in an animal model. If our hypothesis is proven correct, it would lend evidence to the relationship between Pb^{2+} exposure and the GABAergic hypothesis of schizophrenia and elucidate plausible neurobiologic mechanisms whereby Pb^{2+} acts as an environmental risk factor for schizophrenia.

MP177 Phitotoxicity of Sludge from Water Treatment Plant with and without Treated Vinasse

J.R. Ribeiro de Paula Santos, G.M. Silva, University of São Paulo / Escola Superior de Agricultura Luiz de Queiroz; T.G. Messias, G.H. Silva, C.B. De Lima, R.T. Monteiro, University of São Paulo / Centro de Energia Nuclear na Agricultura

The sludge's water treatment plant is a residue constituted by water, organic material, clay, sand and great diversity of chemical elements that came with the river water and also are added during flocculation/coagulation treatment process leading to a great power polluter. Large amount of it, is dumped back in the rivers, or other the sea, sewage treatment plant or sanitary landfill, thereby contaminating the environment. As well as sludge, the vinasse is a residue polluter characterized by having high BOD, COD and large amount of organic material from alcohol industry distillation. *Pleurotus* fungi have an enzymatic complex consisting by laccase and manganese peroxidase capable of degrading many kinds of wastes including vinasse. So, to provide a viable alternative to sludge's fate, the present work aimed to evaluate the effects of mixing the sludge with *Pleurotus sajor caju* treated

vinasse. Two test of toxicology were applied with the soil mixed with the sludge at several concentrations (100, 75, 50 and 25%). The sludges from flocculator (SF) and from decanter (SD) were tested separately. The first test was with *Zea mays*, following the ISO 11269-1 (1993) for germination and plant growth in vessels of 400 g. The second test was performed as the first, using 52 mL of treated sugar cane vinasse instead water for humidify the soil. Both tests were maintained in greenhouse. *Vibrio fisheri* test was prior used to evaluate the toxicity level of the sludges. The SD presented high toxicity (2.2 times) than the SF sludge, showing EC50 30 min of 1.8 and 4.0 mg L^{-1}, respectively. The germination and plant growth after 14 days showed significant difference (Dunnett 0.05%) in the soil with different concentration of sludges, and also the application of vinasse treated with *Pleurotus*. The amendment with treated vinasse showed the best results for germination root and shoot growth and plant weight, improving about 20 times the size and weight compared with the same treatments without vinasse application. One can also infer that the 50% dilution of sludge with soil can be used as the recommended application rate for cultivation of maize, and the amending with treated vinasse improve growth and corn plant development.

MP178 Population modeling of the interaction of toxicant susceptibility and population vital rates: implications for ecological risk assessment

J.D. Stark, Washington State University / Dept. of Entomology

Population modeling is being recognized as a way to improve ecological risk assessment of toxicants. However, there are still many questions that need to be answered before modeling becomes an accepted practice for ecological risk assessment of chemicals. The use of standardized models is attractive to risk assessors because use of these models would reduce the time and expense of producing new models for every chemical and species of interest. There are however, obstacles to the development of standardized models. Differential susceptibility to toxicants may make model extrapolation from one species to another complicated. Even closely related species can exhibit large differences in susceptibility to toxicants. Furthermore, differences among species demographic vital rates may also make extrapolation of model results for one species to others difficult. I will present population modeling results for several species exposed to toxicants based on actual data that show how susceptibility and vital rates can interact to produce unexpected results. The use of uncertainty factors and their potential value for use in population models will also be discussed as well other means to improve modeling approaches for risk assessment.

MP179 Post-Spill Ecological Risk Assessment For An Urban Creek

N. Hausmann, M. Shibata, ERM, Inc.

An ecological risk assessment (ERA) was conducted to assess potential risks to aquatic biota in a freshwater urban creek as a result of potential exposures to residual post-remediation concentrations of spill-related petroleum hydrocarbons. Several lines of evidence were used to assess potential risks to in-creek benthic macroinvertebrates (BMI) including (a) comparisons to sediment threshold effect concentrations, (b) comparisons to PAH compositions and concentrations in local background creeks, and (c) characterizations and comparisons to BMI community structure in local background creeks. Characterization of BMI community structure and function can provide a strong line of evidence to support assessments of potential risks to in-creek benthic biota because they (i) indicate localized conditions given their limited migration patterns or sessile mode of life; (ii) integrate effects of short-term environmental variations; and (iii) describe responses for a broad range of trophic levels and pollution tolerances. Nearby urban creeks were identified to characterize local background conditions of urban creeks not impacted by the spill and were established to discern concentrations and biological responses attributable to the spill. Metrics used to characterize BMI communities in both the affected and local background creeks included species richness, species evenness, diversity, percent EPT, percent chironomids, and Hilsenhoff biotic index. Statistical analyses and results for each line of evidence and the overall finding reached with the State based on the preponderance of the evidence are presented.

MP180 Prediction of physicochemical properties of polybrominated diphenyl ethers (PBDEs) by QSPR models

B. Johnson-Restrepo, University of Cartagena; R. Correa, J. Banquet-Teran, University of Cartagena

Polybrominated diphenyl ethers (PBDEs) are flame retardant used widely to reduce the risk of fire in several consumer products including textile, electronics, plastics, and furniture. PBDEs are ubiquitous in the environmental, persistent, toxic, and bioaccumulative. They are frequently detected in abiotic media such as air, soil, sediments, and water. PBDEs are also detected in the human bodies and wild animals. Assessing the risk in the ecological system and human require the use an array of physical chemical properties which are not always available in the literature for all compounds 209 PBDEs. Several approaches have been published. Quantitative Structure–Property Relationship (QSPR) model is one of the best accurate methods to predict these properties. A set of 1664 molecular descriptors were generated using e-Dragon software for each optimized molecular structure which were grouped in 22 different types: 0D (i.e., constitutional descriptors), 1D (i.e., list of structural fragments), 2D (i.e., topological index), and 3D (i.e., quantum-chemical descriptors, size, steric). Collinear descriptors were considered when the pairwise correlation among descriptors present correlation coefficients $r > 0.9$ retaining the descriptor with the highest correlation with the property while the others were removed. Descriptors were used to build the QSPR models using multiple linear regressions (MLRs), partial least squares (PLS) regression, and artificial neural network (ANN). Genetic algorithm was used to select the variables that resultant of the best fitted models. Resulting models were obtained from the lowest root mean square errors (RMSEs) and the highest correlation confidents. The models were evaluated using leave-one-out cross validation method. Experimental physicochemical properties of PBDEs were taken from the literature for sub-cooled liquid vapor pressures (P_L), *n*-octanol/water partition coefficients (K_{OW}), and Octanol-Air Partition Coefficients (K_{OA}). Multiple linear regressions were established between molecular descriptors and experimental physical chemistry properties which the models obtained shown a high predictive capability with regression coefficients of 0.995, 0.99, and 0.987 for P_L, K_{OW}, and K_{OA}, respectively.

MP181 Regurgitation in Passerine Acute Studies – A Compilation of Results and a Path Forward

W.E. Hillwalker, Exponent / Department of Environmental & Molecular Toxicology; J.M. Stafford, Smithers Viscient / Avian Toxicology Department; L.W. Brewer, Smithers Viscient, LLC / Department of Wildlife Toxicology; M.J. Schocken, Exponent, Inc. / Registration Chemistry Department; J.P. Staveley, Exponent, Exponent, Inc.

Standard guideline toxicity tests with surrogate animal species are commonly used in risk assessments for regulatory decision-making during the pesticide registration process under FIFRA. Surrogate species used in toxicity assessments are selected to represent biological sensitivity and ecological functions. The bobwhite quail and the mallard duck are commonly used avian surrogate species in toxicity tests representing an upland game bird and a waterfowl. In October 2007 the EPA required acute oral toxicity testing for pesticides to include a passerine species (Federal Register, Vol. 72, No. 207). The requirement is based on the concept that increasing the number of species tested addresses interspecies sensitivity and decreases the uncertainty in extrapolating risk to non-target species. The passerine bird order, comprising more than half of all birds, is characterized by unique biological traits that make its members particularly challenging surrogates for laboratory toxicity testing, including small size, high metabolic rate and sensitive regurgitation reflexes. Since the notification in 2007, laboratories have conducted acute tests with diverse pesticide classes on passerine species with varying successes. One of the biggest challenges has been delivering an appropriate oral bolus dose of the test article that does not trigger regurgitation. In response, the EPA published a guidance document that includes a sub-acute 5-day dietary exposure option. When using a 5-day dietary exposure, feed avoidance has been observed and effect values are confounded by fitness issues including starvation. Results from oral and sub-acute dietary studies with passerine species exposed to pesticides from various chemical classes were compiled to evaluate the success of developing toxicity endpoints. We propose a strategy for evaluating passerine acute studies that are hampered by regurgitation, feed avoidance and resulting fitness issues and discuss the applicability of the data in the existing regulatory decision-making framework.

MP182 Relative potency of PCDDs, PCDFs, and dioxin-like PCBs using the H4IIE-luc chemically activated luciferase gene expression assay

S. Hong, Seoul National University / Division of Environmental Science and Ecological Engineering / School of Earth and Environmental Sciences; K. Lee, NeoEnBiz Co / Institute of Environmental Protection and Safety; J. Lee, NeoEnBiz Co; K. Jeong, Sungkyunkwan University; K. Hilscherova, Masaryk University, Faculty of Science, RECETOX; J.P. Giesy, University of Saskatchewan / Toxicology Centre and Department of Veterinary Biomedical Sciences; J. Khim, Korea University / Division of Environmental Science and Ecological Engineering, Seoul National University / School of Earth and Environmental Sciences

While the World Health Organization 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalency factors are useful estimates of relative potencies of mixtures when conducting risk assessments, they are not useful when comparing the results of bioassays such as the H4IIE-luc to concentrations of TCDD equivalents calculated from instrumental analyses. Since there are thousands of dioxin-like compounds (DLCs), one use of screening assays is to determine if all of the aryl hydrocarbon receptor (AhR) active DLCs in a mixture have been accounted for in instrumental analyses. For this purpose, bioassay-specific relative potency (ReP) values are needed. RePs of 21 polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls that exhibit effects mediated through the AhR were determined by use of the H4IIE-luc assay. Different values of RePs are derived, depending on the statistical, curve-fitting methods used to derive them from the dose–response relationships. Here, we discuss the various methods for deriving RePs from in vitro data and their assumptions and effects on values of RePs. Full dose–response curves of 2,3,7,8-TCDD and other representative DLCs were used to estimate effective concentrations at multiple points (e.g., EC20-50-80), which were then used to estimate ReP of each DLC to 2,3,7,8-TCDD.

MP183 Risk Assessment for Terrestrial Vertebrates from Soil-Incorporated Pesticides

C. Habig, Compliance Services International, Compliance Services, Inc.

The USEPA currently uses a revised version of the Hoerger and Kenaga nomogram as the basis for evaluating exposure and risk to birds and mammals through modeling with its T-REX model. The underlying data for the T-REX model is a portion of the data (Day 0 and Day 1 residue data) from the UTAB database. These data underlying the T-REX model represent Day 0 and Day 1 downward-directed foliar applications to a variety different types crops, and some non-crops, such as golf courses. However, these data are not applicable to pesticide products that are applied preplant, at-plant, or as side dressings and are soil-incorporated after application. These types of products may be formulated as granular products, wettable powders, or liquid formulations, and may be broadcast applied followed by incorporation, or may be applied banded with incorporation or in-furrow. While the EPA has a methodology for estimating acute exposures and risks for granular soil-incorporated products, there is no methodology for estimating chronic exposure and risk, and no methodology for evaluating other soil-applied formulations. Residues of these products in plant tissues or invertebrates that may be feed items for birds or small mammals are due to uptake from soil. Some of these products are systemic, and some are not, so predictive estimates are highly uncertain and erroneous. Rather than using modeling estimates that are based on an incorrect application scenario, a more appropriate methodology is to rely on residue data for the product. These data can also provide a valuable index to longer-term exposure of birds and mammals. Data are presented for a high application rate non-systemic soil fungicide that contrast model estimated exposures to exposures based on residue data that indicate estimated exposures based on T-REX modeling overestimate actual residues in plant tissues by two to three orders of magnitude.

MP184 Risk Assessment of a Former Pulp and Paper Mill in Howe Sound, BC

A. Wan, S. Bard, Keystone Environmental Ltd.

A human health and ecological risk assessment of a former pulp and paper mill, located at Woodfibre, BC was undertaken. The risk assessment involved the integration of habitat survey data, soil, surface/marine water, vapour, sediment, groundwater, and porewater chemistry data, fate and transport studies, and human/ecological receptor identification and intake assumptions to arrive at a characterization of risk. Mill operations had taken place from the early 1900s to 2006, when operations ceased and the mill was decommissioned. The main investigative effort occurred in 2006-2008, with supplemental data collected in 2013. Substances quantitatively evaluated in the risk assessment in upland soil and groundwater included a variety of metals and hydrocarbons. Exposures to these constituents of concern by terrestrial ecological receptors, which included plants, soil invertebrates, amphibians, reptiles, birds, and small mammals, were restricted to a single landfill. Outfalls from the former mill operations into Howe Sound resulted in several classes of substances requiring quantitative evaluation in sediments including metals, polyaromatic hydrocarbons, chlorinated organic compounds, and organometallics. Because many of these substances are bioaccumulative, risks were evaluated up several trophic levels within a food chain model, which included the benthic infauna and epifauna community, benthivorous fish community, piscivorous birds and mammals, and humans. Despite the duration of emission, risks were limited to the benthic infauna community as a result of organometallic contamination at isolated portions of the sediment environment, requiring limited amounts of dredging effort. Analysis of various media samples collected recently indicated that many substances of concern had declined, relative to their 2006 levels, likely due to dissipative mechanisms that had taken place.

MP185 Sensitivity of Algae: A Survey of the USEPA's Pesticide Ecological Effects Database

T.M. Blickley, Dow Agrosciences / Ecotoxicology

The standardized algal toxicity studies necessary for global pesticide registrations typically cost between $11,000 and $16,000 per species and are often conducted after several years have already been invested in a molecule's pre-development. Unacceptable adverse effects can lead to costly re-development or cumbersome use restrictions of commercial products, thus screening molecules using the most sensitive algal species could provide early detection of incompatibility issues. Using the USEPA's Pesticide Ecological Effects Database, 1,634 toxicological records were analyzed using SAS JMP (Cary, NC, USA) to determine which algal species (*Psudokirchneriella subcapitata, Navicula pelliculosa, Skeletonema costatum,* or *Anabaena flos-aquae*) was the most sensitive with regard to herbicide, insecticide, and fungicide exposure. Analysis of *Lemna gibba*, an aquatic plant, is also presented for comparison.

MP186 The Personal Care Products Council Environmental Safety Program: Early Progress

I.A. Davies, PCPC (Personal Care Products Council) / Science

The cosmetics industry represents a multi-billion dollar operation in North America alone. Contained within commercial cosmetic formulations are wide arrays of discreet chemically and functionally diverse materials. The Personal Care Products Council (PCPC) is a trade association based in Washington, DC that has been at the forefront of human safety assessment of cosmetic ingredients in North America for a number of decades. Until recently however, the environmental safety of cosmetic ingredients has been widely overlooked. To rectify this, the PCPC has begun early development of an environmental safety assessment program. The first stage of this process has been the development and assessment of *in silico* screening techniques assuming a "down the drain" exposure scenario. Both fate and ecological QSAR models have been developed and applied. As such, factors such as removal by wastewater treatment and toxicity to aquatic organisms have been considered. In addition, experimental data are also being mined to both use for the early screening of cosmetic ingredients and assess ECOSAR output. The end result of early screening efforts will be to prioritize cosmetic ingredients for further assessment based on predicted risk. The data that will be required for this will also be identified by these early screening efforts. Data-gaps will then be filled by a number of techniques, such as standard testing and read-across studies. Allied to these activities are the ongoing efforts by the PCPC and its member companies to assess the exposure of aquatic biota to cosmetic ingredients. The overall outcome of these combined efforts will be to conduct a full environmental risk assessment for high priority cosmetic ingredients. In addition to developing an environmental safety assessment framework, the PCPC has also been tracking and actively participating in the various state-driven environmental safety initiatives, such as those being developed under the auspices of "green chemistry," These efforts will also be discussed within this poster.

MP187 übertool: Web applications for ecological risk assessment

M. Snyder, J. Flaishans, T. Purucker, T. Hong, R. Van Meter, EPA

We have created an integrated web-based tool, the übertool, designed to estimate exposure doses and ecological risks under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Endangered Species Act (ESA). This involved combining a number of individual software models into web applications so they can be more easily parameterized, run, and documented by the EPA regulatory program office as well as federal, industry, and academic researchers outside the agency. These models include a range of aquatic, terrestrial, and atmospheric deposition fate and transport models used to estimate pesticide exposures and effects for a wide range of ecological receptors. Risk assessments based on these models are evaluated when seeking approval for pesticide formulations by the Environmental Protection Agency (EPA). Übertool integration of the ecological risk models creates a unified environment where data inputs and outputs are shared amongst models and saved for each user. This web-based approach takes advantage of new technologies including on-demand cloud computation of models written in a variety of programming languages (e.g., Python, Fortran, C) as well as spreadsheet calculators (e.g., Microsoft Excel) to support complex and screening level models. Übertool's web-based framework has also extended to population dynamic models not currently used by FIFRA and ESA that are publicly available for educational and research purposes. Traditionally, ecological assessment of pesticides is based on the ratio between estimated environment concentrations and extrapolated toxicity effects levels that can be difficult to relate to ecological endpoints that are actually valued and regulated (e.g., populations). By aggregating these models into a virtual dashboard, we enable linkages to be created in the gap between the traditional ecological hazard assessment of screening models (risk quotients) and assessing ecological endpoints at the population level. Closing this gap has been identified in a recent National Academy of Science's report as being necessary to create a common, scientifically credible approach to resolve discrepancies between how FIFRA and the ESA manage ecological risks. The new framework (Untertool) incorporates a variety of models including basic models of population dynamics (e.g., logistic model), intra-colony honey bee population dynamic models, and links to other online models and resources.

MP188 Use of a probabilistic approach to evaluate the certainty of risk estimates for the Lower Passaic River Study Area baseline ecological risk assessment

S. Replinger, Windward Environmental, LLC; B.A. Bergquist, L. Saban, M. Johns, Windward Environmental LLC; R. Law, De Maximis, INC

As part of the preliminary work on the baseline ecological risk assessment (BERA) for the Lower Passaic River Study Area (LPRSA), an exploratory probabilistic analysis is being used to evaluate the sensitivity of initial risk estimates to changes in various exposure parameters such as body weight, site use factor, and diet composition. This analysis results in a better understanding of the sensitivities of the risk estimates, and allows for the transparent presentation of this information for management input. The initial probabilistic analysis output was graphed as a cumulative frequency plot to show the probability associated with various potential risk estimates. The uncertainty associated with risk estimates is often discussed in detail in risk assessments, but may not be captured in ways that are meaningful for making risk management decisions. For example, when determining what action to take based on a hazard quotient (HQ) of 10, it is critical to understand whether that HQ is a good representation of the risk to a given receptor, or whether it is a likely over-estimate based on the use of compounding health-protective assumptions. Analyses similar to these exploratory probabilistic evaluations done for the LPRSA BERA could help inform risk management decisions regarding the evaluation of remedial alternatives and the associated costs.

MP189 Development and Application of a Web-Based Interactive Database for Generating Ecological Species-Specific Protective Concentration Levels

B.S. Yates, West Texas A&M University / Life, Earth & Environmental Sciences; W.H. Mimbs, West Texas A&M University / Department of Life, Earth, and Environmental Science, Oklahoma State University; G.C. Barbee, West Texas A&M University / Life, Earth & Environmental Sciences; L.F. Champagne, TX Commission on Environ. Quality; W.J. Rogers, West Texas A&M University / Life, Earth & Environmental Sciences

This research is the result of a ten year collaborative effort between West Texas A&M University (WTAMU) and the Texas Commission on Environmental Quality (TCEQ) to develop a novel interactive web-based toxicological database capable of generating protective concentration levels (PCLs) for ecological receptors in diverse habitats of the southern United States. Wildlife receptors were selected for each trophic level/guild resulting in 79 receptors in seven primary habitats. Potential contaminants of concern (PCOCs) commonly found in the southern US, particularly at CERCLA sites, and PCOCs from crude oil spills including Corexit oil dispersants, comprised the 135 PCOCs included in the database. A toxicological profile was developed for each PCOC from an exhaustive literature review, which includes fate and transport and acute and chronic toxicity data that is sufficient for developing default Toxicity Reference Values (TRVs). The TRVs, along with the other data gathered, were then used to develop NOAEL / LOAEL-bounded PCLs for each PCOC/receptor pair. This interactive database can generate over 10,000 PCLs each for mortality, reproduction, growth, and population toxicity endpoints for the seven habitats, resulting in a total of over 31,000 PCLs. This tool allows the user to replace default values, such as bioaccumulation factors, with site-specific ones for conducting ecological dose-response calculations. The database also provides a systematic tool to collect toxicological and indicator species data using a web-based data submission form from user contributions. All data submissions are refereed by a technical oversight group headed by the TCEQ. The ability to generate receptor-specific PCLs for entire ecosystems allows for the generation of site-specific terrestrial species sensitivity distributions, which can be an important tool in the weight-of-evidence analysis. While the model focuses on ecosystems of the southern US, it shows the potential to be used as an important tool for practitioners across the country. The user interface is web-based, while the back-end is a MySQL database engine that is used for data storage and retrieval. The WTAMU team has recently added data entry verification and validation features, data search features, and a more user friendly web-based interface and will continue to improve and expand the capabilities of the model.

Terrestrial or Wildlife Toxicology and Ecology

MP190 Accumulation Features of Organochlorine Pesticide Residues in Resident Birds and Their Prey from Ethiopian Rift Valley Region

Y. Beyene, Hokkaido University / Laboratory of Toxicology; Y. Ikenaka, S. Nakayama, Hokkaido University / Laboratory of Toxicology; M. Ishizuka, Hokkaido University

Despite restrictions and bans on the use of organochlorine pesticides (OCPs), many developing countries are still using for agricultural and public health purposes. Among those countries that continue to use OCPs, Ethiopia is one of the major importer and consumer for the past decades with a problem of obsolete pesticides. Moreover, Ethiopia uses approximately 400 metric tons of active-ingredient DDT for indoor residual spraying (IRS) per year for malaria control. In view of this, there is likelihood that the ecosystem in Ethiopia is highly exposed to great amounts of OCPs. Birds have been used as sentinels for biomonitoring of organic pollutants in the environment owing to their higher trophic position, sensitive to environmental changes and they are widespread. To our knowledge, no information is available concerning the bioaccumulation levels of OCPs by bird species in Ethiopia. Therefore, the present study is designed to investigate the contamination status and bioaccumulation features of OCPs in muscle tissues of birds and fish species from the Ethiopian Rift Valley region in association with Stable isotope ($\delta^{15}N$) analysis. Twenty three bird individuals belonging to four species including hamerkop (*Scopus umbretta*, *n*=5), African sacred ibis (*Threskiornis aethiopicus*, *n*=7), marabou stork (*Leptoptilos crumeniferus*, *n*=6) pelican (*Pelecanus onocrotalus, n*=5) and 105 fish specimens of five species (*Oreochromis niloticus*, *Tilapia zillii*, *Carp* spp, *Clarias gariepinus* and *Barbus intermedius*) were collected at Lake Ziway–Ethiopia. Dichlorodiphenyltrichloroethane (DDTs), Hexachlorocyclohexanes (HCHs), heptachlors (HPTs), chlordanes (CHLs) and hexachlorobenzene (HCB) were quantified. DDTs were found to be the predominant OCPs, indicating ubiquitous contamination to the rift valley region. An increasing trend was obtained between concentrations of DDTs and CHLs with trophic level, indicating the biomagnification potential of these compounds. However, HCHs and HPTs exhibited no correlation with trophic level. *p,p′*-DDE, well known for its

adverse effect on the health of wildlife especially birds, was by far the most important compound in all samples. Concentration of *p,p′*-DDE in birds had significantly high burden with a maximum concentration of greater than 1000 µg/g lipid weight in marabou stork bird species, suggesting there might be a possible indication to cause adverse effects on reproduction.

MP191 Adrenal and Endocrine Effects from Inhaling Petroleum-Related Compounds in Laboratory Animals – A Review

C.S. Mori, Industrial Economics Incorporated (IEc); A.B. Rosenstein, Consultant, Industrial Economics Incorporated (IEc); K.M. Colgrove, University of Illinois at Urbana-Champaign / College of Veterinary Medicine

Following oil spills, marine mammals may be primarily affected by inhalation exposures to petroleum-related compounds, due to the time spent at the air-water interface where the greatest concentrations of volatile hydrocarbons are found. Published studies that contain both observed health effects in marine mammals and measured exposure concentrations following oil spills are limited, as are laboratory animal inhalation studies with oil or mixtures of various petroleum products (e.g., gasoline, crude oil, etc.). However, for individual petroleum-related compounds, published laboratory animal inhalation studies are available. We compiled and reviewed these laboratory animal studies and found that endocrine and adrenal effects may be key health parameters related to inhalation exposures. Many of the studies identified were based on toluene exposure, and the range of health effects observed included changes in catecholamine levels, hormone levels, adrenal and thymus weights, and hematological parameters. These findings suggest that toluene may induce a stress response and/or affect the endocrine/adrenal system by inducing secondary hypofunction of the adrenal cortex. Approaches to applying the laboratory animal data to the assessment of health impacts via the inhalation route for marine mammals following oil spills will be discussed.

MP192 Assessment of mitochondrial DNA damage in little brown bats (Myotis lucifugus) collected near a mercury-contaminated river

N.K. Karouna-Renier, USGS Patuxent Wildlife Research Center / Beltsville Lab; C. White, USGS Patuxent Wildlife Research Center; D. Yates, Biodiversity Research Institute

Historical discharges of mercury into the South River near the town of Waynesboro, VA, have resulted in persistently elevated mercury concentrations in sediment, surface water, ground water, soil, and wildlife downstream of the discharge site, to this day. Previous studies have found elevated blood and fur mercury levels in two species of bat, including little brown bats (*Myotis lucifugus*). In the present study, we examined the relationship between mercury levels and genetic damage in little brown bats from the South River. Genotoxicity is among the many known toxic effects of mercury, resulting from direct interactions with DNA or from oxidative damage. In order to determine whether mercury exposure induces sublethal effects on DNA, we compared levels of mitochondrial DNA (mtDNA) damage in bats captured at two mercury contaminated locations to those captured at a reference site using real-time quantitative PCR (qPCR). Because it lacks many of the protective protein structures and repair mechanisms associated with nuclear DNA, mtDNA is more sensitive to the effects of genotoxic chemicals and therefore may be a useful biomarker in chronically exposed organisms. We examined two distinct regions of mtDNA. Significantly higher levels of damage were observed in both regions of mtDNA in adult bats from contaminated locations than in controls. Juvenile bats exhibited greater levels of mtDNA damage at contaminated locations than the reference site in one region of mtDNA. However, levels of mtDNA damage in adults and juveniles exhibited weak correlations with fur and blood mercury levels, suggesting that other factors may play a role in the site-specific differences.

MP193 Behavior and toxicity of copper oxide nanoparticles to Paronychiurus kimi (Collembola) in soil

S. Yu, Y. Lee, Korea Univ.; Y. Kim, Korea University; H. Mo, Division of Environmental Science and Ecological Engineering; K. Cho, Korea University / # 407, Division of Environmental

The metal oxide nanoparticles which have been used widely have potential toxicity due to its high surface area and unstable physicochemical properties. In this study, toxic effects of the copper oxide nanoparticles (CuO-NP) to *Paronychiurus kimi* were evaluated in soil system which has been studied fewer compared with aqueous phase. To investigate the toxic effect, the tests which were to determine the behavior of CuO-NP were also conducted. First, the adsorption capacity of CuO-NP was estimated with Freundlich sorption constants (Kd) and the amount of copper ion and nanoparticles in pore-water were measured. Second, the toxicities of CuO-NP and CuO-NP with dispersant agent (PAH, PAA) to *P. kimi* were assessed in accordance with ISO 11267. After 28 day exposure, LC50 for survival adult and EC50 for reproduction were estimated. To assess biochemical effect, antioxidant enzyme activity of survival adults was measured and protein was profiled using SELDI-TOF MS. Most amounts of CuO-NP and non-nano CuO were adsorbed to soil particle and few copper ions were released to pore-water. PAH and PAA used as dispersant of CuO-NP were ineffective in soil as compared with aqueous base. The result of toxicity on Collembola was not significant for their reproduction and antioxidant enzyme activities. However, protein profiling result showed that the protein pattern of the organisms exposed to CuO-NP was similar to that of CuCl2 treatment which have same copper ion concentration with released from CuO-NP. Based on the results, the cause of the effect on *P. kimi* should be copper ion not related to nanoparticles itself.

MP194 Change in cell morphology and autofluorescence of Chlorococcum infusionum exposed to heavy metals using flow cytometry

S. Nam, Konkuk University / Department of Environmental Science; Y. An, Konkuk University / Department of Environmental Sciences

Heavy metals distributed in environment caused adverse effects of ecosystem. We assessed toxicity of copper and nickel on soil alga *Chlorococcum infusionum*. *Chlorococcum infusionum* was incubated in bold basal medium and exponentially growing culture was used in the toxicity test. *Chlorococcum infusionum* for flow cytometric measurement were collected from the toxicity test after 6 days of treatment. Cell morphology was recorded with forward scatter channel (FSC) and side scatter channel (SSC), and autofluorescence of native pigments was recorded with FL3 channel. As a result, copper and nickel induce changes in cell morphology and autofluorescence of *Chlorococcum infusionum*. Cell size, granularity and autofluorescent intensity of *Chlorococcum infusionum* were reduced. This subject is supported by Korea Ministry of Environment as the GAIA project (2012000540011).

MP195 Climatic Influences on Avian Uptake of Mercury

L. Bryan, University of Georgia / Savannah River Ecology Laboratory; J. Snodgrass, Towson University / Biology department; H. Brant, Savannah River national Laboratory; C. Romanek, University of Kentucky; C. Jagoe, Florida A&M University; G. Mills, University of Georgia / Savannah River Ecology Laboratory

Mercury (Hg) is a global pollutant known for its ability to accumulate and magnify in biota, particularly in aquatic habitats. Wetland habitat types (fresh- vs. salt-water) and characteristics (e.g., hydroperiod, chemistry, and morphology) can influence Hg bioavailability, and thus impact the movement of Hg through trophic levels. Climatic conditions, primarily rainfall amounts and timing, can alter wetland availability and processes/chemistry and thus may influence Hg bioavailability. Avian piscivores are frequently monitored for mercury uptake and can be bio-indicators of mercury contamination. The Wood Stork (*Mycteria americana*) is a large, piscivorous wading bird that forages on aquatic prey in a variety of shallow fresh- and salt-water wetlands in the southern United States, as well as Central and South America. Nestling and adult storks are known to receive Hg through their diet. To examine the possible influence of rainfall on the uptake of Hg by Wood Storks, we collected feathers from nestlings in two Georgia stork colonies (1 coastal/1 inland) from 1997 – 2012 and analyzed them for Hg. In addition, we analyzed these same feathers for stable Carbon and Nitrogen isotopes to determine if rainfall influenced wetland foraging habitat use and/or prey types. Mercury in coastal stork nestlings was strongly influenced by foraging habitat type, with greater use of freshwater wetlands resulting in higher Hg concentrations. Use of freshwater wetlands as foraging habitat by coastal storks was correlated to rainfall amounts. For the inland stork nestlings, the relationship between Hg and rainfall was not as straight-forward, and could be related to rainfall amounts and timing over multiple years. These findings suggest that current and predicted future climatic patterns will likely influence Hg bioavailability and accumulation by piscivorous birds.

MP196 Comparison of atrazine and chlorpyrifos on wild local and commercially available frog species

J.R. Schroeder, Millikin University / Department of Biology

Recent studies have indicated that there may be differences in the biological responses to pesticide exposure from wild frog species compared to those reared in laboratory environments. Due to their exposure in the wild, the physiological response to pesticides for wild frogs is much less severe than under lab conditions as they may have developed adaptations; these adaptations may attribute to some of the inconsistencies in published studies on the effects of pesticide exposure in wildlife. To examine these differences, we have compared the growth and development of locally caught *Hyla versicolor* tadpoles and frogs, to that of commercially available species of frogs. We are examining the size, gross morphological changes, and sex ratios of the species. Future studies will also include a comparison of reproductive capabilities.

MP197 Determining mercury exposure in Western North American grebes by analyzing feathers from museum specimens

M. Perkins, University of Michigan / Department of Environmental Health Sciences; J. Hinshaw, University of Michigan / Museum of Zoology; N. Basu, McGill University / Department of Environmental Health Sciences

Mercury exposure has been shown to cause lethal and sub-lethal effects in birds, and is known to impair physiology, behavior, and reproductive success. Numerous studies have documented mercury exposure in various bird species across North America. However, compared to other areas of the continent, mercury exposure in birds of Western North America has not been well documented. This study intends to increase understanding of mercury exposure in Western North America by analyzing museum-preserved feathers from grebes from the University of Michigan Museum of Zoology collection. Our first objective was to assess mercury variation among feathers across the whole bird and within specific feather types. This would enable us to not only understand how mercury is distributed among grebe feathers, but also to standardize sampling. We analyzed representative flight and body feathers from 20 Clark's Grebe specimens. We determined that the tip of primary 8 most accurately reflects the maximum cumulative mercury exposure for a given individual. Following this, used feathers to quantify mercury exposure in Clark's and Western Grebes over a large geographical area of Western North America. For this study we obtained feathers (primary 8) from preserved skins of Clark's and Western Grebes from the University of Michigan Museum of Zoology. These two grebes were considered the same species until 1967, and because of their similar range, preferred habitat, molt schedule, and food preferences we combined species for most analyses. Specimens were collected in Manitoba (n = 27), Minnesota (n = 14), North Dakota (n = 18), Utah (n = 12), California (n = 75), and Oregon (n = 21) during 1989-1991. Feather total mercury concentrations in individual grebes ranged widely from 1.40 to 32.19 µg/g. We compared grebe feather mercury concentrations among states and province. Grebes collected from California had the greatest mean mercury concentration at 9.27 µg/g. North Dakota had the lowest grebe feather mercury values, with a mean value of 5.29 µg/g, and differed significantly from mean feather mercury values from California and Oregon. The results will be presented in tabular and graphical (map) format, and relationships with other factors (e.g., gender, age class) will be explored. These results indicate that some grebes from Western North America have been exposed to high levels of mercury and that mercury exposure differs geographically across the region.

MP198 Development of a Constructed Willamette Valley Plant Community to Determine Non-Target Effects of Herbicide Drift on Native Plants

D. Olszyk, USEPA / NHEERL/WED; T.G. Pfleeger, USEPA (retired) / NHEERL/WED; E.H. Lee, USEPA / NHEERL/WED; T. Shiroyama, Sen. Environ. Emp. Program; M. Plocher, Dynamac Corporation

As part of its regulation of pesticides, the US Environmental Protection must consider potential environmental effects, including impacts to nontarget plants. Normally the risk assessment to determine these impacts requires simple, individual species, greenhouse, dose-response experiments with growth endpoints. However, the extrapolation of potential risk of pesticide drift from individual species to a plant community should be viewed with caution. More sophisticated field tests may be required, but are rarely conducted. To provide a procedure for a field test to indicate herbicide effects on plant communities, we developed a series of small (0.45 x 0.45 m) plots planted with nine perennial Willamette Valley native plant species: *Eriophyllum lanatum* (Oregon sunshine), *Iris tenax* (Toughleaf Iris), *Prunella vulgaris* var. *Lanceolata (*Self-Heal), *Camassia leichtlinii* (Large Camas), *Festuca roemeri* (Roemer's fescue), *Elymus glaucus* (Blue wildrye), *Ranunculus occidentalis* (Western Buttercup), *Fragaria virginiana* (Virginia/wild strawberry), and *Potentilla gracilis* (Slender Cinquefoil). Plots were established on two Oregon State University farms. One year after establishment of the plots, the effects of aminopyralid and fluroxypyr, alone and in combination, were determined at 0.03 and 0.1 x a Field Application Rate (FAR) of 10.4 grams active ingredient (g.a.i.) for aminopyralid and 147.1 g.a.i. for fluroxypyr. Response endpoints per species were % cover on a periodic basis, # of reproductive structures and seed production. The study demonstrated a useful experimental protocol whereby a plant community can be evaluated for ecological responses. However, there was little if any response to the herbicides for any species at either site in this study, possibly due to the large size of the plants and low levels of herbicides used.

MP199 Differential accumulation of trace elements by lichens growing in the street median as compared to those in a neighboring urban park

L.R. Miller, Christian Brothers University / Department of Biology; L.J. Varriano, University of Tennessee; T.J. Sullivan, Christian Brothers University

Lichens have frequently been used as indicators of pollution. Because of their morphological make-up, they readily absorb many chemicals and/or elements across their surface. Sensitive species only grow in environments with low levels of pollutants whereas more tolerant species can be found in areas with higher levels of pollution. Lichens from Overton Park, Memphis, TN were collected and analyzed for heavy metals and other trace elements using an environmental scanning electron microscope with an electron dispersive x-ray spectroscopy backscatter detector to determine what (if any) elements were being released into the environment by the automobile traffic or industry of this highly urbanized area. Comparisons were made between the lichens collected from the inner area of the park and the lichens found in the median of the heavily traveled roads which border the park. A greater variety of metals and elements were found in the lichens from the median of the road compared to the heavily wooded park. Some of those elements included chromium, erbium, dysprosium, cerium, lanthanum and yttrium. The thickness of the understory within the park may be providing some protection from any airborne particles.

MP200 Effect of soil properties on bioavailability and toxicity of arsenic to Paronychiurus kimi (Collembola) in pore water

Y. Lee, S. Yu, J. Wee, Korea Univ.; Y. Kim, Korea University; K. Cho, Korea University / # 407, Division of Environmental

Soil properties are important factors modifying toxicity result. The different metal toxicity effects in different soils were explained by metal bioavailability of soil. The bioavailability of metal for soil organisms are related to the sorption equilibrium between the soil solid-phase and the pore-water. *Paronychiurus kimi* (Collembola) was selected for toxicity test. Collembola is quite relevant to metal in soil pore water because of ventral tube that is extremely important in fluid balance. In this study, four different forest soils were selected and analyzed the cation-exchange capacity (CEC), clay mineralogy, organic matter and organic carbon contents. The toxicity of arsenic to the soil Collembola *Paronychiurus kimi* (Collembola) was assessed in the forest soils and OECD artificial soil in accordance with ISO 11267. After 28days of exposure to tested metals, LC50 for adult survival and EC50 for reproduction were estimated and arsenic concentration in pore-water and body of Collembola was measured. The toxicity results were different in each forest soil and OECD artificial soil. These results show that soil properties affect the arsenic toxicity. The arsenic concentration in pore-water and body of Collembola considered, soil properties decide metal bioavailability by concentration of available form in pore water.

MP201 Effects of naturally contaminated grain on poultry performance, health and immune function

M. Kautzman, University of Saskatchewan / Toxicology Centre; M.L. Wickstrom, University of Saskatchewan / Toxicology Group; N.S. Hogan, University of Saskatchewan / Toxicology Centre; M. Mostrom, North Dakota State University / Veterinary Diagnostic Lab; T. Scott, University of Saskatchewan / Department of Animal and Poultry Science

Mycotoxins are widespread worldwide and their occurrence in agricultural cereal grain has been increasing steadily. Deoxynivalenol (DON) produced by molds in the genus *Fusarium* is the most common mycotoxin contaminating wheat, durum and barley in Canada. *Fusarium* infection leads to

Fusarium damaged kernels (FDK), which contain lower crude protein (CP) as well as potential mycotoxins. This low protein, high mycotoxin grain is often used as poultry feed and is a major contributor to decreased performance causing significant losses in animal production and economics. Crude protein concentrations in grain can be measured accurately using Near Infrared Reflectance (NIR). This technique has been combined recently with new technology to enable commercial scale sorting of individual wheat, durum or barley kernels from contaminated batches of grain based on CP and by association DON. Preliminary results indicate that the sorted grain fractions containing kernels with the lowest 20% CP had the highest concentrations of DON, and the remaining 80% of the material was substantially less contaminated. Our objective is to evaluate differences in performance, health and immune function of broiler chicks fed diets based on sorted and unsorted grain sources using this technology. Sorted fractions from 3 different grain sources with high proportions of FDK will be analyzed to determine the concentrations of 16 common mycotoxins. Results from the analysis will be used to reconstitute the grain sources into four treatment diets. These diets will contain differing ratios (0:100, 20:80, 40:60 and 60:40) of the lowest 20% CP and top 80% CP fractions. Diets will be fed to broiler chicks for 35 days. Feed intake and body weight will be measured weekly to determine mycotoxin effects on growth and feed efficiency. Potential immunotoxic effects will be evaluated by measuring systemic antibody production in response to antigen challenge, T-cell response to intradermal phytohemagglutinin administration, and histopathology of immune organs. Results of this study may improve knowledge of tolerable concentrations of *Fusarium* mycotoxins in poultry diets.

MP202 Evaluating the stress response of wild birds as a bioindicator of sub-lethal effects of crude oil exposure

C.R. Lattin, H.M. Ngai, L.M. Romero, Tufts University / Department of Biology

The hypothalamus-pituitary-adrenal (HPA) axis allows wild animals to regulate baseline physiology and respond to environmental stressors. However, there is some evidence that the HPA axis may be especially vulnerable to endocrine-disrupting chemicals. This study aimed to systematically quantify the effects of ingested Gulf of Mexico crude oil on the stress response of wild-caught house sparrows (*Passer domesticus*), as well as several other blood chemistry parameters, including plasma glucose, uric acid, and albumin. Crude oil was weathered to ~75% volume by heating at a low temperature and stirring continuously. Oil birds received a dose of 1% oil weight:food weight, determined using preliminary dose-response feeding experiments. Controls received sunflower oil instead of petroleum, and both groups were allowed to feed *ad libitum*. Compared to most blood chemistry parameters, the HPA axis was more sensitive to disruption by toxins in weathered crude oil. After 4 weeks of feeding, we saw significantly reduced stress-induced CORT titers and decreased CORT secretion in response to an injection of adrenocorticotropic hormone in birds ingesting an oiled diet. A reduction in stress-induced CORT titers after ingesting oil has also been seen in ducks, suggesting this effect may occur across all avian species. Our data indicate that this reduction may be due to damage to the CORT-secreting cells of the adrenal gland. These effects are important not only as potential bioindicators of petroleum exposure, but because birds with impaired HPA function show higher mortality when faced with stressors.

MP203 Examining Trends of Methylmercury in Michigan Using Nestling Bald Eagle Feathers

L. Fuentes, University of Maryland / School of Agricultural, Forest, and Environmental Sciences; W.W. Bowerman, University of Maryland / Department of Environmental Science & Technology; W.Y. Chao, Clemson University / School of Agricultural, Forest, and Environmental Sciences; W. Bridges, Clemson University / Department of Mathematical Sciences

Methylmercury (MeHg) contamination of fish has impaired the recreational, economic, and nutritional benefits of freshwater resources. Eighty percent of national fish advisories are attributed to mercury (Hg). Sediment cores from lakes in both hemispheres show that net Hg deposition has increased threefold since preindustrial times due to an increase in anthropogenic inputs. Studies have indicated that Hg methylation is increased by the availability of organic carbon, and wetland environments provide favorable conditions for microbial decomposition of organic matter. Under these conditions, sensitive biota is exposed to increased levels of MeHg. Michigan contains roughly 15% wetland habitat (22,258 square kilometers) and 8,516 square kilometers of Great Lakes shoreline. In 1999, the Michigan Department of Environmental Quality implemented the Michigan Bald Eagle Biomonitoring Project. This long term monitoring effort is a collaborative effort among government and academic entities. The bald eagle (*Haliaeetus leucocephalus*) has been designated as a biosentinel for the aquatic food chain. Analyzed feather samples from nestling bald eagles throughout Michigan provide information about spatial and temporal trends of persistent environmental pollutants such as Hg. Time periods were from 1986-1993 (T1), 1999-2003 (T2), 2004-2008 (T3), and 2009-2012 (T4). One thousand two hundred seventy feather samples were analyzed for total Hg using atomic fluorescence spectrometry. Statewide Hg concentrations were highest during T1 and decreased until the end of T3. There was a small but significant increase in Hg during T4. Geometric means were 7.62, 3.26, 0.79, and 1.49 mg/kg for T1-T4, respectively. Inland regions had significantly higher Hg concentrations compared to Great Lakes shoreline areas. Localized observations showed areas of high Hg concentrations along the Lake Superior shoreline of the Upper Peninsula. As evidenced by the steady increase in the Michigan bald eagle's productivity and expanding population, Hg is currently not negatively affecting breeding success. Accumulation of Hg in feathers still provides managers with information to identify areas that may be of concern due to localized increases in Hg concentrations, and/or having consistently high levels which may translate into risk of nearby fisheries. Sampling efforts should be focused on these areas to further assess any potential risks and to track temporal trends.

MP204 Germination and early plant development of 10 plant species exposed to Nano TiO2 and CeO2

C.P. Andersen, Environmental Protection Agency / Western Ecology Division, NHEERL; M. Plocher, M. Storm, G.A. King, Dynamac Corporation; P.T. Rygiewicz, USEPA / Western Ecology Division, NHEERL

Ten agronomic plant species were exposed to different concentrations of nano-TiO_2 or CeO_2 (0, 250, 500 and 1000 ug/l) and followed to examine effects on germination and early seedling development. For TiO_2, cabbage showed increased and corn decreased percent germination, while the other 8 species showed no significant change in percent germination. However, TiO_2 accelerated the timing of germination in 5 species, and had significant effects on final root length in 9 of 10 species, with four species showing increased and five showing decreased root length. For CeO_2, only one species showed a change (slight decrease) in germination percent, while 5 species showed a decrease in final root length. In a companion study with *Arabidopsis*, CeO_2 was found to alter gene expression more strongly in roots than shoots, consistent with the hypothesis that TiO_2 and CeO_2 may affect roots and shoots differently. Overall, the responses observed did not consistently relate to nanoparticle concentration, suggesting that concentration may not be an appropriate metric for expressing effects with these two nanoparticles. The results suggest that TiO_2 and CeO_2 have different effects on early plant growth of agronomic species, which may alter the timing of specific developmental events during their life cycle. In addition, the standard germination test, which is commonly used for toxicity screening of new materials, may not detect the subtle but potentially more important changes associated with early growth and development in terrestrial plants.

MP205 Great Lakes Restoration Initiative: Reassessment of Wildlife Reproduction and Health Impairments in the Saginaw Bay and River Raisin Areas of Concern

K. Grasman, Calvin College / Department of Biology; S. Fuhrman, S. Hooker, M. Langeland, Calvin College / Biology; L.L. Williams, US Fish & Wildlife Service

This assessment investigated effects of contaminants on reproduction and immunological health of fish-eating birds in the Saginaw Bay and Raisin River Areas of Concern (AOCs) as part of the Great Lakes Restoration Initiative-Fish and Wildlife Service AOC program. In Saginaw Bay, studies were conducted during 2010-13 at two herring gulls colonies (Confined Disposal Facility (CDF) in the southern bay and Little Charity Island in the outer bay), two Caspian tern colonies (CDF and Charity Reef) and one black-crowned night heron colony (CDF). At the River Raisin AOC, herring gulls were studied at the Detroit Edison Monroe Power Plant on the western shore of Lake Erie. Reference sites were located in the lower St. Mary's River (gulls on Pipe Island Twins and terns on Two Tree Island) and on Chantry Island, Lake Huron (herons). Gull nests were marked during egg-laying, and embryonic viability was assessed during late egg incubation using an embryonic viability detector sensitive to heartbeat and movement. Embryonic nonviability rates in herring gulls at the Saginaw Bay and River Raisin

AOCs (multiyear means of 4.3% for the CDF, 7.6% for L. Charity, and 7.3% for Monroe) generally were higher than at reference sites (< 2-4%). Rates on the CDF were elevated in 2010 (6%) but within or close to the reference range in 2012 and 2013 (4.8% and 2.9%, respectively). Infertility and embryonic mortality contributed to noviability at all sites, with infertility accounting for a greater proportion. In 2012 a herring gull chick was found at Monroe with a crossed bill, a deformity associated with PCBs and dioxins. Herring gull chicks in Saginaw Bay had marginally good survival, and Caspian tern chicks had poor to marginal survival, a concern for this state-threatened species. Herring gulls at Monroe experienced poor chick survival during 2010-12, with complete reproductive failure during 2010. Mean phytohemagglutinin skin responses for T-cell mediated immunity were suppressed dramatically compared to reference sites in young herring gulls (50-57%), Caspian terns (48-51%), and black-crowned night herons (39%) in the Saginaw Bay AOC. This immune response was suppressed 57-65% in herring gulls at the River Raisin AOC. Ongoing immunological, developmental, and reproductive impairments in birds at these AOCs are consistent with previous studies on the effects of persistent pollutants, such as PCBs, in Great Lakes wildlife.

MP206 In silico modeling of manganese- and iron-binding properties of a designed peptide inspired by the radioresistant bacterium Deinococcus radiodurans

M.A. Bradley, Department of Environmental Health Sciences; D.M. Faulkner, University of Michigan / Department of Environmental Health Sciences; S.J. Wijeyesakere, University of Michigan / Department of Microbiology and Immunology; D.E. Johnson, University of California, Berkeley / Nutritional Sciences and Toxicology; R.J. Richardson, University of Michigan / Computational Toxicology Laboratory

Recent work has shown that Mn(II)-peptide complexes are an important source of radioresistance in bacterial cells, and radioresistant bacterial species have high cytosolic ratios of Mn(II) to Fe(II) compared to non-radioresistant species. Based on the cytosolic amino acid profile of the radioresistant bacterium Deinococcus radiodurans, a synthetic decapeptide (DEHGTAVMLK) has been found to confer additional radioresistance to the bacteria in the presence of Mn(II). However, the structure of this peptide and the corresponding peptide-metal complexes are unknown, as is its affinity toward metals. To address these gaps in knowledge, we carried out docking and molecular dynamics simulations to gain insights into the structure of the peptide and its modes of interaction with Mn(II) and Fe(II). Because metal binding to peptides is often pH-dependent and extremophilic bacteria can function over wide ranges of pH, we undertook docking studies over a broad pH range (4.4, 7.4, and 10.4). At all pH values, Fe(II) was predicted to bind the peptide more strongly than Mn(II). At pH 7.4, the predicted free energy of binding (ΔG) for Fe(II) was -5.64 kcal/mol, whereas that for Mn(II) was -3.84 kcal/mol. At pH 4.4, the predicted affinities for both metals were lower: -4.34 kcal/mol for Fe(II) and -2.79 kcal/mol for Mn(II). However, the calculated affinities for both metals at pH 10.4 were unchanged from their respective values at pH 7.4. Additional studies assessed the effects of sequential metal ion binding on the predicted binding affinity. Secondary and tertiary ligands bound less strongly, irrespective of the identity of the previous ligand. Fe(II) binding was stronger than Mn(II), irrespective of ligand identity or sequence. Preliminary molecular dynamics simulations suggested that although Fe(II) displays a higher affinity toward the peptide, the Fe(II)-peptide complex dissociates more rapidly than its Mn(II) -bound counterpart does. The structure of the peptide in complex with Mn(II) and Fe(II) is predicted to be α-helical with varying degrees of coordination from the N-terminal residues. These studies are expected to guide future work to experimentally determine the structures and modes of binding of Mn(II) and Fe(II) complexes with the decapeptide as part of an overall effort to understand how these complexes help confer radioresistance.

MP207 Lead poisoning in migratory waterfowls, is this a real threat?

M.E. Pereda, A. Cardenas-Gonzalez, J.H. Martinez-Guerrero, Universidad Juarez del Estado de Durango / Cuerpo Academico de Fauna Silvestre. FMVZ; L.F. Sanchez-Anguiano, J.M. Salas-Pacheco, Universidad Juarez del Estado de Durango / Instituto de Investigaciones Cientificas

Lead poisoning in migratory waterfowls seems to be a solved problem for at least a couple of decades in countries like the US and Canada. However, in developing countries like Mexico this is an ongoing problem that threatens the health of the birds and the aquatic and sub-aquatic ecosystems. To test this hypothesis 26 samples of liver tissue of individuals of snow goose (*Chen caerulescens*) were collected during the 2011-2013 hunting season in a wetland near the city of Durango, Mexico. Quantification of the tissue levels of lead was performed using an atomic absorption spectrophotometer where the levels found (dry weight) for 2011 (n = 8) were 3.06 ppm (ee=0.33, min=1.86, max=4.11) and for 2013 (n=18) 0.85 ppm (ee=0.20, min=0.13, max=3.11). In the histopathological analysis of liver tissue lesions were not found. We found lead shots in the digestive tract in 5% of the birds that underwent necropsy. Was not possible to find a relationship between ingested shot and tissue lead concentrations. According to the recommendations for the interpretation of tissue lead concentrations in waterfowl, the values obtained in this study corresponds to background contamination (< 2 ppm of wet weight in liver). These results suggest that despite the use of lead shot and their presence in the digestive tract of some birds, there is no risk of potential lethal or sub lethal effects due to lead poisoning.

MP208 Metabolism of pesticides after dermal exposure to amphibians

D. Glinski; M. Henderson, USEPA, Ecosystems Research Division; T. Purucker, USEPA; R. Van Meter, Environmental Protection Agency

Understanding how pesticide exposure to non-target species influences toxicity is necessary to accurately assess the ecological risks these compounds pose. Aquatic, terrestrial, and arboreal amphibians are often exposed to pesticides during their agricultural application resulting in potential population disruption. To study these potential pathways, amphibians were exposed to three different classes of pesticides. Atrazine is one of the most commonly used herbicides, triadimefon is a fungicide and fipronil is an insecticide. In spring and summer 2012, southern leopard frogs (*Rana sphenocephala*), Fowler's toads (*Bufo fowleri*), gray treefrogs (*Hyla versicolor*), green treefrogs (*Hyla cinerea*) and barking treefrogs (*Hyla gratiosa*) were collected from the University of Georgia's Whitehall Forest and reared through 60-90 days post-metamorphosis at the USEPA in Athens, GA. Amphibians were exposed to active ingredients through indirect contact with pesticide contaminated soil. After an 8 hour dermal exposure experiment, body burdens (ppm) for both parents and metabolites were quantified using a liquid chromatography-mass spectrometry. Atrazine metabolized into both desethyl atrazine and deisopropyl atrazine which consisted of 11-46% of the total atrazine body burden. The active ingredient triadimefon (TDN) was metabolized into triadimenol (TDL) which consists of two diastereomers a and b (a: 1S,2R; 1R,2S; b: 1S,2S; 1R,2R). All frog species analyzed for TDL exhibited higher body burdens for the less toxic TDL b diastereomer compared to the more toxic diastereomer TDL a. Analyses showed higher concentrations of TDL compared to TDN in the arboreal gray treefrog and the terrestrial toad, whereas all other species had higher concentrations of the parent compound. Although amphibian body burdens between fipronil enantiomers were not statistically significant, there were differences across species. The Fowler's toad exhibited the highest body burdens for fipronil, while the three arboreal treefrog species accumulated the least. Gaining knowledge on species specific differences in metabolism of pesticides will be important in estimating risk to amphibians as non-target organisms considering pesticide metabolites can occasionally be more toxic than the parent compound.

MP209 Persistent Organic Pollutants in Kodiak Brown Bear: Is Their Salmon Diet Too Much of a Good Thing?

L.A. Verbrugge, US Fish and Wildlife Service / Anchorage Fish and Wildlife Field Office; B. Pyle, W. Leacock, US Fish and Wildlife Service / Kodiak National Wildlife Refuge; L. Van Daele, J. Crye, State of Alaska / Department of Fish and Game; D. Verbrugge, State of Alaska / Department of Health and Social Services

Kodiak brown bear (*Ursus arctos middendorffi*) is an iconic keystone species on Alaska's Kodiak Archipelago, and supports world-class sport hunting, wildlife viewing, and photography opportunities, as well as subsistence uses. The diet of these bears varies seasonally, and salmon availability varies widely on different parts of the archipelago. Salmon are biotransport vectors for marine nutrients, persistent organic pollutants (POPs) and mercury to freshwater ecosystems in Alaska, so bears that eat large quantities of seasonally available sockeye salmon may be vulnerable to health effects related to contaminant exposure. To assess exposure to POPs and mercury, we collected adipose tissue and hair from hunter-killed bears (43 bears from the April 1 – May 15, 2012 season, and 30 bears from the October 25 – November 30, 2012 season). We will present a comparison of hair mercury and adipose polychlorinated biphenyl, organochlorine pesticide and polybrominated diphenyl ether concentrations among seasons, and among bears that consume large quantities of sockeye salmon and bears that have limited access to sockeye salmon. Inferred dietary differences based on the geographic site

of collection will be supplemented with stable isotope information from hair samples. POP and mercury results in both groups will be compared to tissue residue concentrations known to adversely impact bears. Management implications of results will be discussed. The Kodiak brown bear population is intensively managed, so Refuge and State of Alaska biologists have a variety of options available to protect its long-term health and abundance.

MP210 Pesticides, Pollinators, and Pathogens: Linking Bee Health Deficiencies to Agrochemical Exposures at the Colony Level

T.D. Anderson, Virginia Tech / Entomology and Fralin Life Science Institute; A.M. Reeves, B.E. Traver, Virginia Tech / Entomology; M. Kakumanu, Virginia Tech / Horticulture; J.R. Williams, Virginia Tech / Entomology; M.A. Williams, Virginia Tech / Horticulture; M.J. Lydy, Southern Illinois University / Zoology; C.C. Brewster, R.D. Fell, Virginia Tech / Entomology

The honey bee is a widely managed crop pollinator that provides the agricultural industry with the sustainability and economic viability needed to satisfy the food and fiber need of our society. The excessive use of agrochemicals is implicated in the reduced number of managed bee colonies available for crop pollination services. However, there are several gaps in our knowledge with respect to agrochemical exposures and the health status of managed bee colonies. Thus, it is necessary to gather information relevant to the areas where knowledge is lacking in order to enhance our ability to predict conditions that are either favorable or unfavorable for bee colony health. Here, we will summarize our research findings related to agrochemical impacts on the microbial community structure and function of managed bee colonies and the resulting nutrition and immune deficiencies that threaten the health of these colonies. These data are being used to model the health profiles for managed bee colonies exposed to agrochemicals in order to provide a theoretical framework to explain bee colony health thresholds and failures. In turn, the information gathered in this study will be translated into utilizable management practices that can reduce the loss of managed bee colonies for both the apicultural and agricultural industry.

MP211 Quantifying Human Exposure to Persistent Organic Pollutants in the Arctic: Developing New Bioaccumulation Models for Narwhal, Beluga Whale and Caribou

M.J. Binnington, University of Toronto Scarborough / Dept. of Physical & Enviro. Science; C. Quinn, University of Toronto Scarborough / Department of Physical and Environmental Sciences; M. Curren, Health Canada / Chemicals Surveillance Bureau, Population Biomonitoring Section; J. Armitage, University of Toronto Scarborough / Department of Physical and Environmental Sciences; J.A. Arnot, ARC Arnot Research & Consulting / University of Toronto Scarborough / Department of Physical and Environmental Sciences; H.M. Chan, University of Ottawa / Department of Biology; F. Wania, University of Toronto at Scarborough / Department of Physical & Environmental Sciences

For aboriginal human populations living in the Arctic, the main route of POP exposure is typically via consumption of high trophic level marine mammals. Accordingly, the ongoing trend of dietary transition away from traditional food items and towards imported foods is an important consideration for historical and contemporary exposure assessments. A previous modeling study conducted by our group detailed a conceptual framework for assessing the relative importance of this dietary transition in determining human POP exposure compared to changes in Arctic environmental levels. In that work, only consumption of fish and ringed seal was considered. However, in order to more holistically model exposure of aboriginal Arctic populations to POPs, the inclusion of additional biota consumed as traditional food in our modeling framework was necessary. Using dietary survey information from four separate regional biomonitoring campaigns performed among aboriginal populations in Canada's North, we identified three Arctic traditional food species which should be included in human food chain modeling: beluga whale (*Delphinapterus leucas*), narwhal (*Monodon monoceros*), and caribou (*Rangifer tarandus*). New bioaccumulation models for these species were developed for inclusion in the Arctic version of the human food chain bioaccumulation model ACC-Human. Here we report on model development and evaluation. Simulation results of the longitudinal exposure of multiple beluga whale, narwhal, and caribou cohorts to PCB congener 153 are converted into population level exposure variables that can be compared with reported field measurements. Ultimately, the expanded ACC-Human model approach will allow us to better characterize the roles of POP emission regulations and aboriginal dietary transitions in influencing historical POP exposures in Northern communities.

MP212 Rapid Screening Techniques to Assess the Impact of Insensitive Munitions in Terrestrial Environments

J.G. Coleman, US Army Engineer Reseach and Development Center / Environmental Laboratory; J. Seiter, US Army Corps of Engineers / Research Physical Scientist; A. Russell, C. Ang, Badger Technical Services; D.R. Johnson, US Army Engineer Research & Development Center / Environmental Laboratory, US Army / CEERD-EP-R; A.J. Bednar, US Army Engineer Research and Development Center / Environmental Laboratory; C. Lounds, N. Melby, Badger Technical Services; M.S. Ansley, University of Mississippi – ETRP / Environmental Toxicology Research Program

Insensitive Munitions (IMs) have received sanction for military use, although little information is available on the implications of these emerging materials in terrestrial environments. IMX-101 has been approved as a main fill ingredient artillery munitions and consists of 40% 2,4-dinitroanisole (DNAN), 20% 3-nitro-1,2,4-triazol-5-one (NTO), and 40% nitroguanidine (NQ). Our objective was to address the interesting challenge of mixture characterization in terrestrial environments through rapid screening techniques which provided data relative to soil retention, toxicity, and bioavailability in whole organisms. Initially, bench-scale soil batch studies were employed to determine environmentally relevant rates of IMX-101 retention in soils. We assessed retention rates of IMX-101 in solution (10-50 ppm) in a field collected and artificial soil over 24 hours (n=3). Following HPLC analysis of solutions in soil reactions, results indicated up to 50% of DNAN retention, while NQ retention was 25 % or less in soils. Next, we conducted a bench scale experiment utilizing a simulated earthworm gut system (SEG) to assess IMX-101 bioavailability. This system utilized hydrophobic membranes within 15 mL centrifuge tubes and enzyme media spiked with IMX-101 to function as a surrogate for uptake in the earthworm gut over a period of 3.5 hours. The HPLC data collected from the SEG system was compared to results of a whole earthworm 10 day dermal exposure, which resulted in accumulation of IMX-101 compounds in both the earthworms and SEG membrane. Additionally, a series of HEPG2 cell exposures was compared with extracted earthworm coelomocytes with a logarithmic dose of IMX 101 (0-1000 ppm) to determine cell impact. The exposures resulted in a dose dependent decrease in cell viability of the HEPG2 cells relative to the earthworm coelomocytes. The present experiments combine rapid soil retention exposures, simulated bioaccumulation, and cellular screening assays with traditional whole organism testing utilizing the earthworm, *Eisenia fetida* to predict rates of uptake and potential impact of IMX-101 in terrestrial environments. Ultimately, the proposed efforts will provide Army researchers and range managers with previously unavailable information on bioavailability, toxicity, and mobility of and IMX-101 in terrestrial systems.

MP213 Reducing Risk Assessment Uncertainty at Small Arms Firing Ranges

S.F. Richardson, AMEC Environment and Infrastructure / Risk Assessment Group

We captured mice and shrews in the downrange shot fall zone of a small arms firing range. We then measured and compared lead concentrations in whole body samples and mineralizing tissue (bone). We found that lead partitioned preferentially into mineralizing tissue with concentrations greater in femur tissue (77.2 ppm) than whole body tissue samples (35.7 ppm). The results of this study indicate that exposure point concentrations based on whole body lead levels overestimate risk for receptors that do not digest bone, such as owls and canines (e.g., foxes).

MP214 Soil temperature and aging time can alter the glyphosate toxicity to Paronychiurus kimi (Collembola)

J. Wee, Division of Environmental Science and Ecological Engineering; Y. Lee, S. Yu, Korea Univ.; Y. Kim, Korea University; H. Mo, Division of Environmental Science and Ecological Engineering; K. Cho, Korea University / # 407, Division of Environmental

Climate change is a global phenomenon and has the potential to have large effects to ecotoxicology. Temperature is an important factor on fate of toxic compound and responses of organism. A greater metabolic activity at the higher temperature results in an increase in the rate of uptake and degradation of toxic compound. Therefore, the research of temperature effect on toxicity is needed to understand the change of toxic effect under climate change. Glyphosate-based herbicides are one of the most widely used pesticides in the world and have been known to be easily degraded by microorganisms in soil. Many studies concern the effect on non-target species. In

this regards, the response of *Paronychiurus kimi* (Collembola) to glyphosate-based herbicide (Geunsami™) were evaluated at different temperatures (20°C, 25°C) and aging time (15, 30 days). Survived adults and hatched juveniles were counted after 28-day exposures in artificial soil spiked with 0.1, 0.5, 1, 5, 50 mg/kg of herbicide in different temperature conditions. In addition, soils spiked with 1, 5, 50 mg/kg of herbicide were tested after 15, 30 days to confirm aging effect. The toxicity results were different in each temperature and aging time. This result shows that soil temperature and aging time affect the response of *P. kimi* and degradation of herbicides.

MP215 Survey of Southern US Quail for Organochlorine Pesticides

C.E. Baxter, Texas Tech University / Environmental Toxicology; R.J. Kendall, Texas Tech University / Department of Environmental Toxicology

Numerous population surveys have shown a marked decrease in northern bobwhite quail (*Colinus virginianus*) and scaled quail (*Callipepla squamata)* in the Rolling Plains ecoregion of Texas and Oklahoma. This decrease presents a serious problem for the ecological health and hunting industry of the area. Causes for this decrease in quail numbers are many in theory, but so far there is no evidence available to highlight a particular phenomenon. Suggestions include drought, habitat loss, parasites, viruses, predation, heavy metals, and pesticides, all of which are currently under investigation. As part of this effort, our study focused on conducting a survey of common organochlorine pesticides in quail tissues, with the focus being whether found levels presented a risk to quail that may help explain the observed decline. This study represents a unique and comprehensive evaluation of a game/ground bird population over multiple years and sampling sites. Samples were collected during the late summer and early fall of 2011 and 2012 from different locations in the Rolling Plains of west Texas and Oklahoma. Several different tissues, primarily thigh muscle but also liver and brain, were analyzed using the QuEChERS method with GC-ECD/ECD quantitation. Most residues were below the detection limit (generally below 10 ng/mL), and residue profiles differed between tissues. DDE, Aldrin, and Endosulfan I were among the organochlorines detected most often. The results of this study suggest that quail are at low direct risk of organochlorine toxicity, probably because they subsist at the lower levels of the food chain. However, it is possible that the effects of low-level burdens, such as immune impairment, may contribute to quail decline through a decreased ability to deal with other, more intense, stressors like parasites. The results of this study provide valuable information describing ground bird organochlorine exposures that may be integrated with developing knowledge of other stressors and their potential roles in quail population decline.

MP216 The effect of applied abraded dusts from neonicotinoid treated seeds in field plots of Phacelia tanacetifolia on honeybees (Apis mellifera L.)

M. Kriszan, Eurofins Agroscience Services EcoChem GmbH; U. Heimbach, J. Pistorius, JKI; O. Klein, S. Knaebe, Eurofins Agroscience Services EcoChem GmbH

Drift of abraded dust of insecticidal seed treatments resulted in bee poisoning incidents in the past. For risk assessment purposes, tests with realistic applications of defined amounts of dust are needed, e.g., to determine NOEC or LOEC values. However, tests with dusts are much more difficult than tests with liquid substances. Due to solid state and the varying particle size it is challenging to develop standard ways of applying dust in situ and in vitro. In the field it is even more problematic to apply the low rates required in a practical way over a larger area. For this purpose we developed a method to apply defined amounts of dusts together with a dilution material in the field, to determine the effects of exposure on honeybees (Apis mellifera L.).

MP217 The relevance of Pontoscolex corethrurus as a bioindicator of soil quality under geogenic stress conditions

L. Cunha, Universidade dos Açores / Biology; H. Arruda, Universidade dos Açores; C. Parelho, University of Azores / Department of Biology; CITA-A – Azorean Biodiversity Group, Departamento de Ciências Agrárias, University of the Azores; M. Barreto, R. Camarinho, Universidade dos Açores; P.V. Garcia, University of Azores / Department of Biology, CITA-A – Azorean Biodiversity Group; P. Kille, A. Morgan, Cardiff University; A.S. Rodrigues, University of Azores / Department of Biology, (CVARG) Center of Volcanology and Geological Risks Assessment

Furnas volcanic soils exhibit elevated heavy metal availability, low oxygen/high CO_2 contents and a high temperature (~37ºC), being therefore under permanent conditions of geogenic stress. In such particular soils, the use of an appropriated bioindicator species could be routinely used to obtain and evaluate consistent evidence on the past, present and future situation of the environment and therefore enable an effective management of soil quality. The presence and high abundance of this species in Furnas geothermal field may indicate its relative tolerance to the multifactorial stress challenge posed by the active volcanic environment. Furthermore, the amount of ecophysiological and ecotoxicological observations on this species is very limited. Most of the analysed elements in the soils were also present in earthworm's tissues; however, the proportion between concentration in soil and earthworm tissues varied greatly among the analysed chemical elements. In fact, the presence of some elements in the body tissue of *P. corethrurus* that were not detected in the soil reveals that species can be highly sensible to detect these elements even at very low concentrations. The epidermis of earthworm's from the geothermal field in Furnas was significantly 30% thinner than the conspecifics at the pineapple culture houses. Thus we propose that *Pontoscolex corethrurus* should be considered as a relevant bioindicator species for soil quality, since it reflects with accuracy the composition of a given soil, with a valuable independency of the surrounding environmental factors due to its environmental tolerance and plasticity.

MP218 Use of the Japanese quail model for ecologically based Pb-soil dietary exposure

P.F. Henry, N. Beyer, US Geological Survey / Patuxent Wildlife Research Center; B.A. Rattner, USGS-Patuxent Wildlife Research Ctr; Y. Chen, Department of Fisheries and Wildlife Sciences, USGS Patuxent Wildlife Research Center, Virginia Tech / Department of Fisheries and Wildlife Conservation; D. Sprague, USGS / Patuxent Wildlife Research Center

The Japanese quail (*Coturnix japonica)* has become a model species for avian toxicity testing in many countries and is currently being proposed for screening transgenerational exposure to potential endocrine active xenobiotics. The life cycle of a Japanese quail can be completed in less than 3 months and it is easily maintained under controlled laboratory conditions across all stages of development. For this reason, the quail model was selected to generate data on toxicity effects, bioavailability, and tissue Pb concentrations from dietary exposure to surface soils collected from mining and smelting operations. Four week old male quail were exposed for 6 weeks to diets mixed with increasing percentages of Pb-contaminated soils ranging from 0 to 12%. Diet-soil mixes were formulated and pelletized specifically for the quail and proved to be successful in ensuring quail were ingesting the added soil at the nominal rates. Concentrations of Pb measured in the blood, liver, and kidney were higher with increasing % soil present in the diet; all were significantly different from controls. Changes in both ALAD and protoporphyrin in the erythrocytes supported toxicity associated with low and increased blood Pb concentrations. No other toxic effects were observed. The linear slope relating blood to dietary Pb concentrations from the quail study was similar to the slopes generated from regressions based on published data for several alternate wildlife species. Further use of the quail model and the relationship between blood Pb levels, biochemical effects, and dietary uptake of Pb from soil for ecological risk assessments are discussed.

MP219 Investigating whether biosolids-derived triclosan adversely effects the growth of three crop species

R.S. Prosser, School of Environmental Sciences; P.K. Sibley, University of Guelph / School of Environmental Sciences

Biosolids used in the amendment of agricultural fields have been shown to regularly contain triclosan (TCS), a broad-spectrum antimicrobial chemical, at relatively high concentrations. There is little data on the toxicity of TCS to terrestrial plants. Soybean (*G. max*), carrot (*D. carota*), and radish (*R. sativus*) plants were grown under controlled environmental conditions in TCS-spiked soil or soil amended with TCS-spiked biosolids. Seedling emergence was monitored. Shoot length, root length, shoot dry and wet weight, and root dry and wet weight were measured midway through the life cycle and at maturity for each plant species. The yield of soybean plants (number of seed pods, number of seeds, total mass of pods, and total mass of seeds) at maturity was also measured. Seedling emergence was significantly lower in some triclosan-spiked soil and triclosan-spiked biosolids treatments compared to control treatments ($p< 0.05$). Triclosan exposure did not significantly adversely affect any of the growth parameters in soybean, carrot, or radish plants relative to control plants ($p>0.05$). In general, growth parameters were higher in biosolids treatments but only significantly higher in one biosolids treatment of soybean plants ($p< 0.05$). There was no significant difference in measures of soybean plant yield across treatments

(p>0.05). No concentration-response relationship was observed across triclosan treatments. Results indicate that biosolids-derived triclosan do not have a significant adverse effect on the growth of the three species of plants examined in this study.

MP220 Reduction of hexavalent chromium by trehalose-producing soil bacteria

K.S. Haislop, University of Southern Maine; D. Finocchietti; P. Woodruff, University of Southern Maine

Chromium is a genotoxic carcinogen in its hexavalent form. Industrial use of chromate leads to environmental contamination. Chromium is found in the environment in the form of hexavalent (Cr(VI)) and trivalent chromium (Cr(III)). Hexavalent chromium is soluble and can contaminate the water supply. The soil bacterium *Pseudomonas fluorescens* has previously been engineered to clean up napthalene in the environment. P. fluorescens is also known to reduce toxic hexavalent chromium to its less nontoxic trivalent state.However this reduction creates hydroxyl radicals as a byproduct, damaging *P. fluorescens* in the process. Compatible solutes are small molecules that accumulate in many organisms as a response to stress. We are engineering *P. fluorescens* to produce trehalose, a compatible solute, to protect against damaging hydroxyl radicals. By protecting cell viability, more toxic hexavalent chromium can be reduced to the insoluble, nontoxic, trivalent chromium.

Procedures, Models and Techniques

MP221 A comparison of culture agitation techniques for Anabaena flos-aquae to decrease variability in environmental testing

M. Staggs, Smithers Viscient, LLC

Major regulatory agencies (e.g., USEPA and OECD) provide guidance for conducting environmental toxicity tests with chemical substances and/or mixtures. Within each of the guidelines, certain environmental conditions are recommended. The OCSPP 850.4550 Cyanobacteria (*Anabaena flos-aquae*) toxicity guideline recommends that "culture and test vessels should be shaken by hand once or twice daily. Continuous shaking usually results in clumping of the cells." On the other hand, the OECD 201 guideline recommends continuous mechanical shaking, which may increase cell clumping and variability in cell density data consequently making the control performance criteria in the OECD 201 guideline difficult to achieve. Based on data provided by Crop Life International in February 2008, 88% of 36 existing *A. flos-aquae* studies evaluated failed the section-by-section growth rate criterion and 75% failed the average specific growth rate criterion. Therefore, the objective of this investigation is to compare the variability of *A. flos-aquae* cell densityusing the two suggested agitation techniques in an attempt to reduce data variability and, if possible, meet OECD 201 control performance criteria. Preliminary data indicate hand shaking does provide less variable cell counts than mechanical shaking.

MP222 A multi-tiered approach to assessing biological effects of contaminants of emerging concern on fishes in Great Lakes tributaries

H.I. Schoenfuss, L. Thomas, St. Cloud State University; Z. Jorgenson, US Fish and Wildlife Service / Aquatic Toxicology Laboratory / Environmental Contaminants; A. Trowbridge, US Fish and Wildlife Service

There is growing concern over the presence, distribution, and possible biological effects of Contaminants of Emerging Concern (CECs) in the Great Lakes. To assess the risks of CECs on aquatic wildlife the St. Cloud State University (SCSU) Aquatic Toxicology Laboratory is collaborating with the US Fish and Wildlife Service in collecting, analyzing and interpreting fish samples across several Great Lakes tributaries. In conjunction with feral fish sampling at each field site SCSU deployed caged sunfish for 14 days. Automated temperature data loggers were placed near the site of the caged fish exposures to provide a continuous record of conditions during the caging. Feral and caged fish were assessed for a range of biomarkers including indications of morphometric, physiological and pathological changes to fishes consistent with exposure to estrogenic CECs. Enzyme-linked immune-assays (ELISAs) were used to quantify plasma vitellogenin concentrations in four fish species (white sucker, smallmouth bass, largemouth bass, sunfish) and histological assessments determined reproductive status and presence of pathologies in gonadal and liver tissues. To provide greater control in assessing the effects of CECs on fish health, we applied a predator avoidance assay previously shown to be sensitive to CEC exposure in larval fathead minnows. Larvae were exposed to field site-specific water (50% daily static renewal) for 21 days. Following the 21-day exposure, predator avoidance performance of larval fathead minnows was assessed using a high-speed camera set at 1000 frames/second. For each individual the following performance parameters were measured: larval growth, reaction time, escape velocity, and total escape response. By integrating biological results with analytical chemistry and land use characteristics, linkage will be generated between field sites and the effects of CEC exposure that will inform resource managers across the Great Lakes and assist in reducing the environmental impacts of CECs.

MP223 A Single-Parameter Non-Linear Isotherm Model for Predicting Adsorption of Multiple Solutes onto Granular Activated Carbon

C.W. Davis, University of Delaware / Department of Civil & Environmental Engineering; D.M. Di Toro, University of Delaware / Dept. of Civil & Environmental Engineering

The ability to predict partition coefficients for sorption to various media, e.g. organic carbon, from sorbent characteristics, e.g. octanol-water partition coefficient or LSER parameters, is a well -established procedure. However, the comparable capability for predicting the parameters for non-linear isotherms models, e.g. Freundlich sorption isotherms, is not available. The purpose of this presentation is to introduce a new model, log-normal Langmuir (LNL) isotherm, for describing adsorption behavior. It is based on a log normal distribution of the Langmuir binding constant. The model parameters correspond to physical and chemical characteristics of the sorbates and sorbent. The LNL isotherm is able to accurately describe both saturating and non-saturating adsorption behavior using a single solute parameter – the median Langmuir binding constant, and two global sorbent parameters – the total site density and the standard deviation of the log normal distribution. The LNL isotherm model is fit to four data sets comprising multiple chemicals sorbed to graphite, charcoal, and activated carbon with root mean square errors from 0.08 to 0.18 log units. It is also shown that the sorbate median LNL binding constants can be predicted using an Abraham poly-parameter linear free energy relationship (LSER). This model provides a solution to the problem of predicting the sorption isotherm for a new chemical once the sorbate parameters are known.

MP224 A toxicity profile approach to determine multiple modes of action in weakly estrogenic compounds: nonylphenol and octylphenol as a case study

C.A. Staples, Assessment Technologies, Inc.; K. Coady, The Dow Chemical Company; T. Osimitz, Science Strategies, LLC; B. Losey, RegNet Environmental Services

The development of sensitive assays that can measure endpoints such as estrogen receptor binding or induction of vitellogenin in male fish (*in vivo*) or tissues (*in vitro*) provide sensitive tools for assessing a compound's potential estrogenic activity. Traditional aquatic toxicity testing guidelines measure apical endpoints related to survival, growth and development, and reproduction following exposure at sensitive windows of development or throughout an organism's lifecycle. Assays measuring endocrine activity are being added to traditional testing guidelines, and some may consider that measurable estrogenic activity, for instance following exposure to a compound, is sufficient to warrant categorization of the compound as an endocrine disruptor. This approach to chemical categorization and potential regulation fails to account for the significant differences that exist between potent endocrine active substances, such as endogenous sex steroids (i.e., estradiol, E2) or pharmaceuticals (i.e., ethinylestradiol, EE2), and many chemicals of commerce widely used in consumer and industrial products that have much lower estrogenic potencies and act with different or multiple modes of action. The aquatic toxicity profiles of branched para-nonylphenol (NP) and 4-tert-octylphenol (OP) will be compared to those of E2 and EE2 with a focus on both apical and non-apical endpoints directly influenced by estrogenic activity. The available data show that the span of concentrations over which the potent estrogens cause reproductive effects in fish are millions of times lower than those concentrations causing a true narcosis effect, i.e., death. These highly potent estrogens exhibit a toxicity profile in aquatic organisms that clearly distinguishes exposures that result in hazards due solely to an estrogenic mode of action. In contrast, apical effects of NP and OP on aquatic organisms ranging from reproductive effects to overt toxicity and death, occur over a much narrower range of exposure concentrations (ca. a 50-fold span). For NP and OP, the overall aquatic toxicity data present a toxicity profile that suggests that there are multiple modes of

action associated with observed adverse effects in aquatic organisms occurring within the same range of exposure concentrations. This in turn suggests that adverse apical effects from weakly estrogenic compounds, such as NP and OP, are not clearly caused by an endocrine mode of action.

MP225 An Inexpensive, Temporally-Integrated System for Monitoring Occurrence and Biological Effects of Contaminants in the Field

M. Kahl, USEPA / National Health and Environmental Effects Research Laboratory; D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; K.E. Stevens, USEPA Mid-Continent Ecology Division, ORISE; K.M. Jensen, USEPA / National Health and Environmental Effects Research Laboratory; B. Holmen, USEPA, EMR, Inc.; E.J. Durhan, US-EPA / Mid-Continent Ecology Division; J.E. Cavallin, USEPA, ORISE / National Health and Environmental Effects Research Laboratory; J.P. Berninger, USEPA; E. Eid, A. Schroeder, USEPA / Mid-Continent Ecology Division; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory

Assessing potential biological impacts of complex mixtures of contaminants in aquatic environments is an ongoing challenge for ecotoxicologists. Instrumental analysis of site waters alone can identify contaminants but provides only limited insights as to possible adverse effects, due to factors such as the presence of unknown/unmeasured chemicals, mixture interactions and uncertainties in bioavailability. As a consequence, it is necessary to complement analytical determinations of the occurrence of contaminants with different measures of possible biological effects. Our lab currently is conducting studies associated with the Great Lakes Restoration Initiative (GLRI) to develop effects-based methods for assessing the effects of contaminants at different Great Lakes Areas of Concern (AOCs). A component of this work involves caged fish (fathead minnow) exposures. Previous caging studies with the fathead minnow have used a wide variety of test systems, depending on variables such as study objectives, water body characteristics, available materials, etc. For our GLRI studies we wanted to develop and implement a relatively standardized test system suitable for the wide range of habitat/deployment situations encountered at Great Lakes AOCs. In addition to a caging system for the fish, we sought to develop an automated device for collection of composite water samples which could be simultaneously deployed with the cages, and would reflect a temporally-integrated exposure of the animals. The water samples then could be used for targeted analysis of specific chemicals of interest, and/or determination of biological "activities" of concern (e.g., estrogenicity) using *in vitro* systems. A description of the *in situ* caging systems and a relatively simple and inexpensive (< 500 USD) time- integrated water auto-sampler will be presented. The fundamental design, construction and use of the composite sampler, along with cage and sampler performance, fish survival and recovery, site conditions and adaptability will be discussed. This abstract does not necessarily reflect official Agency EPA Policy.

MP226 Comparison of SEA Ring and laboratory marine bioassays in controlled aqueous and sediment exposures

M.A. Colvin, SDSURF/SPAWAR; G.H. Rosen, SPAWAR Systems Center Pacific / Environmental; B. Chadwick, SPAWAR Systems Center San Diego; R. Dolecal, SDSURF/SPAWAR; J.M. Guerrero, Space & Naval Warfare Systems Center; C. Stransky, AMEC Earth and Environmental / Aquatic Sciences, AMEC Environment & Infrastructure; A. Cibor, Nautilus Environmental; R. Darlington, Battelle

The bioavailability and toxicity of contaminated sediment and water samples are traditionally assessed using laboratory tests on field collected samples. Although widely used and well established, laboratory-based assessments of sediment and water quality do not necessarily represent the realistic nature of organism exposure to contaminants in the field. This may result in inaccuracies in data interpretation, leading to inappropriate management decisions. A series of experiments were conducted to verify the ability of the in situ-based Sediment Ecotoxicity Assessment Ring (SEA Ring) to provide comparable data to traditional EPA and ASTM-approved laboratory methods, under controlled laboratory conditions. Side by side static-renewal testing of marine samples in beakers and SEA Rings were conducted: 1) in aqueous exposures with copper using juvenile mysid shrimp (*Americamysis bahia*) and Pacific topsmelt (*Atherinops affinis*); and 2) in three sediment types, using marine clams (*Macoma nasuta*), polychaetes (*Neanthes arenaceodentata*), and amphipods (*Eohaustorius estuarius*). Test acceptability criteria were met for all exposures. For both aqueous and sediment exposures, dose responses and survival, respectively, were similar between SEA Ring and laboratory tests. Bioaccumulation of polychlorinated biphenyl (PCB) congeners varied among sediment samples, but was not statistically different between SEA Ring and laboratory treatments for any species. Growth rates for polychaetes differed significantly between the SEA Ring and laboratory methods, with the former resulting in greater mean wet weights. This was likely attributed to several factors including enhanced water flow and exchange rates in the SEA Rings, and associated dilution of water soluble contaminants in the SEA Rings relative to that in static test chambers. Constant water flow to varying degrees is more representative of conditions that naturally occur in the environment. Overall, the SEA Ring technology produced results highly comparable to standard laboratory-based methods, when conducted under similar exposure conditions. With appropriate quality assurance measures, in situ testing using the SEA Ring can provide a viable alternative to standard laboratory-based tests to provide more realistic and meaningful data towards accurate site-specific assessments of water and sediment quality.

MP227 Development of an abbreviated in vivo bioconcentration factor test in common carp (Cyprinus carpio)

M. Cantu, D. Hala, University of North Texas; L.H. Petersen, University of North Texas / Institute of Applied Science; D.B. Huggett, University of North Texas / Department of Biological Sciences

Bioconcentration factor testing serves as the most valuable surrogate for the assessment of bioaccumulation. The assessment of potentially harmful chemicals is crucial to not only the health of aquatic environments, but to humans as well. Chemicals that possess the ability to persist in the environment or that have the potential to bioaccumulate, pose a greater risk to organisms that are exposed to these chemicals. The Organization for Economic Cooperation and Development Guideline 305 outlines specific protocols to run an accurate and reliable aquatic flow-through test. However, since its adoption in 1996, very few changes have been made to accommodate the endeavor to lowering the amount of test species to run one of these said tests. Running an aquatic flow-through test, according to 305, takes much time and money as well as numerous amounts of fish. Such burdens can be eliminated through simple modifications to the standard protocols. In this study, we propose an abbreviated study design for aquatic bioconcentration testing which effectively alleviates the burdens of running a flow-through test. Four chemicals were used individually to evaluate the usefulness of the proposed shortened design; 4-Nonyphenol, Chlorpyrifos, Musk Xylene, and DDT. The study consisted of exposing *Cyprinus carpio* for 7 days followed by 7 days of depuration, for a total of a 14-day study. Our results for each of the four compounds are consistent with literature values, thus, demonstrating that BCF_k can be accurately predicted in an abbreviated *in vivo* test.

MP228 Evaluation of Chronic Invertebrate Studies

J.L. Brill, The Procter & Gamble Co. / Environmental Stewardship and Sustainability; S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization; G. Carr, The Procter & Gamble Company; A.(. Beasley, Middle Tennessee State University

Environmental risk assessments of chemicals are broadly supported by chronic toxicity testing with *Daphnia magna*. Recent revisions to other aquatic toxicity test guidelines have demonstrated the importance of understanding both statistical power as well as sound evaluations of effect information using point estimate and hypothesis testing approaches. To address these needs a comprehensive database was developed based on internal P&G studies of a large number of diverse chemicals. The database covered all relevant aspects of *Daphnia magna* chronic toxicity tests based on full disclosure of study designs and findings from the selected studies. These highly detailed summarizations allowed the assessment of all relevant experimental variables were used to develop recommendations for appropriate study design and toxicity endpoints encouraging good experimental technique with minimal error. Based on statistical analyses of the summarized data we are able to demonstrate less variability in outcome measures thus increasing our confidence in the interpretation of chronic results. A by-product of this investigation was a deeper analysis of the strengths and weaknesses of point estimate (ECx) versus hypothesis test (NOEC) outcomes. We conclude that point estimates (EC10 estimates with 95% confidence intervals) remain preferable to hypothesis tests (NOECs) as long as point estimates lie above the lowest test concentration, confidence intervals are not overly large, and that other validity criteria for the test are met for adult survival and number of offspring produced.

MP229 First-time application of a modified luminescent bacteria test for the initial ecotoxicity assessment of beta-blockers after phototransformation

J. Menz, T. Rastogi, C. Leder, M. Schneider, K. Kuemmerer, Leuphana University Lüneburg / Faculty of Sustainability, Institute of Sustainable and Environmental Chemistry

In this study, the environmental toxicity of phototransformation products (PTPs) resulting from direct photolysis of the known aquatic micropollutants Atenolol (ATL), Metoprolol (MTL) and Propranolol (PPL) was preliminarily assessed. The standardized and commercially available luminescent bacteria test according to ISO 11348 (e.g., Microtox®, LUMIStox®) has been frequently applied for the analysis of complex reaction mixtures resulting from direct photolysis or advanced oxidation processes (AOPs). Unfortunately, its application can lead to an underestimation of the potential environmental risk, as the standard method is not suitable for the assessment of chronic effects. To overcome this limitation, a modified luminescent bacteria test (modified LBT) for the combined analysis of acute and chronic effects towards the luminescent bacterium *Vibrio fischeri* was developed. Phototransformation experiments with ATL, MTL and PPL were carried out at different concentrations (100 mg/L – 400 mg/L) using a 150 W medium-pressure Hg-lamp. Primary elimination of the parent compound and mineralization of carbon during UV irradiation was monitored by HPLC-UV and dissolved organic carbon (DOC) analysis, respectively. For the toxicity testing of PTPs, photolytic mixtures were collected within 256 min of irradiation and subsequently analyzed with the modified LBT at different dilutions. After 256 min of UV-treatment, ATL, MTL and PPL were completely primarily eliminated but not mineralized according to HPLC and DOC analysis, which indicated the formation of PTPs to a high extent. In the modified LBT, a distinct increase of acute and chronic toxicity because of direct photolysis was observed for all the three beta-blockers. In case of MTL and PPL, the toxicity steadily increased with the irradiation time. In contrast, PPL showed a toxification maximum already after 8 min of irradiation. For the first time, the modified LBT was successfully applied to the toxicity testing of PTPs, revealing a high toxic potential of the investigated PTPs compared to the parent compounds. Acknowledgements: This study was funded by the German Federal Ministry of Education and Research, funding code 03X0094C. Jakob Menz received a scholarship for part of this work from the Innovations-Inkubator Lüneburg (Teilmaßnahme 1.4 Graduate School).

MP230 Human low density lipoprotein as a substrate for in vitro steroidogenesis assays with fathead minnow ovary explants

K.E. Stevens, USEPA Mid-Continent Ecology Division, ORISE; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; J.E. Cavallin, USEPA, ORISE / National Health and Environmental Effects Research Laboratory; N. Garcia-Reyero, Mississippi State University / Institute for Genomics Biocomputing and Biotechnology; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory; A. Schroeder, D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; L.C. Wehmas, Oregon St. University / Mid-Continent Ecology Division, University Of Oregon

Gonad explant steroidogenesis assays are used as part of a multifaceted strategy to detect endocrine active chemicals capable of altering steroid hormone synthesis. An in vitro steroidogenesis assay used in our laboratory involves exposing fathead minnow (FHM) gonad explants to medium 199 supplemented with 25-hydroxycholesterol for 12 hours, and then measuring steroid production. Data from this assay have been used to support the development of computational models of steroidogenesis, and assess the impacts of a variety of chemicals on steroid synthesis. However, it is thought that the 25-hydroxycholesterol used in the assay may bypass steriodogenic acute regulatory protein (StAR), which is known to be an important, rate limiting step in the production of steroid hormones, as well as a target of environmental contaminants. Therefore, to develop a more physiologically realistic assay, experiments were undertaken to determine whether commercially-available human low density lipoprotein (LDL), could be used as a viable alternative substrate in the assay. Using standard, non-stimulated conditions, steroid production by ovary explants was not detectable after 12 h of incubation in medium supplemented with LDL. Adding human chorionic gonadotropin to the LDL containing medium failed to stimulate detectable steroid production. However, after supplementing with 8-bromo cyclic adenosine monophosphate (8 Br-cAMP), steroid production using LDL as a substrate was similar to that obtained using 25-hydroxycholesterol as a substrate (without 8 Br-cAMP). To further elucidate the impact of 8 Br-cAMP treatment on the FHM ovary tissue in culture, we examined in vitro expression of key steroidogenesis-related genes following 8 Br-cAMP treatment. The 8 Br-cAMP significantly increased the abundance of transcripts coding for cyp19a1a (aromatase) and decreased the abundance of transcripts coding for follicle stimulating hormone receptor. On-going work is aimed at evaluating the sensitivity of the modified in vitro steroidogenesis assay protocol (i.e., 8 Br-cAMP stimulated, LDL as substrate) for detecting the effects of steroidogenesis inhibiting compounds, compared to the conventional assay and utilizing the modified protocol to support further model development. The contents of this abstract neither constitute nor necessarily reflect USEPA Policy.

MP231 Increasing the usefulness of biomarkers in fish for environmental management by introducing limit values

N. Hanson, EnviroPlanning / Department of Plant and Environmental Sciences, University of Gothenburg / Plant and Environmental Sciences, EnviroPlanning; A. Larsson, L. Forlin, University of Gothenburg

Biomarkers are sub-organismal measurements that can show if an organism is exposed to or affected by chemicals. Biomarkers are also frequently considered as early warning signals for effects on the population level. The method is often used in fish to determine if a recipient is affected by chemical discharges. The results of the biomarker analyses can then be used for environmental management, for example to demand better waste treatment of an industry. A problem with biomarkers is that they, like so many other biological endpoints, tend to vary between sites and over time even in the absence of human impact. This means that there is a risk that undisturbed sites are considered as disturbed, and vice versa. These risks should be considered and dealt with, but are often ignored. To reduce the risk for errors, reference sites are needed. The reference sites should be chosen to be as similar as possible to the examined site, and they should be sampled close in time. Adding more reference sites means that there is more confidence in the conclusions, but it also means an increased cost. Since 1988, about 25 biomarkers (*Perca fluviatilis*) have been analyzed annually in perch at a reference site on the Swedish Baltic coast. With time, the program has expanded to include three reference sites. This gives valuable information about the normal variation of these 25 biomarkers. It has been suggested that this data could be used to develop upper and lower limits for biomarkers. These limits can then be used to determine if a site is impacted, without the need for reference sites. However, there are a number of problems with developing such limits. For example, there are time trends in a number of biomarkers, suggesting that "normal" changes over time. It is also possible that spatial differences are too large to allow reference sites to cover larger regions. During 2013, biomarker limits for perch were developed and tested on data from both impacted sites and reference sites. The use of limits was compared to traditional statistical methods with reference sites, including ANOVA and normal variation. Preliminary results show that the use of limit is not more likely than traditional methods to result in erroneous conclusions. This means that equally good, or better, guidance for environmental management can be achieved without the need for reference sites.

MP232 Incremental improvements to the trout S9 biotransformation assay

P.N. Fitzsimmons, USEPA / Mid-Continent Ecology Division; A.D. Hoffman, USEPA / ORD, NHEERL, Mid-Continent Ecology Division; J.W. Nichols, USEPA / Mid-Continent Ecology Division

In vitro substrate depletion methods have been used in conjunction with computational models to predict biotransformation impacts on chemical accumulation by fish. There is a consistent trend, however, toward overestimation of measured chemical residues resulting from controlled whole-animal exposures. One possible reason for this discrepancy is that in vitro methods underestimate in vivo rates of metabolic activity. The goal of this effort was to evaluate a well-known in vitro assay employing the trout liver S9 fraction, and determine whether and how assay performance can be improved. This work was performed using several polycyclic aromatic hydrocarbons (PAHs) as model substrates. In vitro intrinsic clearance was inversely related to substrate concentration, consistent with saturable (Michaelis-Menten) kinetics. However, the activity of the S9 system was found to decrease steadily over time. Combining these results, it is suggested that first-order clearance in assays performed over longer time periods (> 1 hr) may be an artifact of these competing influences. These factors may also explain the curvilinear nature of data reported by several authors. Acetone used as a spiking solvent had negative effects on PAH clearance

at concentrations greater than 0.5% (v/v), as did alamethicin (25 µg/mL), which is often added to support Phase II UGT activity. Decreasing the S9 protein content increased intrinsic clearance, normalized for protein content. This result could be explained in part, although not entirely, by changes in substrate bioavailability, as characterized by solid phase microextraction (SPME). Collectively, these results underscore the importance of conducting preliminary studies to optimize assay performance. Intrinsic clearance rates are maximized by decreasing S9 protein content, substrate concentration, and the amount of spiking solvent; however, these factors must be balanced by limitations on analytical sensitivity and the need for measurable activity. This abstract does not necessarily reflect USEPA policy.

MP233 Inter- and intra-species chemical sensitivity: A case study using dinitroanisole

A.J. Kennedy, US Army Engineer Research and Development Center, CEERD-EPR; P. Gong, Specpro; S.M. Brasfield, J. Laird, US Army Engineer Research and Development Center; C. Lounds, N. Barker, Badger Technical Services

Standard aquatic toxicity test species are employed to determine the potential for adverse impacts due to chemical manufacturing effluents. Such species include the fathead minnow *Pimephales promelas* and cladocerans (e.g., *Ceriodaphnia dubia*, *Daphnia pulex*). Test organisms can be obtained from various vendors and sources (e.g., laboratory cultures, field-collection). The current study investigated how selection of different species and their sources may impact toxicity test results, using the insensitive munition constituent dinitroanisole (DNAN). The relative sensitivity between species was determined using standard chronic toxicity test methods; a three brood test was adopted for *D. pulex* since no standardized chronic toxicity method was available. Five different genotypes of *D. pulex* were obtained from various laboratory and field-collected sources, as confirmed using 20 microsatellite markers. Chemical concentrations that induced median lethal effects (LC50) and 25% inhibition in sublethal endpoints (IC25) were compared between the three species and within five *D. pulex* genotypes. Chronic LC50 values for *P. promelas* and *C. dubia* were 10.0 (8.8 – 11.2) and >24.2 mg/L, respectively, while the mean *D. pulex* LC50 value was 14.3 mg/L, ranging from 7.9 to 21.6 mg/L depending on genotype. Thus, fish and *D. pulex* survival endpoints were significantly more sensitive than *C. dubia* to DNAN. The sublethal IC25 values for *P. promelas* growth and *C. dubia* reproduction were 10.4 (8.2 – 14.3) and 8.2 (7.4 – 8.7) mg/L, respectively, while the mean *D. pulex* IC25 was 1.6 mg/L, ranging from 0.4 to 3.1 mg/L between genotypes. Thus, the cladoceran reproduction endpoint was generally more sensitive to DNAN than the fish growth. It is important to consider that field-collected organisms may be stressed in laboratory conditions. However, this study demonstrates that the selection of test species and the source of that test species (genotype) can have significant implications on results, which could impact effluent discharge limits. This reinforces the need for the multiple species approach and prior discussion and consensus on suitable testing methods. Further work involves confirming intra-species trends and determining a mechanistic explanation for differences in chemical sensitivity.

MP234 No point estimates: analysis of distributions from concentration-response curves for understanding toxicity and in making risk management decisions

A.F. Johns, Western Washington University / Institute of Environmental Toxicology, Huxley College of the Environment; W.G. Landis, Western Washington University / Institute of Environmental Toxicology

Recently there has been an intense discussion regarding the proper analysis tools for the description of the exposure-response relationship for environmental risk assessment. We have developed an alternative analysis that relies on curve fitting and generates a distribution around a selected ECx value where x is the boundary of the unacceptable effect size. We have explored a variety of exposure-response datasets from two laboratories and with a diverse group of chemicals. The DRC package in the R programming environment was used to calculate the regression and the 95 percent confidence intervals. At concentrations at and surrounding what would be estimated as a 20 percent effect a distribution was derived to capture the likelihood of effects. A triangle distribution was created via Monte Carlo with the mode the point corresponding the estimated exposure-response and the upper and lower limits corresponding to the range of the confidence interval. Analysis of a variety of exposure-response curves illustrated the importance of the slope of the curve and the breadth of the confidence interval. For example, the concentrations of malathion corresponding to an EC_{20} value for immobilization of *Daphnia magna* was bounded by effects levels from 9 to 38 percent. The description of the EC value becomes a distribution bounded by the upper and lower bounds of the effect axis, the upper and lower bounds along the concentration axis and the likelihood of each exposure and effect combination. This kind of probabilistic information is not available from point estimations from conventional hypothesis testing or reliance on the determination of a point EC_x. We present several examples to demonstrate the critical nature of having the exposure-response curve, its confidence bounds and the exposure-effects distribution in estimating risk.

MP235 Optimization of Culture and Test Procedures for Two Species of Copepods

E. Ussery, M.J. Foster, S. Garcia, University of North Texas; H. Krueger, Wildlife International; D.B. Huggett, University of North Texas / Department of Biological Sciences

Copepods comprise an ecologically important role in freshwater and marine ecosystems, which is why they are often considered an important ecotoxicological model organism. The International Organization for Standardization's (ISO) 14669 protocol is the only guideline for the determination of acute toxicity in three European marine copepod species: *Acartia tonsa*, *Tisbe battagliai*, and *Nitrocra spinipes*. The goal of this project was to assess the feasibility of establishing and maintaining cultures of *Acartia tonsa* and *Tisbe biminiensi,* as well as to refine current culturing and egg separation methods. Initial culture methodology proved difficult for consistent production of eggs and collection of nauplii. The development of an airlift system for the separation of eggs from nauplii and adults, based on size, successfully increased the availability of eggs, nauplii and adults. The addition of *Chaetomorpha sp.* to the culture system greatly improved copepod survivability and egg production. The sensitivity and relative conditions of the two copepod species was assessed by running a series of 48h acute toxicity tests with the reference toxicant 3,5-dichlorophenol, as well as several other toxicants. The acute 48h 3,5-dichlorophenol EC50s of *A. tonsa and T. biminiensis* were 0.13 mg L^{-1} and 0.04 mg L^{-1}, respectively.

MP236 Proposal for a Test Design for a Dietary Bioconcentration Study in Fish According to OECD Guideline 305: Points to Consider

R. Burri, Harlan Laboratories Ltd. / Animal Metabolism; M. Gassen, Harlan Laboratories / Metabolism & Environmental Fate

The revised OECD guideline 305 was adopted on October 2, 2012. The guideline contains an additional section dealing with the dietary bioconcentration study in fish. This poster presents a possible design, describing a minimal number of samples to be taken and measurements that have to be performed. Before starting with a test, it is essential to know test item parameters such as solubility in water and $logP_{OW}$. Then pre-tests dealing with fish toxicity, stability and homogeneity of the test item in the diet as well as leaching behavior of the test item into water have to be considered. The homogeneous mixing of the test item into the diet is essential. We present data from different mixing experiments using corn oil as carrier. Furthermore, according to guideline, the data have to be lipid normalized and it is important to measure reliably lipid contents in fish and fish diet. We present and discuss lipid content measurements with two different techniques. The study can be done with either radiolabelled or non-labelled test item. We compare advantages and disadvantages of the two options. Lipid normalized and growth corrected biomagnification factors (BMF) will be obtained by the test procedure. Presently, the relation of BMF values to bioconcentration factors (BCF) obtained by the aqueous exposure test is not clear and should be clarified in the future. Alternatively, BMF values could be used as independent factors for, e.g., PBT assessments.

MP237 Revisions to USEPA's Approach for Modifying Aquatic Life National WQ Criteria to Protect Site-Specific Assemblages of Species from Toxic Pollutants

C.G. Delos, USEPA / Mail Code 4304; T.K. Linton, C. Voros, Great Lakes Environmental Center

The Recalculation Procedure is used to edit the taxonomic composition of the toxicity dataset used to construct the Species Sensitivity Distribution (SSD) upon which a site-specific aquatic life water quality criterion is based, in order to better match the assemblage that resides at the site. The underlying premise of the Recalculation Procedure continues to be that taxonomy has value in predicting sensitivity to a particular toxicant, such that a site-specific SSD can be adjusted to reflect the taxonomy of species that reside at

a site. Because the national criterion already incorporates all reliable toxicity data available at the time the criterion was published, the core of the procedure is the Deletion Process, which involves removing certain tested species from the SSD. As with previous versions of the procedure, the newly recommended procedure allows deletion of nonresident tested species if and only if they are not appropriate surrogates of resident untested species – based on taxonomy. That is, because some tested species might be needed to represent untested species that occur at the site, the deletion procedure does not provide for simplistic deletion of all species that do not occur at the site. Rather the concept is to consider which tested species are most closely related to those occurring at the site, and delete those for which another tested species would better represent the species occurring at the site. This paper compares the behavior of new revised procedure with the old, and discusses issues of relating sensitivity to taxonomy.

MP238 Revisiting Dissolved Oxygen Thresholds of the Protection of Freshwater Aquatic Life

G.N. Saari, Baylor University / Environmental Science; B.W. Brooks, Baylor University / Department of Environmental Science

Freshwater systems are undergoing extraordinary change due to heavy exploitation of their ecosystem services. Water quantity and quality in streams and rivers in arid to semi-arid regions of the United States has become particularly important because of their increased reliance due to water shortages as a result of drought and human consumption. Growing populations are creating a need for increased agricultural practices that can potentially lead to increased nutrient inputs to water bodies. Instream flows of many surface waters in Texas are dominated and even dependent on discharges from wastewater treatment plants. Altogether these point and nonpoint sources of nutrients can result in depressed dissolved oxygen (DO) creating stress on water quality. Under the mandates of the Clean Water Act, states have set water quality standards and criteria to protect water quality for its designated uses. Dissolved oxygen is a particular important stressor to water quality because of the increased frequency of hypoxia worldwide and its harmful impacts on aquatic organisms. Hypoxia and low dissolved oxygen levels has received much study along marine and coastal systems but relatively limited investigation in inland waters. Depressed DO in inland waters of Texas consistently results in listing of impaired systems on the Texas 303(d) list due to noncompliance with DO water quality criteria. Low DO can adversely affect fish activity, survival, growth rates, and reproduction. An extensive literature search has been completed to compile and review experimental data used to create the US Environmental Protection Agency (EPA) Ambient Water Quality Criteria for Dissolved Oxygen (1986). Aquatic hazard assessments were then performed to examine DO thresholds for cold and warm water fish and invertebrates relative to existing regulatory criteria. Inconsistent experimental methods and disproportionate selection of aquatic organisms from past studies used to derive DO water quality criteria introduce uncertainties during surface water quality assessments and may limit desired ecosystem protection goals.

MP239 Sub-Lethal Ceriodaphnia Toxicity As a Result of Ion Imbalance

M.S. Bowersox, Tetra Tech, Inc. / Laboratory Department; J. Gilliam, P. Siwinski, R. Shaw, J.M. Diamond, Tetra Tech, Inc.

An industrial effluent was observed to cause chronic sub-lethal toxicity to *Ceriodaphnia dubia*. Using EPA's Toxicity Identification Evaluation procedures, multiple lines of evidence were established that indicated an ion imbalance as the cause of the observed sub-lethal toxicity. Effluent hardness was observed to be in excess of 500 mg/L as $CaCO_3$ and typically included a 3 to 1 ratio of magnesium to calcium. Through the development of a simulated effluent, which resulted in similar toxic response as the site effluent, it was concluded that the concentration of cation/anions in the effluent may be the cause of reduced *Cerodaphnia* reproduction. Simulated effluent had similar concentrations of calcium and magnesium and resulted in a significant effect on reproduction. By rebalancing the ratio of magnesium to calcium or adding total organic carbon, the sub-lethal toxicity is eliminated. When the effluent was adjusted to a ratio of approximately 1:1 Ca:Mg, sub-lethal toxicity is reduced. The observed reduction of sub-lethal toxicity with the addition of organic carbon is likely due to the ability of negatively charged organic carbon to complex excess magnesium resulting in a more optimal ion balance for *Ceriodaphnia*.

MP240 Testing the Toxicity Toolbox: A Thorough Assessment of Water Quality in the Sacramento River at Hood, CA

C. Callinan, K.M. Jeffries, L. Deanovic, M. Stillway, B.M. DeCourten, R.E. Connon, S.J. Teh, University of California Davis / UC Davis School of Veterinary Medicine, Anatomy, Physiology and Cell Biology

Laboratory toxicity testing is a critical component of aquatic health monitoring across the US and world-wide. However, it is unknown how well such test methods characterize the overall health of aquatic systems over space and time. Standard toxicity testing methods assess the lethal effects of a water body by measuring mortality in test species exposed to individual grab samples of water. The purpose of this study was two-fold, 1) to compare the sensitivity among aquatic monitoring tools and 2) to examine the presence and effects of chemicals in the Sacramento River downstream of the city of Sacramento at Hood (CA). Standard toxicity tests were employed in concert with ex-situ monitoring, that included assessing molecular and biochemical responses in an EPA certified fish species, *Pimephales promelas*. Ex-situ exposures contained replicates of 145-300 free-swimming fish and 5 buoyantly caged fish across four 60 liter flow-through tanks, and were compared with laboratory-based control tanks. Two consecutive 14d ex-situ exposures occurred over two separate 28d monitoring events. At the termination of each 14d ex-situ exposure, surviving *P. promelas* were sampled for enzyme and molecular analyses. Significant mortality in free swimming *P. promelas* occurred during the first monitoring event in the ex-situ exposure, but not in the traditional toxicity tests, and there was no significant toxicity during the second monitoring event. Tissue and molecular analyses suggest that pathogens associated with exposure to river sediment may have affected survival during the first monitoring event. Additionally, preliminary qPCR results suggest that fish were exposed to compounds that affected expression of genes involved in endocrine-related processes. Chemical analyses of water samples combined with assessments of molecular and biochemical responses will help to identify the causes of toxicity at the site, and may uncover additional stressors undetected via traditional toxicity testing methods. Results from this study provide a basis of comparison among various bioanalytical tools and will enable managers to select optimal monitoring methods for important water bodies and ecosystems.

MP241 The Atlantic and Gulf Killifish as Key Models for Determining the Effects of Environmental Stressors on Immune Function in Estuarine Fish

R. Silva, Clemson University / Biological Sciences; M.L. Rodgers, Clemson University / Department of Biological Sciences; C.D. Rice, Clemson University / Biological Sciences

Atlantic and Gulf killifish, *Fundulus heteroclitus* and *Fundulus grandis*, respectively, occupy a key ecological position in high marsh habitats along the entire east coast of North America and Gulf of Mexico of USA. Both species of killifish have a small home range fidelity, thus their entire life cycle and extending generations are confined to local physical and environmental factors. These two killifish are very popular models in developmental biology, environmental physiology, and environmental toxicology. High marsh habitats are subject to highly fluctuating environmental factors lending estuarine killifish as excellent models to determine the effects of estuarine stressors, including contaminants, on immune functions that may impact disease susceptibility. A cell-based method was used to generate monoclonal antibodies (mAbs) against IgM and eosinophilic granular cells (EGC) of *F. grandis* by immunizing mice with lymphoid cells. *F. grandis* were immunized with *V. anguillarium* and bled for immune plasma which was then used as a source of antigen-specific IgM. Resulting hybridomas were screened for antibodies specific to both cell-surface (B-cells) and plasma IgM. Hybridomas were also screened for mAbs specific only to EGC. This all-at-once approach to mAb production resulted in mAb 2C11 for EGCs and mAbs D58 & IC9 for IgM heavy and light chains, respectively. This lab previously generated mAb M24-2 against Atlantic killifish lysozyme, which seems to be a pan-fish lysozyme-specific antibody. Together, these mAbs allow for detailed studies on the immunobiology of killfish. mAbs D58 and IC9 allow for co-localization of splenic germinal centers and CYP1A (with mAb C10-7), thus allowing quantification of B-cell numbers and distribution, as well as antibody responses. mAbs 2C11 and M24-2 allow for quantification of EGC cells and phagocytes. Moreover, these mAbs are highly cross-reactive between killifish species, thus extending the use of killifish as a model species in environmental immunotoxicology within estuaries of both the Atlantic and northern Gulf of Mexico. (NIH R15-ES016905-01; R15-ES010556-01)

MP242 Toxicity of ambient waters for three aquatic organisms and characterization of toxicants in highly populated areas in Japan

J. Morita, Y. Yasuda, I. Tamura, Graduate School of Integrated Arts & Sciences, University of Tokushima; N. Tatarazako, National Institute for Environmental Studies; H. Yamamoto, Institute of Socio, Arts, and Sciences, Graduate school of the University of Tokusima

Various chemical substances are used in our industrial, agricultural, and daily activities, and are continuously discharged into the aquatic environment. Since the mixture effects of these compounds have become growing concerns, direct measurement of adverse effects of effluents or ambient waters on aquatic organisms and toxicity identification evaluation has been frequently used in the US and other countries. We conducted short term chronic toxicity tests using algae, daphnia and algae referred to theUSEPA's whole effluent toxicity (WET) test methods to evaluate over 30 water samples in our previous studies. Since no specific toxicant was identified in the investigation, we collected four samples at rivers in highly populated areas including Tokyo metropolitan area and Osaka/Kyoto. The potential toxicants were characterized using the procedure of Phase I of Toxicity Identification Evaluation such as pre-treatments using solid phase extraction cartridges, and a chelating agent. As a result, cations such as metal ions were found to be the major contributors to the growth inhibition of algae for the selected ambient waters. For daphnia, both cations such as metals and organic compounds are found to be major contributors to the inhibition of the reproduction or survival, while organic compounds are major contributors to the inhibition of hatching and survival of fish

MP243 Use of EPA's Revised Deletion Process to Modify National Recommended Criteria to Protect Site-Specific Assemblages of Freshwater Species from Ammonia

T.K. Linton, C. Voros, Great Lakes Environmental Center; C.G. Delos, USEPA / Mail Code 4304; L.F. Huff, USEPA / Office of Water; K. Gallagher, USEPA / Office of the Science Advisor, Risk Assessment Forum

The US Environmental Protection Agency (EPA) allows the use of its Recalculation Procedure to edit the taxonomic composition of the toxicity dataset used to construct a species sensitivity distribution (SSD) upon which a site-specific aquatic life water quality criterion can be based, in order to better represent the assemblage that resides at the site. The underlying premise of the Recalculation Procedure is that taxonomy has value in predicting sensitivity to a particular toxicant, such that a site-specific SSD can be adjusted to reflect the taxonomy of species that reside at a given site. The core of the Recalculation Procedure is the Deletion Process, which involves removing certain tested species from the SSD based upon taxonomic relatedness, and where nonresident tested species are deleted if and only if they are not appropriate surrogates of resident untested species. The concept is to consider which tested species are most closely related to those occurring at the site, and delete those for which other tested species would better represent the species occurring at the site. Because EPA's 2013 ammonia criteria are applicable nationally, taking into account the latest toxicity information for freshwater species, including sensitive unionid mussels and non-pulmonate snails, application of the new Deletion Process of the Recalculation Procedure is an available option for states, tribes, and territories to develop site-specific criteria based on the distribution of such species. This poster focuses on application of the revised Deletion Process for recalculation of the ammonia criteria at several sites across the conterminous United States with taxonomic assemblages of interest to both EPA and the regulated community. The resultant changes in the magnitude of the recalculated criteria will be provided, and the overall utility of the revised Deletion Process for modifying National Recommended Aquatic Life Water Quality Criteria to protect site-specific assemblages of freshwater species exposed to ammonia will be discussed.

MP244 Developing a multi-compartment perfluorooctane sulfonate (PFOS) uptake and depurination model for fish

A.D. Olson; M.A. Funkhouser, Texas Tech University / TIEHH; C.J. Salice, Texas Tech University / Environmental Toxicology

Perfluorooctane sulfonate (PFOS) was used in Scotchgard products and fire-fighting foams until 2002 and has been measured in aquatic systems close to where the chemical was used. Perfluorooctane sulfonate is a persistent perfluoroalkylated acid that has been found to accumulate and biomagnify throughout food webs. Aquatic organisms are of particular interest with regard to the ecotoxicology and fate of PFOS because these organisms have two potential pathways for PFOS exposure – via diet and water. Fish are especially important in aquatic systems because many represent higher trophic levels within the aquatic food web and serve as a potential exposure pathway to humans. However, considerable uncertainties remain regarding the extent that PFOS accumulates and depurates in different fish species. This project presents results from a preliminary three-compartment bioaccumulation model using a kinetic bioaccumulation factor for uptake and depuration over time of PFOS in fish. The three compartments the model uses are muscle tissue, blood, and liver. The uptake and depuration kinetics data used in the model were obtained from peer-reviewed sources as well as studies we have recently conducted on several different fish species. The ultimate objective is to develop a refined fish bioaccumulation model that can be used with field measurements of PFOS concentrations in dietary items and water to predict PFOS concentration in fish. The current model predicts a hyperbolic accumulation of PFOS over time that matches patterns observed in uptake studies. According to the model, PFOS tissue concentrations approach an asymptote after approximately 12 days, at a concentration 10,000 times greater than aqueous exposure levels, assuming constant PFOS exposure. The model indicates that accumulation in the blood and liver is approximately 5 times greater than accumulation in the carcass. The model also suggests that exposure to contaminated water is of greater importance to accumulation than from contaminated diet. The model predictions are consistent with data in the literature. Future efforts will be aimed at refining the bioaccumulation model with several lab and field-validation exercises.

Pharmaceuticals

MP245 A kinetic modeling study of sources of variability in measurements of absorption efficiency of chemicals by fish

R. Xiao, Stockholm University / Department of applied environmental science; J.A. Arnot, ARC Arnot Research & Consulting / University of Toronto Scarborough / Department of Physical and Environmental Sciences; M.S. McLachlan, Stockholm University; M. MacLeod, ITM – Stockhom University / Department of applied environmental science

The dietary absorption efficiency (E_D) of chemicals is a measure of the fraction of chemical in food that is taken up via the gastro-intestinal tract (GIT). An accurate and consisent E_D measurement not only helps one understand mechanisms of the uptake process in the GIT, but also serves as a basis to develop models of the biomagnification factor (BMF) and bioaccumulation factor (BAF) of chemicals. There have been many studies investigating E_D for hydrophobic chemicals by fish, but the reported values vary significantly. For example, the reported E_D for 2,2',5,5'-tetrachlorobiphenyl (PCB 52) from 19 studies ranged from 0.38 to 0.98. Much of the variation in reported E_D can be attributed to variability in individual biological response and different experimental conditions, such as dosing strategies and exposure duration. The objective of this study is to develop and apply a kinetic model in fugacity format to describe chemical transport between the GIT and fish tissue, and ultimately describe time-dependent E_D. Based on the decrease in fugacity capacity of chemicals along the digestive tract, the GIT is divided into two compartments, GIT1 (from buccal cavity to pyloric ceca) and GIT2 (from pyloric ceca to anus). The model accurately described the observed concentration time course for ^{14}C labelled PCB 52 in feces and fish tissue, which was obtained from the Nichols et al. study (2004). We hypothesize that the model can reduce variability in reported E_D values in two ways: 1) by allowing test data reported in the literature to be benchmarked against E_D values for well-studied chemicals, and 2) by assessing the potential for depuration from fish back to GIT to cause variability in E_D values reported from tests with different exposure durations.

MP246 Acute to Chronic Ratios of Pharmaceuticals to Support Environmental Risk Assessments

J. Small, Merck & Co., Inc.; D.J. Caldwell, Johnson & Johnson; L.A. Constantine, Pfizer, Inc. / Pharmacokinetics, Dynamics and Metabolism; T. Davidson, Bristol-Myers Squibb / Centre for Materials and Coastal Research; V. D'Aco, Quantum Management Group, Inc.; D.G. Dolan, Amgen; R.E. Hannah, Temple University / CEHS – RS 1105; G.H. Panter, AstraZeneca / Brixham Environmental Lab; N. Parke, Eli Lilly and Company; K. Kappler, Johnson & Johnson / Environmental Engineer; J. Ryan, GlaxoSmithKline / Corporate Environment, Health, Safety & Sustainability; J. Straub, F.Hoffmann-La Roche Ltd

/ Group Safety & Environment Protection; J.G. Tell, Merck & Company, Inc. / Global Safety & the Environment; P.W. Wilson, Sanofi US, Inc. / HSE

A multi-pharma company team was formed to evaluate the relationship between acute EC_{50} and chronic NOEC ratios for a broad range of active pharmaceutical ingredients (APIs) in various therapeutic areas and with diverse modes of action. Additionally, the team attempted to characterize API toxicity using both mass and molar concentration in comparison to the *n*-octanol/water partition coefficient. The ten member companies provided internal, blinded, acute and chronic daphnia, fish and algae data and log Dow data for approximately 113 compounds in order to compare these various endpoints. All compounds were classified according to the American Hospital Formulary Service (AHFS) classification scheme at the primary level. Compounds were grouped into like classes and trends of toxicity and acute : chronic ratios were assessed. In the majority of cases, acute : chronic ratios remained below 100, consistent with current tiered regulatory schemes in the United States and in Europe. Additionally, most chronic NOEC values fell above the US and EU regulatory Action Limits for all three species and all compounds that fell below the more conservative EU Action Limit of 0.01 ppb were hormones. Moreover, correlations were found between increasing toxicity and increasing log Dow.

MP247 Bioconcentration of Pharmaceuticals and Personal Care Products in liver of Zebrafish (Danio Rerio)

F. Chen, National University of Singapore / Graduate School of Integrated Sciences and Engineering; Z. Gong, National University of Singapore / Department of Biological Sciences; B.C. Kelly, National University of Singapore

The occurrence of pharmaceuticals and personal care products (PPCPs) in the environmental has received increasing interest in recent years. The bioconcentration behavior of PPCPs in aquatic organisms is not well known. This study involves laboratory investigations to assess the bioconcentration behavior of PPCPs in zebrafish (*Danio rerio)*. We conducted a continuous flow-through exposure experiment to assess the bioconcentration kinetics of several PPCPs in liver of adult female zebrafish. Bioconcentration experiments involved 6 days of aqueous exposure, followed by an 7-day depuration phase, at high and low exposure concentrations. For exposure experiment, fish were collected at six time-points during uptake phase and five time-points during the depuration phase. Liver were collected from individual fish and pooled into composite samples (five fish per composite). The liver samples were extracted and cleaned up by sonication and solid phase extraction (SPE). Determination of test compound concentrations was conducted by analysis using liquid chromatography tandem mass spectrometry (LC-MS/MS). Observed bioconcentration factors (BCFs) varied among test compounds and ranged from approximately 0 to 116 for the various PPCPs investigated. The results are further evaluated to assess the role of key biological constituents (proteins, phospholipids) and influence of octanol-water and protein-water distribution coefficients (Dow, Dpw) on bioaccumulation potential of PPCPs in aquatic organisms.

MP248 Common Effects of Ibuprofen Exposure on Underyearling Rainbow Trout and Caenorhabditis elegans

L.L. Brown, Environment Canada / Molecular Biology and Biochemistry / Environmental Toxicogenomics, University of Simon Fraser / Molecular Biology and Biochemistry; R. Lo, Simon Fraser University; F. Brinkman, Simon Fraser University / Molecular Biology and Biochemistry

Ibuprofen is a commonly used nonsteroidal anti-inflammatory drug (NSAID) and one of the most abundant pharmaeuticals found in sewage and surface water (detected at levels up to 2.7 ug/L) worldwide. Recent toxicology studies have shown that ibuprofen exposure negatively impacts the spawning behaviour of medaka and egg hatching in *Daphnia magna*. Ibuprofen is known to inhibit prostanglandin synthesis by targeting cyclooxygenase enzymes; however, the mechanisms of action are not well understood. Underyearling rainbow trout were exposed to ibuprofen for 96 hours (plus a 24 hr recovery) at environmentally relevant (0.5 ug/L, 1.0 ug/L) and higher concentrations (30 ug/L, 1000 ug/L). RNA-Seq was conducted on fish liver mRNA and differential gene expression was examined at the gene and pathway level. Both expected and novel gene expression changes were observed in rainbow trout in response to ibuprofen – findings subsequently validated by qPCR. As expected, there were statistically significant changes in prostaglandin pathway-associated genes. More surprisingly, vitellogenin gene expression was down-regulated in male fish. This analysis of rainbow trout RNA-Seq data highlighted the benefits and challenges of using RNA-Seq in the context of ecotoxicogenomics. This work also illustrated the benefit of using genetic markers to identify sex-specific responses to toxicants. In addition to fish bioassays, *Caenorhabditis elegans* worms were exposed to ibuprofen at environmentally relevant concentrations (0.002 ug/L to 2 ug/L) for 3 days. This experiment was repeated more than 6 times and had consistently showed decreased egg-laying within 24 hrs of exposure at concentrations as low as 0.02 ug/L. These observations suggest that ibuprofen may have negative impacts on egg production and further experimentation with both rainbow trout and *C. elegans* is needed. This also highlights the potential benefits of studying the effect of toxicants not just on large animals such as fish, but also on more microscopic organisms, as part of efforts to better assess toxicity on an entire ecosystem.

MP249 Diphenhydramine bioaccumulation by Mugil cephalus in urban Texas estuaries

S.P. Haddad, Baylor University / Environmental Science; B. Du, Baylor University / The Institute of Ecological, Earth, Environmental Sciences / Department of Environmental Science, Center for Reservoir and Aquatic Systems Research; W.C. Scott, Baylor University / Department of Environmental Science; C. Breed, Baylor University / Department of Environmental Science, Center for Spatial Research; M. Kelly, L. Broach, TCEQ; K. Chambliss, Baylor University; B.W. Brooks, Baylor University / Department of Environmental Science

Though bioaccumulation of pharmaceuticals has received attention in inland waters, studies of pharmaceutical bioaccumulation in estuarine systems are limited. Further, an understanding of pharmaceutical bioaccumulation across size classes of organisms displaying ontogenetic feeding shifts is lacking. The flathead (stripped) mullet *Mugil cephalus* is a versatile species with a wide range of occurrences in tropical, subtropical, and temporal coastal waters in all major oceans. *M. cephalus* is strongly euryhaline, which lends to the large range occupied by this species and the life history strategy employed. During the larval phase of life, *M. cephalus* are primarily planktonic feeders. The juvenile fish undergoes a shift in diet, first feeding on small invertebrates in the water column, and then transitioning to benthic organisms. Eventually, as the mullet increases in size and reaches adulthood they will feed more frequently on detritus and inorganic matter (sand). Smaller individuals selectively browse while the larger individuals are opportunistically feeding on detritus in the benthos. Our primary objective was to identify whether diphenhydramine bioaccumulates in *M. cephalus* residing in several Texas estuaries. Our secondary objective was to determine diphenhydramine BAF values and identify if trophic position influenced such observations in mullet. We determined trophic position using isotope ratio mass spectrometry with $\delta^{15}N$, which accumulates up a food chain at ~3.3% per trophic level. Preliminary results suggest that as *M. cephalus* increases in size there is a trophic position shift, where larger individuals have a higher $\delta^{15}N$ than smaller individuals and thus larger individuals occupy a higher tropic position. Further, preliminary data also suggests that the size of the individual may affect the uptake and accumulation of diphenhydramine in urban Texas estuaries.

MP250 Early-Life Stage Toxicity of Fathead Minnows, Pimephales promelas, to the Synthetic Progestin Levonorgestrel

M.D. Overturf, Department of Biological Sciences; D.B. Huggett, University of North Texas / Department of Biological Sciences

Pharmaceuticals are routinely detected in the environment; and several of these compounds have been extensively researched due to their potential impacts to the endocrine system of aquatic organisms. Synthetic progestins have only recently been investigated and shown to cause negative reproductive consequences in fish at ng/L exposure concentrations. Our first study utilized the OECD 210 Early Life Stage (ELS) study to investigate the impacts of levonorgestrel (LNG), a synthetic progestin, on fathead minnow (FHM) survival and growth. After 28 days post-hatch (dph), survival of larval FHM was impacted at 462 ng/L, while growth was significantly reduced at 86.9 ng/L. Further analysis was conducted by measuring specific endocrine related mRNA transcript profiles in FHM larvae following the 28 d ELS exposure to LNG. Transcripts of 3β-HSD, 20β-HSD, and FSH were significantly down-regulated following 28 d exposure to both 16.3 and 86.9 ng/L LNG. Also, CYP19a expression was significantly down-regulated at 86.9 and 2392 ng/L LNG. Subsequently, a second study examined time periods that may be most sensitive (e.g., windows of sensitivity) for FHM larvae exposed to LNG. Larvae were exposed to a single concentration of LNG (i.e., $LOEC_{growth}$ of 86.2 ng/L as determined in the 28 d ELS study)

for different time periods starting with fertilized egg through 28 dph. Growth and mRNA expression of the four differentially expressed transcripts from the first study were measured. Regardless of the duration of exposure, LNG significantly decreased growth in the FHM larvae at day 28. For both 20β-HSD and CYP19a, mRNA expression was decreased following exposure to LNG; however, these transcripts returned to baseline levels after removal of LNG. 3β-HSD and FSH showed similar trends after exposure to LNG with 7-14 d and 14-28 d exposures exhibiting a decrease in expression; however, FSH expression returned to baseline once removed for LNG exposure. Based on these data, 3β-HSD was the only transcript to remain down regulated after LNG exposure. Together these data suggest LNG can negatively impact FHM larval survival and growth, with significant alterations in endocrine related responses. Future research is needed to understand what impacts these changes have on adult stages of development.

MP251 Effects of Progesterone and Norethindrone on Female Fathead Minnow (Pimephales promelas) Steroidogenesis

L.H. Petersen, University of North Texas / Institute of Applied Science; D. Hala, D. Carty, M. Cantu, University of North Texas; D. Martinovic, University of St. Thomas; D.B. Huggett, University of North Texas / Department of Biological Sciences

The widespread use of oral contraceptives contributes to the near ubiquitous presence of synthetic progestins in the aquatic environment. Currently, there is limited information on the potential endocrine disrupting effects of synthetic progestins in the aquatic environment. The objective of the present study was to compare the effects of a synthetic (norethindrone or NET, 100 ng/L) and endogenous progestin (progesterone or P4, 100 ng/L) on female fathead minnow (*Pimephales promelas*) steroidogenesis. In order to test for effects, steroid hormone productions were quantified using LC-MSMS with steroidogenic enzyme and receptor gene expressions quantified in brain and ovary tissue using q-PCR. From our studies it was evident that the two progestins (NET and P4) differentially modulated steroid hormone productions. Analyses revealed progesterone to act as substrate for pregnenolone and 17α-hydroxyprogesterone productions. In ovaries, HSD11β and CYP19 were up-regulated following NET exposure, whereas both genes were down regulated following P4 exposure. In contrast, HSD3β gene expression was reduced in NET exposure and up-regulated in P4 exposure. NET exposure further caused a statistically significant down-regulation of membrane PR-β and ER-β expressions in ovaries, whereas P4 exposure only decreased membrane PR-α and PR-β isoforms. In the brain, both NET and P4 down-regulated (although not statistically significantly) LH levels in exposed fish, but neither progestin influenced FSH expression. Overall this study showed differential modulations of steroidogenesis under synthetic and natural progestin exposures.

MP252 Effects of two progestins, norethindrone and levonorgestrel, on reproduction in a marine fish, Tautogolabrus adspersus

D.B. Horowitz, USEPA, ORD, NHEERL / Population Ecology Divison; G. Zaroogian, USEPA, ORD, NHEERL, AED / PEB Branch; B. Rashleigh, USEPA / Research Ecologist; B. Riffle, USEPA, ORD, NHEERL, Toxicology Assessment Divison; S. Laws, USEPA, ORD, NHEERL, Toxicology Assessment Division; L.J. Mills, USEPA, ORD, NHEERL, AED / Population Ecology Division

Endocrine-active pharmaceuticals that enter the aquatic environment through sewage effluent may have unintended impacts on reproduction in fish, which in turn may affect the sustainability of exposed populations. Laboratory experiments were conducted with the marine fish cunner (*Tautogolabrus adspersus*) to evaluate whether norethindrone (NOR) and levonorgestrel (LNG) affected reproduction in spawning adults. Both progestins are used in human contraceptive formulations and have been detected in low (ng/L) concentrations in aquatic environments. Synthetic progestins in aquatic environments are of special concern because some fish use natural progesterones as pheromones to coordinate reproduction, and evidence suggests progestins may be selectively taken up through the gills in some species. Reproductive endpoints of egg production, viability and fertility were assessed daily in spawning cunner treated with NOR or LNG (nominal concentrations of 0, 0.075 or 0.75 mg/kg) by oral gavage on days 0, 4, 8, 12 and 16 of the experiment. All fish were sacrificed on day 17 and gonadosomatic index (GSI) was determined. In NOR-treated fish, egg production per gram female was significantly reduced relative to controls at both concentrations, while egg fertility and viability was notably decreased, although not significantly, only in the 0.75 mg/kg treatment. GSI was significantly reduced in both males and females from the 0.75 mg/kg treatment. Female mortality in this treatment group was more than twice that in controls, indicating an increase in male aggression. In LNG-treated cunner, no significant effect was seen on egg production, fertility, viability, or GSI compared to control fish. Results indicate some progestins can impact fish reproduction, even in short-term exposures. Research is planned to determine if these fish selectively take up progestins from the aquatic environment. This abstract does not reflect USEPA policy.

MP253 Effects on steroidogenesis in female fathead minnows (Pimephales promelas) via sertraline exposure utilizing transcriptional analysis

D. Carty, H. Mohamed, University of North Texas; M. Foster, S. Garcia, K. Lamichhane, D. Hala, L. Petersen, D. Huggett, University of North Texas / Department of Biology

Sertraline is a selective serotonin reuptake inhibitor (SSRI) that is widely used for the treatment of depression. Due to the abundant human therapeutic use of sertraline, it has been detected in municipal wastewater effluents suggesting that aquatic organisms may be exposed to low levels. The purpose of this study was to evaluate the reproductive and/or steroidogenic effects of sertraline on female fathead minnows (FHM), *Pimephales promelas.* Fathead minnows were exposed to 0, 3 or 10μg/L of sertraline for 7 days with the brain and gonad removed at exposure termination. Fathead minnow brain and gonads were analyzed via qRT-PCR for relative expression levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), 11β-Hydroxysteroid dehydrogenase (11β-HSD), aromatase enzyme (CYP19), and 20β-Hydroxysteroid dehydrogenase (20β-HSD), as well as thyroid receptors alpha and beta (TRα. TRβ). At 10μg/L, 11β-HSD expression in the FHM brain was significantly increased, while no changed was observed in the FHM gonad. Similarly, in FHM brain tissue, CYP19 and 20β-HSD expression levels were significantly higher in fish exposed to 10μg/L sertraline. The significance of these findings with respect to survival, growth and reproduction are currently unknown, but represent future research needs.

MP254 Evaluating the performance of a mechanistic screening level model for estimating the bioaccumulation of Active Pharmaceutical Ingredients in fish

J. Armitage, University of Toronto Scarborough / Department of Physical and Environmental Sciences; J.A. Arnot, ARC Arnot Research & Consulting / University of Toronto Scarborough / Department of Physical and Environmental Sciences; F. Wania, University of Toronto at Scarborough / Department of Physical & Environmental Sciences

It is now well-established that certain Active Pharmaceutical Ingredients (APIs) are present in aquatic organisms at readily detectable levels, particularly in biota inhabiting water bodies subject to substantial releases from wastewater treatment plants. Consequently, implementation of monitoring programs and development of tools for characterizing and assessing the ecological relevance of long-term exposure to such compounds is of increasing interest to the scientific and regulatory community. We recently developed and evaluated a one-compartment mechanistic model for estimating whole-body bioconcentration factors (BCF, L/kg) of ionogenic organic compounds (IOCs) in fish. The main objective of the current study is to apply the same modelling approach to selected APIs and evaluate the performance of the model to the extent possible, given the availability of empirical BCF data. This exercise is warranted as i) the majority of APIs are organic acids or bases (i.e., are IOCs), ii) the BCF model was primarily evaluated using empirical data for industrial chemicals and pesticides, not APIs and iii) some APIs may undergo active transport processes that substantially influence uptake and elimination kinetics whereas the model only considers passive diffusion processes. Model performance is discussed with respect to key uncertainties (e.g., estimation of biotransformation rate constants) and general applicability in the context of hazard (i.e., categorization), exposure and risk assessment.

MP255 Evaluation of Bioaccumulation and Therapeutic Hazard of Contaminants of Emerging Concerns in Fish Collected in Texas Estuaries

B. Du, Baylor University / The Institute of Ecological, Earth, Environmental Sciences / Department of Environmental Science, Center for Reservoir and Aquatic Systems Research; S.P. Haddad, Baylor University / Environmental Science; K.A. Connors, Baylor University / Institute of Biomedical Studies; W.C. Scott, B.W. Brooks, Baylor University / Department of Environmental Science;

C.S. Breed, Baylor University / Department of Environmental Science, Center for Reservoir and Aquatic Systems Research; L. Broach, M. Kelly, TCEQ; B. Byars, Baylor University / Center of Spacial Research; C.K. Chambliss, Baylor University / Department of Chemistry and Biochemistry; B.W. Brooks, Baylor University / Department of Environmental Science, Center for Reservoir and Aquatic Systems Research

Though pharmaceuticals and other contaminants of emerging concern (CEC) have been reported to occur in inland surface waters and accumulate in aquatic organisms, an understanding of hydrologic loading of many CECs and associated risks to aquatic life in different coastal systems remain poorly understood. We examined occurrence of 24 target analytes in different fish species collected from estuaries alongTexasCoastover two years and further assessed the therapeutic hazard of these CECs to fish. Species-specific occurrence pattern was observed among 8 fish species collected at Buffalo Bayou in Houston,TX, USA. Interestingly, we also observed the plasma concentration of diltiazem to actually be higher than the human therapeutic level, which suggested that fish may be experiencing similar effects of this heart medication as found in humans. Our findings highlight the importance of pH on the bioaccumulation of ionizable CECs and the necessity of characterizing trophic transfer among study compounds to better understand the bioaccumulation of select CECs in aquatic organisms in effluent-dependent coastal systems. Such considerations will be important during future ecological risk assessments of ionizable CECs.

MP256 Gene Expression Profiling of Zebrafish (Danio rerio) Exposed to the Steroidogenisis Inhibitor Ketoconazole

M. See, D.C. Bencic, R. Wang, USEPA / National Exposure Research Laboratory; D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; A. Biales, USEPA / National Exposure Research Laboratory

Diverse environmental contaminants, including pesticides and pharmaceuticals, can alter the reproductive function of fish through inhibition of sex steroid synthesis. This work is part of a larger, integrated project to examine the response of the fish hypothalamic-pituitary-gonadal axis to endocrine active chemicals (EACs) using model small fish species. The goal is to identify new molecular indicators of exposure to various classes of EACs and to establish links between biomarkers and whole organism outcomes. Ketoconazole (KTC)is a pharmaceutically available fungicide and a model imidazole and triazole pesticide. It is a broad inhibitor of the cytochrome P450 (CYP) superfamily and is known to inhibit xenobiotic metabolism and steroid biosynthesis in vertebrates. The purpose of this research was to examine the transcriptomic response of adult zebrafish (*Danio rerio*) to KTC exposure. Reproductively mature zebrafish were treated up to 96 h with 400 µg/L KTC in a flow-through system. Total RNA was isolated from brain, liver and gonad and gene expression changes were identified using high density (4x44 K) commercially available zebrafish microarrays. Tissue- and sex- specific gene expression changes ($p< 0.05$; FDR< 5%) were observed, typically 120-220 differentially-expressed (> 1.3 fold change) genes per treatment. Data were analyzed by gene ontology term clustering, gene set enrichment analysis and Ingenuity Pathway Analysis. These analyses provide insight into the molecular mechanisms altered by ketoconazole exposure. Understanding the modes of action of endocrine disrupting compounds can aid in establishing functional links between biomarkers and whole organism endpoints.

MP257 How does sewage effluent exposure affect the pharmacokinetics of non-steroid anti-inflammatory drugs (NSAIDs) in fish?

L. Gunnarsson, University of Gothenburg / Department of Infectious Diseases, Institute of Biomedicine; J. Fick, Umea University / Department of Chemistry; M. Axelsson, L. Hofgaard Brattstrom, A. Grans, University of Gothenburg; J.D. Larsson, University of Gothenburg / Department of Infectious Diseases, Institute of Biomedicine

A meta-analysis of studies investigating bioconcentration of non-steroid anti-inflammatory drugs (NSAIDs) in fish, either exposed to NSAIDs in sewage effluents or in pure water suggests that ketoprofen, naproxen and ibuprofen bioconcentrate more in fish exposed to effluent. The aim of this study is to assess how sewage effluent exposure affects the pharmacokinetics of NSAIDs in fish. Exposure of rainbow trout (*Oncorynchus mykiss*) to NSAIDs in presence or absence of sewage effluent under otherwise as similar conditions as possible is ongoing. A pharmacokinetic study with ketoprofen has been performed with the aim to start addressing why NSAIDs appear to bioconcentrate more in fish exposed to sewage effluent. The processes involved in uptake was eliminated by intra vascular administration of deuterium-labelled ketoprofen in fish pre-exposed to either clean water orsewage effluent and potential differences in distribution, metabolism and/or excretion were assessed.The results show that fish exposed to sewage effluent had a significantly increased blood plasma half-life of ketoprofen compared to fish kept in pure water. Additional studies of potential *in vitro* differences in hepatic elimination rates are ongoing. Increased understanding of how complex mixtures can affect pharmacokinetics and consequently the toxicity of pharmaceuticals has the potential to direct improvements of environmental risk assessment procedures of pharmaceuticals. Relating toxicity to internal concentrations, as often done in human toxicology, rather than to water concentrations, may be a valuable way to reduce uncertainties arising from differences in bioconcentration of chemicals under different exposure situations.

MP258 In vitro biotransformation and bioconcentration of a model benzodiazpine, diazepam, in channel catfish (Ictalurus punctatus)

C. Overturf, D.B. Huggett, University of North Texas / Department of Biological Sciences; A. Maldonado, University of California, Riverside / Environmental Science; D. Schlenk, University of California-Riverside / Department of Environmental Sciences

Recently, there has been an increase in the detection of pharmaceuticals in surface waters across the world. Pharmaceuticals have known mechanisms of action in target organisms and are typically found in the environment at concentrations well below therapeutic levels in humans; however, their mechanisms of action and biologically active concentrations are largely unknown in non-target organisms (e.g., teleost species). Thus, the *in vitro* metabolism of a model benzodiazepine, diazepam, was investigated in channel catfish (*Ictalurus punctatus*). Following exposure to diazepam (5 mm) *in vitro*, the concentrations of the parent compound as well as biologically active metabolites (nordiazepam, temazepam and oxazepam) were examined. The results indicated that approximately 8% of diazepam was converted to active metabolites, with approximately 3% and 5% representing nordiazepam and temazepam, respectively. Oxazepam was below detection in all analyses, indicating that channel catfish may not possess the specific CYP isoform(s) needed for its formation. Due to the low rate of diazepam metabolism observed in channel catfish, the *in vivo* bioconcentration potential of diazepam and distribution of metabolites was also investigated. 7d tissue specific kinetic bioconcentration factor values (BCF_k) were calculated for diazepam. The BCF_k resulted in values of 146.3, 9.8, 2.1, 45 and 15 for plasma, liver, muscle, gonad and brain, respectively, indicating that a large portion of absorbed diazepam remains in the blood. Metabolite formation was low (< 1 ng/g wet weight) or below detection in all tissues examined following *in vivo* exposure, confirming the results obtained from the *in vitro* metabolism assays. Due to the low metabolic capability of channel catfish to remove diazepam and the potential for bioconcentration, channel catfish may have an increased risk for adverse effects from exposure to benzodiazepine drugs.

MP259 In vitro pharmaceutical metabolism with fathead minnow (Pimephales promelas) liver S9

K.A. Connors, Baylor University / Institute of Biomedical Studies; B. Du, Baylor University / The Institute of Ecological, Earth, Environmental Sciences / Department of Environmental Science, Center for Reservoir and Aquatic Systems Research; P.N. Fitzsimmons, USEPA / Mid-Continent Ecology Division; K. Chambliss, Baylor University; J.W. Nichols, USEPA / Mid-Continent Ecology Division; B.W. Brooks, Baylor University / Department of Environmental Science

The occurrence of pharmaceuticals in the environment represents an area of emerging concern. Chemical and biological properties of therapeutics present unique challenges to ecological risk assessment. Unlike other industrial chemicals, existing pharmaceutical safety data and pharmacology information may be leveraged through biological "read-across" to aid environmental assessments. However, few approaches and robust empirical datasets exist, particularly for comparative pharmacokinetic applications. The present study examined in vitro biotransformation rates of several select pharmaceuticals using conventional substrate depletion methods in fathead minnow liver S9. The results indicate that fathead minnows are able to biotransform study pharmaceuticals at a rate approximately 4 to 34 times lower than clearances rates previously measured in rainbow trout. Contrary to observations in rainbow trout, fathead minnows are capable of modest diphenhydramine

depletion. Further, enantiomer-specific clearance rates were not consistent between fish species. Additional study is required to describe the relative metabolic enzyme activity in fathead minnows and trout. This information could be used to facilitate more accurate cross-species extrapolations.

MP260 Influence of Diel pH Variability on Predicted Fish Plasma Concentrations of Ionizable Pharmaceuticals in Texas Estuaries

W.C. Scott, Baylor University / Department of Environmental Science; B. Du, Baylor University / The Institute of Ecological, Earth, Environmental Sciences, Baylor University / Department of Environmental Science, Center for Reservoir and Aquatic Systems Research; S.P. Haddad, Baylor University / Environmental Science; K. Chambliss, Baylor University; B.W. Brooks, Baylor University / Department of Environmental Science

Tidally influenced rivers are dynamic aquatic systems subject to significant spatial and temporal fluctuations in water chemistry. Approximately 70% of all pharmaceuticals are ionizable and subject to bioavailability alterations from pH. Spatiotemporal variability in ambient pH can influence the site-specific bioconcentration of ionizable pharmaceuticals leading to uncertainty in ecological risk assessments. For this study, we examined diel pH variability in the surface and bottom water of four Texas estuarine systems. Furthermore, we attempted to relate spatial and temporal fluctuations in pH to predicted fish plasma concentrations (FPC) of two pharmaceuticals, diphenhydramine and diltiazem, using measured water concentrations and pH adjusted logD. Between the four tidally influenced rivers, median surface water pH ranged from 6.93 to 8.27. Existing site-specific considerations for monitoring aquatic contaminants does not make recommendations on dealing with spatial or temporal gradients in pH. During this study, the median change in pH between surface and bottom water ranged from 0.27 to 1.23 pH units, while the pH in a river varied by as much as 0.57 and 0.46 over 24 hours in surface and bottom waters respectively. Resulting ranges of mean site-specific predicted FPC of diphenhydramine and diltiazem were 5.8 – 95.2 and 0.5 – 31.1 ng/L respectively. We then established effects ratios (ER), comparing human therapeutic plasma concentrations of the pharmaceuticals to these predicted FPC, which varied as much as < 500 to >3000 at a given geographic coordinate. This study highlights the uncertainty that spatiotemporal pH variability introduces to predictions of FPC of diphenhydramine and diltiazem in Texas tidally influenced rivers.

MP261 Investigating the Reproductive Effects of Triclocarban on Japanese Medaka (Oryzias latipes)

I. Flores, University of California-Davis / Veterinary Medicine: Anatomy, Physiology and Cell Biology; T. Kurobe, S. Teh, University of California, Davis

Triclocarban (TCC; 3,4,4'-trichlorocarbanilide) is a common antibacterial additive in personal care products that has been extensively used. Now, it is one of the most frequently and widely detected chemicals in aquatic systems yet our knowledge of its effects on animals and the environment are still limited. Studies are emerging to assess the toxicity of this chemical to animals and in particular, its potential to cause endocrine disruption. Due to the prevalence of TCC in the aquatic environment, there is a need to determine the reproductive effects in fish. Therefore the objective of this study is to investigate the reproductive and developmental effects of fish from chronic TCC exposure. Using Japanese medaka (*Oryzias latipes*) as a fish model, a full life cycle chronic toxicity test was conducted. 1-day-old fertilized medaka embryos were bath exposed to 5 µg/L of TCC or a vehicle control and were continuously exposed until fish became reproductively active. Health indices such as condition factor, hepatosomatic and gonadosomatic indices were measured. Liver and gonad development were assessed using histology to determine abnormalities and pathologies associated with long-term TCC exposure. Vitellogenin, a female specific protein, was measured as a biomarker for estrogenic effects. Expression of genes pertaining to endocrine disruption was measured using Reverse Transcription- quantitative PCR. Finally, fertility and fecundity of crossbred fish was used to assess reproductive performance. The multiple endpoints evaluated in this study will allow us to fully understand the effects of endocrine disruption of TCC since more than one pathway can lead to a physiological change in the reproductive ability of fish. Results from this study will help in expanding our knowledge of the environmental impact of TCC. A better understanding of TCC toxicity can be used to initiate monitoring of TCC in the aquatic environment and steps can be taken to mitigate TCC contamination.

MP262 Lifetime Exposure of the Least Killifish (Heterandria formosa) to 17 α-Ethinylestradiol: Organism-and Population-Level Effects

L.M. Jackson, University of Louisiana at Lafayette / Biology; P.L. Klerks, University of Louisiana at Lafayette / Department of Biology

The presence of endocrine disrupting compounds (EDCs) in the environment has gained worldwide attention because of the possibility that EDC's may be affecting the reproductive health of wildlife and humans. The synthetic estrogen 17α-ethinylestradiol (EE2) is a potent endocrine modulator and is present at biologically active concentrations in aquatic ecosystems. To investigate impacts of EE2 in the aquatic environment, a full life cycle in vivo study was done to examine the potential effects of EE2 in the least killifish (*Heterandria formosa)* at both the individual and population levels. I tested the effects of an environmentally relevant concentration of waterborne EE2. Newborn fishes were exposed to 5 ng/L EE2 or a solvent control (ethanol) in a static-renewal system with replacement every 72 hours. Effects of EE2 on length, weight, sex ratio, survival rate, and time to sexual maturity in the developing fishes were examined at the individual level. At the population level, population growth rates and changes in population were examined. My preliminary studies indicated that at 5 ng/L, EE2 causeda significant reduction in growth of females, female-biased sex ratio, decreased survival, and longer times to sexual maturity. There was also a trend of EE2-exposed males exhibiting faster growth than control males. At four months, there were notably fewer individuals in the exposed populations compared to the control populations. The control populations grew at a rate of 1.6% per month while the exposed populations exhibited no growth at all. Results for population growth rates and individual measurements for an entire breeding season will be displayed at the meeting. This ongoing study adds to the growing body of evidence of significant risks to aquatic organisms from exposure to environmentally-relevant concentrations of EE2.

MP263 Multi-generational effect of propranolol at environmentally relevant concentration on Daphnia magna

T. Jeong, S. Kim, Gwangju Institute of Science and Technology; D. Kim, Kongju National University; J. Ra, GIST

The identification of pharmaceutically active compounds is an important emerging global health issue. The human pharmaceutical which is highly reactive compound is a potential hazardous material to aquatic organism because of its continuous inflow into aquatic system. However, the effects of pharmaceuticals may be underestimated during single generation exposure. Although several researches revealed subtle effect on invertebrate at environmentally relevant exposure concentration, they have been limited in a range of investigation of temporarily harmful effect leading subsequent recovery or subtle effect of biomarker that is a partial change not linked to phenotypic change. This study is a part of the research plan aiming to evaluate human pharmaceutical's impact on *Daphnia magna* during dozens of generations and to anchor its impacts to various levels of biological organization. The purpose of this study is to determine the multigenerational chronic effects of the beta blocker, propanolol, on *D. magna* over multi successive generations at sublethal concentrations. The reproduction and morphological parameters are investigated at different concentrations and generation numbers. The exposure concentration of propanolol is 5ng/L, 500ng/L, and 50µg/L, which are detected from surface waters. Ten *D. magna* are exposed to chemical at each test chamber with four replicates. One generation exposure is maintained for 21d. The somatic growth, reproduction size related to population growth and the physiological endpoints are investigated. We also quantified malformation/abortion rate and body size of neonate to cover developmental effect on embryo. The malformation/abortion rate and reproduction size are regularly checked three times a week and other endpoints are observed at the end of a generation. From our previous study on tetracycline, the population effect of *D. magna* induced by the organismal responses is expected.

MP264 Oxidative stress produced by ketorolac on brain and liver of the common carp C. carpio

M. Galar-Martinez, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional / Laboratory of Aquatic Toxicology, Department of Pharmacy; S. Garcia Medina, C. Razo Estrada, I. Perez Coyotl, D. Mendoza Monroy, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional; L. Gomez Olivan, Facultad de Química, Universidad Autónoma del Estado de México

Ketorolac is a pharmaceutical product belonging to the group of non-steroidal antiinflamatory drugs (NSAIDs), used as an analgesic in acute and postsurgical pain, and in México do not require a prescription for sale,

favoring self-medication of the population. Several studies have shown that some NSAIDs such as diclofenac, ibuprofen, acetaminophen and naproxen among others, cause oxidative stress in both mammals and aquatic species including the common carp *Cyprinus carpio*. However, there are not reports regarding the toxic effects of ketorolac in any species and it has been detected at concentrations between 0.2 and 60 ug/L in hospital effluents, so it can become a health risk for the hydrobionts. In this study, the oxidative stress caused by sublethal exposure to ketorolac on *C. carpio* was assessed, evaluating the degree of lipid peroxidation (LPX), hydroperoxides (HX) and oxidized proteins (PX) content, as well as the activity of the antioxidant enzymes superoxide dismutase, catalase and glutathion peroxidase, in liver and brain of organisms exposed for 12, 24, 48, 72 and 96 h to 1 and 60 ug/L of the drug. The obtained results showed that ketorolac increases the values of damage biomarkers (LPX, HX and PX) and modifies the antioxidant status of the organisms, being oxidative stress dependent of drug concentration, exposure time and organ assessed.

MP265 Prioritizing and selecting pharmaceuticals to test the read-across approach: using human clearance rates to predict biotransformation in fish

J.P. Berninger, USEPA; K.A. Fay, USEPA / ORD, NHEERL, Mid-Continent Ecology Division; C.A. LaLone, USEPA; D.A. Dreier, Baylor University / Department of Environmental Science; E.J. Durhan, E.A. Makynen, D.L. Villeneuve, J.W. Nichols, USEPA / Mid-Continent Ecology Division; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory

Many active pharmaceutical ingredients (APIs) have been detected in aquatic systems around the world. These systems typically receive continual municipal sewage inputs, which results in pseudo-persistent exposures of aquatic animals to APIs, thus enhancing their bioaccumulative potential and possibility for adverse effects. Thousands of APIs exist, so full chemical and toxicological assessments are impractical and present a cost and logistical impasse. Further, limited information on bioaccumulation of APIs in fish exists. We developed an effects-based prioritization scheme for APIs leveraging available mammalian pharmacokinetic (PK) data to predict biotransformation rates in fish. We collated a database of referenced mammalian PK data to be used strategically for our prioritization method. The method can be used to prioritize drugs based on a single or multiple PK parameters. To predict biotransformation rates in fish, human clearance values were identified for 875 APIs. Human clearance values for the selected APIs ranged from 0.0037 to 1070 ml/min/kg. APIs with the slowest clearance were assumed to present the greatest potential for bioaccumulation in fish. Human clearance values were rank-regressed to form a probabilistic distribution. To evaluate the read-across assumptions (slow clearance = high bioaccumulation potential) three groups of APIs were derived. These groups represent high (>90th centile; slowest clearance: 0.0037 – 0.4 mg/min/kg), median (45th – 55th centile; median clearance: 2.9 – 4.4 mg/min/kg) and low (< 10th centile; fastest clearance: 31 – 1070 mg/min/kg) hazard potential based on centile. Each group initially contained ~100 APIs, which was reduced in a step-wise process. First the APIs with reported renal clearance in humans were excluded, to ensure more direct read-across. Next, priority was given to those compounds with known interactions with hepatic xenobiotic metabolizing enzymes (CYP1A or 3A) in humans relative to the presence of similar enzymes in fish. This resulted in 12 APIs grouped based on hazard potential for biotransformation studies: high – dutasteride, fluconazole, phenobarbital, phenytoin; medium – dexamethasone, spironolactone, gemfibrizol, chloroquine; and low – propofol, praziquantel, dextromethorphan, hydralazine. As described in a companion poster, predictions of bioaccumulative potential will be validated for the selected APIs using trout S9 substrate depletion method.

MP266 Regulatory Approaches to the Bioaccumulation of Drugs: A Polar Bare Walks Into A Bar...

G. Rattray, Health Canada / Environmental Impact Initiative Division; M.A. Bonnell, Environment Canada / Ecological Assessment Division; G. Stringer, Health Canada

A regulatory framework has been developed for specifically for active pharmaceutical ingredients (APIs) in products regulated by the Canadian *Food and Drugs Act* (F&DA) to assess their potential environmental effects. Designed to harmonize with the drug approval process stipulated by the *Food and Drugs Act* (F&DA) and its regulations, the framework assesses the potential impacts of APIs in human pharmaceuticals and veterinary drugs on exposed aquatic and terrestrial organisms in soils, sediments and surface waters. Similar to other jurisdictions, the framework incorporates a stepwise assessment of the persistence, bioaccumulation and inherent toxicity (PB(T)) profile of the substance to attempt to evaluate the "risk" an API may pose to the environment prior to extensive distribution or exposure in the environment (cf., listing in Annex XIII of REACH guidance); however the use of these properties to guide a risk assessment are grounded in the assessment of legacy neutral organic chemicals that are often, but not always, of global concern (e.g., UNEP POPs). Current bioaccumulation criteria, assessment approaches and models have relied on the passive diffusion hydrophobicity-lipophilicity paradigm for the purpose of determining the potential for adverse effects from exposure to contaminants. We will examine the purpose of current bioaccumulation criteria and the extent to which these criteria and approaches should be applied to pharmaceuticals as ionizing bio-active substances. We will also examine additional bioaccumulation metrics that could be used in place of or in addition to current criteria or approaches to bioaccumulation assessment and how the proposed regulatory framework might utilize these differing approaches.

MP267 The Effects of Hydroxypropyl-β-Cyclodextrin on the American Flagfish (Jordanella floridae) Over One Complete Life-Cycle

J.C. Anderson, University of Ontario Institute of Technology / Faculty of Science; L. Beyger, University of Ontario Institute of Technology; J. Guchardi, University of Ontario Institute of Technology / Faculty of Science, Aquatic Toxicology; D.A. Holdway, University of Ontario Institute of Technology / Faculty of Science, Aquatic Toxicology

Understanding the impacts of pharmaceuticals and personal care products (PPCPs) on aquatic ecosystems has become important in the field of aquatic toxicology. Many PPCPs have been shown to cause effects on aquatic biota within detected environmental ranges. Of particular interest are the potential toxicological effects of hydroxylpropyl-β-cyclodextrin (HPβCD) on fish. HPβCD is amphiphilic, toroidal in shape, and able to form non-covalent inclusion complexes with a variety of guest molecules. HPβCD has been shown to reduce volatility as well as improve the aqueous solubility of apolar guest compounds. As such, the use of HPβCD in the pharmaceutical and personal care industry has dramatically increased, for example, it is the active ingredient in Febreeze®. With the potential for entering the environment through wastewater treatment plant (WWTP) effluent, HPβCD poses an unknown risk to non-target aquatic biota. As a result, a 145-day chronic full life-cycle exposure using American flagfish (*Jordanella floridae*) was completed using flow-through concentrations of 0 – control, 5, 16, 50,160, 500, and 1600 μg/L of HPβCD maintained via a peristaltic pump. Reproductive output and egg quality of exposed fish were not significantly different from controls. No significance differences were observed in growth, condition factor (K), and hepatosomatic index (HSI) of HPβCD exposed fish compared to the controls ($P \leq 0.05$). An increase in gonadosomatic index (GSI) was observed in females exposed to HPβCD, significant at 50 μg/L of HPβCD ($P \leq 0.05$). An acute copper toxicity challenge experiment using second generation HPβCD exposed larvae was performed and altered sensitivity to copper toxicity was monitored. Copper toxicity results are pending analysis and will be presented.

MP268 Tracking the uptake and assimilation of TNT and RDX in coastal marine organisms using stable isotopic tracers

M. Ballentine, University of Connecticut / Department of Marine Sciences; R. Smith, University of Connecticut; T.S. Ariyarathna, University of Connecticut / Dept. of Marine Sciences; P. Vlahos, University of Connecticut / Departments of Marine Sciences and Chemistry; C. Tobias, University of Connecticut

2,4,6 – trinitrotoluene (TNT) and hexhyro – 1,3,5 – trinitro – 1,3,5 – triazine (RDX) are munitions compounds used widely at Department of Defense facilities, including coastal military installations. TNT and RDX and their derivatives are Environmental Protection Agency priority pollutants and have known toxicity in a variety of terrestrial and aquatic species. While both have been shown to persist in freshwater environments and soils, less is known of the fate and transport in marine systems. As part of a multi-scale marine ecosystem fate and transport study, a series of small mesocosms complete with sediments and marine organisms representing multiple trophic levels were spiked with nitrogen-15 (^{15}N) labeled TNT and RDX in separate pulse addition experiments. Yields of dissolved and biomass extractable explosives were monitored over a period of 11 days, along with isotopic enrichment (d15N) of bulk tissue. TNT was detected along with 2 major reduced derivatives 4 amino-dinitrotoluene (4-ADNT)

and 2 amino-dinitrotoluene (2-ADNT), which were often observed to be 2 times higher than the total TNT in the organism. The ratio of 4-ADNT to 2-ADNT averaged 3:1 respectively. The mass of ^{15}N derived from TNT and its derivatives increased with time and the percent of munitions that could account for ^{15}N decreased over time. In winter flounder ^{15}N increased from 0 to 475 μg ^{15}N g^{-1} dry weight while the percent due to explosives decreased from 13% to 4% indicating the nitrogen derived from TNT was being assimilated, metabolized, or entered other unknown pathways. δ ^{15}N uptake via TNT in macroalgae was 10 times higher than the other species with 10,000‰. RDX uptake profiles differed from those of TNT. RDX and its four main though only one was detected with any consistency. δ ^{15}N that could be accounted for by RDX ranged from 20 to 30‰ in majority of the species while reaching a high of 127‰ in flounder. The percent of ^{15}N due to RDX often remained relatively flat with no more than 10% in most species, indicating a stability of the RDX or a steady state uptake and clearance. Further studies will test the amino acid ^{15}N values to better understand the pathways and ultimate fate of the munitions within coastal marine organisms of different trophic levels.

MP269 Use of human clearance rates to predict the biotransformation of pharmaceuticals by fish: A test of the read-across approach

K.A. Fay, USEPA / ORD, NHEERL, Mid-Continent Ecology Division; J.P. Berninger, C.A. LaLone, USEPA; D. Dreier, Baylor University; E.J. Durhan, US-EPA / Mid-Continent Ecology Division; M. Lee, USEPA; E.A. Makynen, D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; J.W. Nichols, USEPA / Mid-Continent Ecology Division

Pharmaceuticals are increasingly found in aquatic environments near wastewater treatment plant discharge, and may be of particular concern to aquatic life given their pseudo-persistence. The large number of detected pharmaceuticals necessitates a prioritization method for hazard assessment in fish. Because pharmaceuticals undergo extensive testing during development, it may be possible to leverage existing mammalian pharmacokinetic data to predict certain hazards. In this study, reported human clearance rates were used as a read-across to prioritized drugs for assessing bioaccumulative potential in fish. Based on this parameter, we selected 12 chemicals predicted to be of high (dutasteride, fluconazole, phenobarbital, phenytoin), medium (dexamethasone, spironolactone, gemfibrizol, chloroquine) and low (propofol, praziquantel, dextromethorphan, hydralazine) bioaccumulative concern. To evaluate this read-across, *in vitro* intrinsic clearance rates were obtained using a substrate depletion method with trout S9 fractions. Preliminary experiments were conducted to establish reaction conditions resulting in linear depletion kinetics. Chemical free fractions were measured using equilibrium dialysis to obtain binding information for the *in vitro* system as well as trout plasma. Trout hepatic clearance estimates were then determined for each drug using the measured *in vitro* depletion rate constants, binding information and scaling factors. With few exceptions (i.e., dexamethasone), extrapolated clearance values were in good qualitative agreement with observed human values, aligning with our predictions of high, medium and low clearance. These results suggest human pharmacokinetic data may be useful for identifying pharmaceuticals of bioaccumulative concern in fish.

Ecotoxicology of Fungicides

TP001 Acute toxicity of antifouling booster on copepod Nitokra sp.

L. Diniz, T.S. Pinto, São Paulo State University- Institute of Chemistry; M.D. Spadoto, São Paulo State University- Institute of Chemistry / Sao Luis; L.T. Cappelini, São Paulo State University- Institute of Chemistry; T.C. Franco, Maranhao Federal University; E.M. Vieira, São Paulo State University- Institute of Chemistry

This study was aimed at investigating the acute toxicity of biocide antifouling diuron under the copepods *Nitokra* sp. The sensitivity bioassay with zinc sulfate, reference substance, presented $LC_{50\text{-}96h}$ mean was$LC_{50\text{-}96h}$1.69 mg L^{-1} with a coefficient of variation 0.67% and with confidence limits between 1.0 mg L^{-1} and 2.96 mg L^{-1}. The acute toxity of diuron for test-organisms presented $LC_{50\text{-}96h}$means was 0.97 mg $L^{-1}$with a coefficient of variation 0.08% and with confidence limits between 0.82 mg L^{-1} and1.16 mg L^{-1}. The results indicated the reproducibility of the method and sensitivity of the organism to the biocidal highlighting the negative effect of unregulated use of this substance in marine environments. The several activities developeded on port regions contribute to environmental pollution caused by antifouling paints in the coastal zone. Used to prevent the growth and development of organisms, such as bacteria, macroalgae, mussels and barnacles on ships and port structures, the paints ensure an effective protection from fouling offering greater resistance infrastructures.The negative effects of the coastal ecosystems are related to antifouling booster that can be inorganic, organic or organometallic of reasonable toxicity.These substances are used separately or in mixtures, in the latter case to enhance the biocide action of painting.With the ban on organotins, diuron started being used also as booster antifouling paints third generation and despite its more intense agricultural use, the presence of diuron in samples of seawater may indicate its source is associated with your use as antifouling agen. In 2006, approximately 50% of antifouling paints commercially available possessed diuron in its composition. Its mechanism of action is associated to inhibition of photosystem II (PSII) of plants and photosynthetic microorganisms. Studies have demonstrated the toxic effects of diuron on species relevant to primary and secondary productivity of coastal ecosystems in the concentration ranges between ng L^{-1} and μg L^{-1}.

TP002 Chronic toxicity of Azoxystrobin in water-only toxicity tests with select freshwater invertebrates

J.L. Kunz, US Geological Survey / Columbia Environmental Reserach Center; C.G. Ingersoll, US Geological Survey / Columbia Environmental Research Center; J. Teerlink, USGS / California Water Science Center; K.L. Smalling, K.M. Kuivila, US Geological Survey; A.A. Elskus, US Geological Survey / S.O. Conte Anadromous Fish Research Laboratory, Maine Field Office

Fungicides are an understudied group with respect to evaluating sublethal effects on non-target organisms. Given the basic modes of action through which fungicides exert toxic effects, one might expect these chemicals to be potent toxicants for a variety of species. Recent studies report effects of exposure to some fungicides on fish and invertebrates at low, environmentally relevant concentrations. Azoxystrobin was selected for chronic toxicity testing based on previous acute toxicity tests that showed: (1) acute toxicity to multiple test organisms and (2) photoactivated toxicity to several of the test organisms in the presence of UV. The objective of the current study is to evaluate the toxicity of the fungicide azoxystrobin in chronic water-only toxicity tests with the cladoceran *Ceriodaphnia dubia*, the amphipod *Hyalella azteca*, the midge *Chironomus dilutus*, and the mussel *Lampsilis siliquoidea*. Exposures were conducted up to six weeks under ambient laboratory lighting conditions (low ultraviolet radiation; UV) to 0, 12.5, 25, 50, and 100μg/L (nominal) with all four species. Endpoints included effects of azoxystrobin on survival, weight, biomass (amphipods, mussels, and midge), emergence (adult midge), or reproduction (amphipods and cladocerans). Sublethal endpoints were more responsive than lethality and amphipods were more sensitive than the other three species. The highest exposure (100μg/L) caused lethality to amphipods but did not reduce survival of cladocerans, midge, or mussels. Both survival and reproduction of amphipods was reduced at the lower exposure concentrations (12.5-25 μg/L; preliminary chemical analysis indicates measured concentrations were about 60% of the nominal concentrations). Cladoceran reproduction was reduced (20%), and emergence of adult midge was reduced at the highest nominal concentration of 100 μg/L compared to control. Analysis of weight and biomass for amphipods, mussels, and midge is still underway. At the end of the cladoceran and mussel exposures, the surviving organisms were placed in clean water and exposed for 4 hours to ultraviolet (UV) representative of environmental levels to evaluate the potential lethality associated with photoactivated azoxystrobin. No lethality of cladocerans or mussels were observed after a 4-hour exposure to elevated UV. Results of this study indicate that sublethal effects on the amphipod were observed at reported environmentally relevant concentrations of azoxystrobin.

TP003 Effects of azoxystrobin on microbial and macroinvertebrate leaf decomposition by the amphipod Hyalella azteca under laboratory conditions

A.A. Elskus, US Geological Survey / S.O. Conte Anadromous Fish Research Laboratory, Maine Field Office; K. Capps, University of Maine / Wildlife Ecology; C.G. Ingersoll, US Geological Survey / Columbia Environmental Research Center; J. Longcore, University of Maine

Aquatic fungi contribute significantly to the decomposition of stream leaves, a key ecosystem function, but the effects of fungicides on fungi and macroinvertebrates involved with leaf decomposition are understudied. The objectives of this study are to (1) develop and optimize laboratory conditions under which fungicides could be tested for their effects on fungal and macroinvertebrate leaf decomposition, and (2) to examine the effects of a widely used fungicide, azoxystrobin, on microbes and leaf shredders involved with leaf decomposition. We are using a commonly tested organism, the amphipod leaf shredder, *Hyalella azteca*, to examine the effects of azoxystrobin on microbial flora (bacterial and fungal) and amphipod growth and survival. Stream-conditioned red maple (*Acer rubrum*) leaves are exposed to environmentally relevant concentrations of azoxystrobin (0, 10, 150 and 5,000 ng/L), or to vehicle (acetone), in the presence and absence of *H. azteca* for 2 weeks at 25 C under constant darkness in artificial water optimized for performance of *H. azteca*. Unconditioned maple leaves are used as negative controls. Dosing water is changed every two days to maintain azoxystrobin concentrations and optimal water quality. The endpoints evaluated include: 14 day amphipod survival and biomass, fungal biomass (ergosterol, which does not include Chytridiomycota, which may be an important part of the fungal biomass), bacterial biomass (fluorescence microscopy), microbial respiration and leaf decomposition rate. Changes in leaf decomposition and biological effects on amphipods are sensitive indicators, which can be used as early warning, biomonitoring tools of potential fungicide effects on ecosystem function. Supported by the USGS Toxic Substances Hydrology Program.

TP004 Effects of fungicides on non-target gut fungi (trichomycetes) and accumulation in their insect larvae hosts

K.L. Smalling, US Geological Survey; E.R. Wilson, Boise State University; T.J. Reilly, US Geological Survey; M.M. White, Boise State University

Fungicides are moderately hydrophobic and have been detected in water and sediment, particularly in agricultural watersheds. However, many of the newly registered fungicides are not included in routine water quality monitoring efforts despite their increasing use and frequent application to combat fungal pathogens. The efficacy of fungicides on fungal pathogens is well documented but little is known about their effects on non-target fungi, including gut fungi. These symbiotic microorganisms found in the guts of many aquatic macroinvertebrates and have the ability to assist in assimilating nutrients for their host in times of stress. Field surveys were conducted from April through December 2010 on four streams with varying pesticide inputs (two agricultural and two reference sites) in southwestern Idaho to assess the possible non-target impact of fungicides on gut fungi, or trichomycetes. Larval black flies were chosen as candidate hosts because they play important roles in stream ecosystems. In addition, tissues of larval black flies (Diptera: Simuliidae), which are hosts to many gut fungi, were analyzed for pesticide accumulation using gas chromatography tandem mass spectrometry. Larval black flies from agricultural sites had decreased prevalence, spore production, and density of gut fungi. Measurable levels of pesticides were observed in both black fly larval tissue and surface water from these sites. The fungicides azoxystrobin, boscalid, imazalil and pyraclostrobin were detected in greater than 60% of black fly tissue samples from the agricultural sites at concentrations up to 0.84 μg/g wet weight. In surface water the fungicides, azoxystrobin and boscalid, were detected in 92% of the samples collected from the agricultural sites at concentrations less than 50 ng/L. No fungicides were detected in black fly tissue or water from the reference sites. Results from the current study indicate that fungicides have the potential to affect non-target fungal communities in surface water systems, with possible far-reaching consequences for higher trophic level organisms within

aquatic ecosystems. This study also highlights the need to monitor aquatic ecosystems for fungicides as demonstrated by the potential impact on non-target fungi. Future laboratory and field based experiments should focus on elucidating the complex relationship between applied fungicides, larval black flies, and their gut fungi.

TP005 Effects of fungicides on shredding detritivore responses under varying temperature regimes

M.M. Willming, Texas Tech University / Department of Environmental Toxicology; J.D. Maul, Texas Tech University / The Institute of Environmental and Human Health / Department of Environmental Toxicology

The presence of contaminants such as fungicides in aquatic environments may impact non-target bacterial and fungal communities and the invertebrate detritivores responsible for the decomposition of allochthonous organic matter. Additionally, in some aquatic systems daily water temperature fluctuations may influence these processes and alter contaminant toxicity, but such temperature fluctuations are rarely examined in conjunction with contaminants. In this study, *Hyalella azteca* served as a model shredding detritivore and organisms were exposed in a series of experiments to the fungicide pyraclostrobin. Experiments were performed at a constant temperature (23°C), fluctuating temperature regime (18-25°C) based on field-collected data from the S. Llano River, TX, or a fluctuating temperature regime (20-26°C) adjusted based on possible climate change predictions. Endpoints included *H. azteca* mortality, growth, and leaf processing. Ergosterol content of leaves was measured to estimate fungal biomass. Generally, fungicide effects were greater than temperature effects for leaf shredding, and data suggest little effect on growth. There was a significant interaction between temperature treatment and pyraclostrobin concentration on *H.azteca* mortality. This study provides information on the influence of realistic temperature variation on fungicide effects in aquatic systems, which is important for understanding how future alterations in temperature due to climate change may influence the assessment of ecological risk of contaminants.

TP006 Effects of organic and inorganic current-use fungicides and their mixtures on the feeding of a key shredder species

J.P. Zubrod, P. Baudy, R. Schulz, Institute for Environmental Sciences; University of Koblenz-Landau; M. Bundschuh, Department of Aquatic Sciences and Assessment; Swedish University of Agricultural Sciences

Recent research indicates that environmental concentrations of fungicides and their mixtures may affect heterotrophic ecosystem processes like leaf litter breakdown in streams, which is mainly mediated by detritivorous macroinvertebrates (i.e., shredders). However, during environmental risk assessment, toxicity of fungicides is routinely assessed using representatives of autotrophic (i.e., photosynthesis-based) food webs. In this context, the present study investigated direct effects of these substances on the feeding activity of the key shredder *Gammarus fossarum*. For this purpose, a set of organic (azoxystrobin, carbendazim, cyprodinil, quinoxyfen and tebuconazole) as well as inorganic (three copper-based substances and sulfur) fungicides and two model mixtures were tested. Quinoxyfen and cyprodinil resulted in the strongest effects on gammarids' feeding among all tested organic fungicides, followed by carbendazim, azoxystrobin, and tebuconazole. Although all organic fungicides used have dissimilar (intended) modes of actions, a mixture assessment using a fixed-ratio design and "independent action" as reference model revealed a slight but significant synergistic effect. This synergism may be explained by the documented synergizing effect of azole fungicides (i.e., tebuconazole). While the tested copper-based fungicides resulted in considerable effects but differed markedly in their toxicity, sulfur (up to ~5 mg/L) did not cause any effect. However, sulfur acted antagonistically on copper's toxicity in a mixture assessment using an A-in-the-presence-of-B design. This antagonism may be driven by physiological reactions within the test organisms since sulfur can act as dietary antagonist of copper in animals. The present study indicates for most of the tested substances, applying an assessment factor of only 10 to the fungicides' EC20-values, a risk to *Gammarus* and thus leaf litter breakdown at environmental concentrations. Moreover, this research clearly shows that fungicides do not act independently resulting in unexpected deviations of mixture effects from model predictions. A protective environmental risk assessment for fungicides should thus acknowledge this fact by, e.g., increased assessment factors.

TP007 Toxicity of Pyraclostrobin and Trifloxystrobin to Amphibians and Aquatic Invertebrates

J.B. Belden, S.T. McMurry, Oklahoma State University / Department of Zoology; L.M. Smith, Oklahoma State University; S.A. Morrison, E.A. Hooser, Oklahoma State University / Department of Zoology

The use of strobilurin fungicides has increased substantially in the last decade. Typically, these fungicides are applied aerially, which may lead to exposure to nontarget organisms within fields or in nearby habitat. In regions with wetlands that are frequently embedded in the agricultural landscape, such as the Central Plains of the US, risk of exposure could be especially high. In a series of experiments, the toxicity of pyraclostrobin and trifloxystrobin was examined to a nontarget amphibian (*Bufo*) and an aquatic invertebrate (*Hyalella*). Both compounds were found to be acutely toxic to both organisms at environmentally relevant levels. In aquatic systems, the toxicity of formulations was similar to that of the active ingredients, suggesting toxicity is primarily due to the active ingredient. Aquatic toxicity tests conducted with sediment/water systems confirm that sediment would ameliorate some of the expected toxicity. However, toxicity still occurred at exposure levels that are expected following a direct overspray event over shallow water. Finally, chronic toxicity tests with *Bufo* tadpoles suggested limited effects on growth at concentrations significantly lower than the lethal levels. Based on results to date, improper use of these fungicides poses risks to nontarget organisms. Further research is needed to evaluate mechanisms of toxicity and relevant levels of exposure in terrestrial systems, sublethal endpoints in aquatic systems, and partitioning in aquatic systems under field conditions.

Organic Flame Retardants Beyond PBDEs: What Have We Learned?

TP008 Beyond concentrations: what flame retardant inventories tell us

A. Golnoush, University of Toronto / Geography; E. Goosey, University of Toronto / Earth Sciences; A. Saini, University of Toronto / Department of Physical and Environmental Sciences; M.L. Diamond, University of Toronto / Department of Earth Sciences; A. Buser, ETH Zürich; A.G. Soehl, Great Lakes Commission; M.W. Murray, National Wildlife Federation / Great Lakes Field Office

A dynamic inventory of PBDE-containing products was estimated according to the consumption pattern and assuming that the lifespans of the products in the use phase follow a Weibull distribution that defines the fraction of each product leaving the use phase at a certain time. The mass of electronic products entering the use phase was obtained from consumption patterns from 1970 to 1980, and sales data from 1980 to 2011. The same rate of change in product consumption from 2007 to 2011 was assumed after 2011. The discrete dynamic SFA was based on the material flow analysis developed by Morf et al. (2008). Our geographic system boundary was defined as the borders of North America (US and Canada) and our temporal boundary was 1970 to 2020. The three main mixtures of PBDEs were quantified for four product categories; electronics and electrical equipment (EEE), personal vehicles, and textiles and foam furniture. We estimated that the total inventories of penta, octaand decaBDE were 45 000, 20 000 and 250 000 tonnes, respectively. The greatest product usage was in foam furniture (60% of penta), vehicles (90% of octa) and vehicles, textiles, and electronics (30, 25 and 25% of deca, respectively). 90% of foam furniture (and hence most of penta) was estimated to be landfilled in North America by 2020. In comparison, 45% of deca in electrical and electronic equipment was expected to be exported off-shore as "used" equipment destined for dismantling and questionable disposal. The remaining 29 and 17% of deca in electrical and electronic equipment was estimated to be landfilled and recycled in North America, respectively. Whereas most of the penta- and octa-containing products will cease to enter the waste stream by 2020, the mass of deca-containing products entering the waste stream will continue to increase after 2020. These estimates represent an optimistic scenario since they are based on single sale life spans and hence neglect resale or reuse as "used" product life spans which is common with furniture and some vehicles.

TP009 Developing an Environmentally Sustainable Flame Retardant

J.W. Davis, Dow Chemical Company; I. Beulich, H. Hollnagel, C. Lukas, C. Rowlands, The Dow Chemical Company

Flame retardants (FR) are routinely used to delay ignition and to slow the spread of flame. The growing use of brominated flame retardants (BFR) in consumer products has resulted, in part, from the increased stringency

in national fire safety regulations. Recently some BFRs such as selected polybrominated dipehnyl ethers have been banned or discontinued due to issues surrounding their environmental persistence, bioaccumulation and toxicity (i.e., PBT) characteristics. Currently the BFR hexabromocyclododecane (HBCD), which is used in polystyrene insulation foam boards, is facing a phase-out from commercial use in Europe in August 2015 (REACH Annex XIV Authorization) and is under consideration in the Stockholm POP convention. In order to develop more environmentally sustainable flame retardants to replace HBCD we have developed a tiered approach for assimilating data and information on the environmental partitioning/ persistence, bioaccumulation, and toxicity of potential candidates. During the development of viable candidate FR, they were initially screened using quantitative structure–activity relationships (QSARs) models to estimate persistence, bioaccumulation potential and aquatic toxicity. The use of QSARs aids in prioritizing chemicals for laboratory testing and promotes the more efficient use of chemical testing resources. FR candidates exhibiting positive environmental fate characteristics of low persistence, bioaccumulation potential and aquatic toxicity were then identified as possible candidates for further development including developing a base set of environmental and health information using laboratory testing protocols. These laboratory tests were conducted on candidate FRs specifically to generate information which provided refinements of the initial QSARs estimates of the environmental fate such as partitioning, persistence and bioaccumulation. Through the combination of *in silico* modeling and laboratory testing a new class of brominated polymeric flame retardants (PFR) has been developed that can provide effective flame retardant performance in polymer systems that previously relied on HBCD. This polymeric material exhibits improved sustainability and has superior environmental profile to that of HBCD and other lower molecular weight FR molecules.

TP010 Bioaccumulation of Selected Halogenated Organic Flame Retardants in the Lake Ontario food web

B.P. de Jourdan, Department of Environmental Biology / School of Environmental Sciences; D.C. Muir, C. Teixeira, J. Epp, H. Embers, X. Wang, Environment Canada / Aquatic Ecosystem Protection Research Division; M. Keir, Environment Canada / Water Quality Monitoring and Surveillance; S. Backus, Water Quality Monitoring and Surveillance Division, Science and Technology Branch / Environment Canada

In this study the bioaccumulation and concentrations in surface waters of a wide range of non-legacy halogenated organic compounds (HOCs) was determined in Lake Ontario food web. The same HOCs were also determined in lake waters and zooplankton from Lake Erie and a large, remote, inland lake (Lake Opeongo in Algonquin Park, ON). Large volume samples of surface waters (100 L; 4 m depth) were collected over the period 2006-2010 at mid-lake sites in each lake by direct pumping through a glass fiber cartridge followed by primary and secondary XAD-2 resin columns. Zooplankton (>100 um) and mysids were obtained by vertical hauls at the same sites as water while forage fish and lake trout from Lake Ontario were obtained in fall collections. Extracts were screened for 27 individual BDEs (Br3-Br10) and 30 other HOCs (Br3-Br6 compounds/ PBDE replacements and chlorinated flame retardants) using GC-electron capture negative ion mass spectrometry (GC-EC NIMS) with a HP-5MS and RTX-1614 capillary columns. Pentabromoethylbenzene (PBEB), 1,3,5-tribromo-2-methoxy-4-methylbenzene (Br3MeBz), 2,4,6-tribromophenyl allyl ether (TBP-AE) and 2,4,6-tribromophenyl ether 2,3-dibromopropyl (TBP-DBPE) were the most prominent non-PBDE HOCs in Opeongo lake waters; present at sub-pg/L concentrations. A larger suite of HOCs were detectable in Lake Ontario and Lake Erie waters including Dechlorane Plus (DDC-CO), Br3MeBz, PBEB, TBP-DBPE, 1,2,3,4,5-pentabromobenzene (PeBBz), penta- and hexabromotoluene, and 2-ethyl-1-hexyl 2,3,4,5-tetrabromobenzoate (EH-TBB) although concentrations were near or at MDLs. A wide range of Br3-Br6 compounds were detected in zooplankton and mysis from Lake Ontario including Br3MeBz, 1,3,5-tribromobenzene (TBB), tetrabromoxylene (TBX), bis(tribromophenoxy)ethane (BTBPE), PBEB, BPTE, pentabromocyclododecene (PBCDD), undecachloropentacyclooctadecadiene (aCl11DP), as well as 4,4'-dichlorodiphenyl sulfone (BCPS). Lake Opeongo zooplankton had a more limited suite, with Br3MeBz, TBP-AE and PeBBz present at low pg/g (wet wt) concentrations. BDE47, BDE153, Br3MeBz and PBCDD had the highest trophic magnification factors in the Lake Ontario food web based on the slope of the log (lipid wt) concentrations versus trophic level. BCPS, TBX, 1,3,5-TBB and BTBPE showed trophic dilution while other compounds, e.g., BDE202 (and other hepta- and octaBDEs) were detectable in most samples but showed limited increases with trophic level.

TP011 Organophosphorous flame retardants in components of a Lake Ontario food web

L. Bestvater, Freshwater Institute / Department of Chemistry; G. Tomy, University of Manitoba; C. Marvin, G. MacInnis, Environment Canada / Water Research and Technology Directorate; S. Backus, Environment Canada / Water Research and Technology Directorate; R. Letcher, Environment Canada; E. Sverko, Environment Canada / Water Research and Technology Directorate

Organophosphate flame retardants (OPFRs) have been used for many decades; however, due to the recent ban of PBDEs, their use has increased greatly in the past couple of years. Increasing use has led to their detection in many environmental compartments including ground water, surface water, wastewater, air, sediment, dust, and human biological samples, both in Europe and North America. Considering these FRs are now ubiquitous, there is very little information on their fate in the environment and their effects on wildlife. We developed a method for the analysis of 21 OPFRs based on HPLC electrospray negative ion tandem mass spectrometry and applied the method to the analysis of biota recently collected from Lake Ontario. The OPFRs that were most consistently detected within all levels of the food web were TEP, TCEP, TCPP, TDCP, TPP, TBP, TBEP, and EHDPP.

TP012 Degradation of flame retardants by anaerobic bacteria in sewers

L.A. Rodenburg, Rutgers University / Department of Environmental Sciences; S.L. Capozzi, Rutgers, the State University of New Jersey; J. Guo, Rutgers, the State University of New Jersey / Department of Environmental Science; A.J. Murphy, Rutgers, the State University of New Jersey; D. Fennell, Rutgers University / Department of Environmental Sciences

Many organic flame retardants are halogenated compounds that are persistent, toxic, and bioaccumulative. They are usually resistant to aerobic biodegradation. However, like other organochlorine and organobromine compounds, they are susceptible to microbial dehalogenation under anaerobic conditions. Recently, we used monitoring data on concentrations of polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) in wastewater treatment plant influents and effluents to demonstrate that these classes of contaminants are extensively dechlorinated within the wastewater collection system. Here we present evidence that dechlorane plus, brominated diphenyl ethers (BDEs), and hexachlorocyclododecane (HBCD) are dehalogenated by bacteria present in sewers. We constructed simulated sewers using sewer sediment that was kept under methanogenic conditions. The sewer sediment was spiked with dechlorane plus and HBCD, both of which were dehalogenated within 35 days with no apparent lag time. Evidence of native BDE debromination was difficult to discern due to matrix effects, but debromination products such as BDE17 were detected in our simulated sewer system. This suggests that these persistent organic pollutants are transformed in the sewer, and that the sewer serves as an anaerobic pretreatment system that destroys recalcitrant organics before they reach the aerobic wastewater treatment plant.

TP014 Hexabromocyclododecane Flame Retardant Contamination Near Antarctic Research Stations

D. Chen, Carleton University / Chemistry, Ecotoxicology and Wildlife Health Division, Wildlife and Landscape Directorate, Science and Technology Branch, Environment Canada, National Wildlife Research Centre, Southern Illinois University Carbondale / Cooperative Wildlife Research Laboratory; R.C. Hale, M.J. La Guardia, Virginia Institute of Marine Science / Environmental & Aquatic Animal Health; D. Luellen, H. Geisz, Virginia Institute of Marine Science; S. Kim, Moss Landing Marine Laboratories

To date, little data on the occurrence of the current-use flame retardant hexabromocyclododecane (HBCD) in Antarctica are available. Most studies regarding persistent organic pollutants (POPs) in Antarctica are based on the premise that contamination is low and arises from long-range transport, rather than local human activities. Indeed, historical POPs are banned from Antarctica under international treaty. However, we observed 226 ng/g dry weight (dw) of ∑HBCD (sum of a-, b- and g- diastereomers) in indoor dust from the US research base, McMurdo Station and 109 ng/g in Scott Station (New Zealand) dust; rivaling those reported in dust from their host countries. Station wastewater serves as a conduit for contaminants to the local outdoor environment, especially in lieu of exhaustive treatment. Sewage sludge collected from the recently completed McMurdo

and Scott treatment facilities exhibited ∑HBCD of 45 and 69 ng/g dw, respectively. Near McMurdo, ∑HBCD levels in surficial marine sediments and aquatic biota (invertebrates and fish) were as high as 2350 ng/g (total organic carbon basis) and 554 ng/g (lipid weight: lw) in marine sponges. Levels declined dramatically with distance from McMurdo. Overall, marine organisms collected within 25 km of McMurdo wastewater outfall contained HBCD levels rivaling those reported in Arctic wildlife. Adélie penguins also contained HBCD levels (2.7-124 ng/g lw) comparable to Arctic birds. Surprisingly, g-HBCD was the major diastereomer in several Antarctic biota, including penguins. Our results illustrate that, despite efforts to eliminate contaminant releases and restrict POP importation, Antarctic research stations still serve as local sources to the pristine Antarctic environment.

TP015 Alternative halogenated and organophosphate flame retardants: estimated phys-chem properties and persistence in indoor and outdoor environments

D. Serodio, University of Toronto / Department of Earth Sciences; A. Krol, University of Toronto / Department of Chemical Engineering and Applied Chemistry; X. Zhang, University of Toronto Scarborough / Chemistry / Environmental Sciences; M.L. Diamond, University of Toronto / Department of Earth Sciences; A. Blum, V. Singla, M. Dedeo, Green Science Policy Institute

In the wake of the manufacturing ban and discontinuation of penta- and octaBDEs and the pending discontinuation of decaBDE, an increasing number of alternative halogenated flame retardants (FRs) are being used in products. Some replacement FRs are being found in indoor dust, urban watersheds and remote Arctic air. Though currently in use, there is very little information regarding the physical-chemical properties, environmental fate, and persistence of these chemicals. As such, we compiled a list of over 70 compounds that are used or marketed as halogenated flame retardants, including 58 that are brominated, 10 chlorinated, 5 containing Br and Cl, and 7 containing Br and/or Cl with phosphorus. Their physical-chemical properties were obtained using various QSAR models available through the public domain and privately-owned estimation software. Values of $\log K_{OW}$ ranged from 0.85 for 2,2-Bis(bromomethyl)propane-1,3-diol (DBNPG) to 16.89 for 1,2,4,5-tetrabromo-3,6-bis(2,3,4,5,6-pentabromophenoxy)-benzene (4'-PeBPOBDE208). Values of $\log K_{AW}$ ranged from -18.83 for N-N-Ethylene-bis(tetrabromophthalimide (EBTEBPI) to -1.48 for Mirex. Values of $\log K_{OA}$ ranged from 4.80 for Tris(2-chloroethyl) phosphate (TCEP) to 28.63 for EBTEBPI. Their overall persistence (P_{ov}) and their long-range transport potential (LRTP) were modeled using the OECD Pov & LRTP Screening Tool. Their fate and residency in indoor spaces were estimated using an adapted Multi-media Indoor Model (MIM). For comparative purposes, tetra-, penta-, octa-, and decaBDE were also modelled. The following chemicals were recognized to have the potential for long residence times in the indoor and outdoor environment: Dechlorane 602 (DDC-DBF), Dechlorane 604 (HCTBPH), Dechlorane Plus (DDC-CO), EHTBB, 4'-PeBPOBDE208, Bis(2-ethylhexyl) tetrabromophthalate (BEH-TEBP), EBTEBPI, 1,2-Bis(2,4,6-tribromophenoxy)ethane (BTBPE), tetrabromobisphenol A diallyl ether (TBBPA-BAE), tetrabromobisphenol A dimethyl ether (TBBPA-BME), ethoxylated tetrabromo bisphenol A diacrylate (TBBPA-BHEEBA), octabromobisphenol S (TBBPS-BDBPE), octabromotrimethylphenyllindane (OBTMPI, or OBIND), and decabromodiphenylethane (DBPDE).

TP016 Two "new" organophosphorus flame retardants detected in the indoor and outdoor environment

S. Brandsma, VU University, Institute for Environmental Studies; C. De Wit, U. Sellstrom, Stockholm University; P. Martinez Moral, VU University, Institute for Environmental Studies; P. Leonards, VU University, Institute for Environmental Studies / Chemistry & Biology

Some brominated flame retardants (BFRs) have unintended negative effects on the environment and human health. During the last decade an increasing number of reports have presented evidence of these negative effects caused by some BFRs. Less toxic alternatives appear to be available already but comprehensive information on their possible toxicological effects and exposure are lacking. The European Commission-funded project ENFIRO investigated halogen-free substitution options for some BFRs resulting in a comprehensive dataset on viability of production and application, environmental safety, risk assessment, and life cycle assessment. In total 15 halogen-free flame retardants (HFFRs), consisting of metal-, organic-, and nano-based FRs, as alternatives for decaBDE, TBBP-A, and brominated polystyrenes were selected. One of the tasks was to investigate the exposure of the HFFRs. Field monitoring of organic HFFRs was conducted in several European countries to assess the environmental levels arising from known sources (e.g., a wastewater treatment plant) as well as background contamination in a region. To assess indoor contamination, sampling was performed in microenvironments where products containing FRs are used (e.g., homes, offices). The focus of this study was on the analysis of the alternative flame retardants resorcinol bis (diphenylphosphate) (RDP) and bis phenol A bis (diphenylphosphate) (BDP) in dust, sediment and sewage sludges. RDP and BDP were detected in various dust samples from Sweden, The Netherlands and Greece. BDP was the most predominant FR in the dust samples with the exception of dust samples taken from leather sofas and the highest concentrations were found on electronic equipment (flat screen TVs, PCs, laptops). The highest concentrations were found in the dust collected on the electronic equipment with BDP levels up to 700 000 ng/g. The levels of RDP were 10-200 times lower than the BDP levels. Sediment samples from The Netherlands, Germany, Belgium, France and Norway showed that RDP and BDP were present in all sediment samples and sewage sludge. To our knowledge this is the first time that RDP and BDP have been detected in the outdoor environment. Interesting to note is that the ratio of RDP/BDP is different between the locations, and RDP is the predominant FR in sediment in contrast to dust were BDP is predominant.

TP017 Aryl Phosphate Esters Within a Major PentaBDE Replacement Product Induce Cardiotoxicity in Developing Zebrafish Embryos: Potential Role of the AHR

D.C. Volz, University of South Carolina / Department of Environmental Health Sciences; S.P. McGee, University of South Carolina; A. Konstantinov, Wellington Laboratories; H.M. Stapleton, Duke University

Firemaster 550 (FM550) is an additive flame retardant formulation of brominated and aryl phosphate ester (APE) components introduced as a major replacement product for the commercial polybrominated diphenyl ether mixture (known as PentaBDE) used primarily in polyurethane foam. However, little is known about the potential effects of FM550-based ingredients during early vertebrate development. Therefore, we first screened the developmental toxicity of each FM550 component using zebrafish as an animal model. Based on these initial screening assays, we found that exposure to the brominated components as high as 10 μM resulted in no significant effects on embryonic survival or development, while exposure to triphenyl phosphate (TPP) or mono-substituted isopropylated triaryl phosphate (Mono-ITP) – two APEs comprising almost 50% of FM550 – resulted in targeted effects on cardiac looping and function during embryogenesis. As these cardiac abnormalities resembled aryl hydrocarbon receptor (AHR) agonist-induced phenotypes, we then exposed developing embryos to TPP or Mono-ITP in the presence or absence of an AHR antagonist (CH223191) or AHR2-specific morpholino. Based on these studies, we found that CH223191 blocked heart malformations following exposure to Mono-ITP but not TPP, while AHR2 knockdown failed to block the cardiotoxic effects of both components. Finally, using a cell-based human AHR reporter assay, we found that Mono-ITP (but not TPP) exposure resulted in a significant increase in human AHR-driven luciferase activity at similar nominal concentrations as a potent reference AHR agonist (β-Naphthoflavone). Overall, our findings suggest that two major APE components of FM550 induce severe cardiac abnormalities during early vertebrate development.

TP018 Do TBB and TBPH affect Steroidogenesis in Female Rainbow Trout (Oncorhynchus mykiss)?

L. Bestvater, Freshwater Institute / Department of Chemistry; L. Peters; K. Dangerfield, University of Manitoba; S. Mittermuller, Freshwater Institute; G. Tomy, University of Manitoba / Department of Chemistry

Firemaster 550 (FM 550) is one of the major replacement mixtures for the recently banned pentaBDE. The two main components of FM 550 are 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) and bis (2-ethylhexyl) tetrabromophthalate (TBPH). Recently, these two chemicals have been detected in various environmental matrices. TBB and TBPH have been detected in house dust, biosolids from waste water treatment plants, marine mammals, and the atmosphere. The increased use of these novel FRs raises concerns about their release into the environment and the toxicological effects it may have on the exposed organisms. This study investigates the possible effects of TBB and TBPH on the *in vitro* steroidogenic activity of rainbow trout (*Oncorhynchus mykiss*) ovarian follicles. Our preliminary results show that

there were no significant differences in oocyte estradiol (E2) or estrone (E1) production in the female trout with increasing TBB or TBPH concentrations under both basal and hCG stimulated conditions. However, there was an evident increase in hormone levels from the basal conditions to the hCG stimulated conditions in all hormones tested. The hormone concentrations of E2 and E1 under stimulated conditions for the two compounds were comparable to each other and ranged from 700-950 pg/follicle and 25-40 pg/follicle, respectively. While there was no overall dose-response effects we did observe a significance difference in E1 concentrations compared to the control at 100 pg/uL TBB treatment under hCG stimulated conditions.

TP019 In ovo exposure to an organophosphate flame retardant, tris(2-butoxyethyl) phosphate, in zebra finches

M.L. Eng, Simon Fraser University; R.J. Letcher, Environment Canada / Science and Technology Branch, Ecotoxicology and Wildlife Health Division; T.D. Williams, Simon Fraser University; J.E. Elliott, Environment Canada

Tris(2-butoxyethyl) phosphate (TBEP) belongs to a group of chemicals collectively known as organophosphate (triester) flame retardants (OPFRs). OPFRs are widely used as flame retardants and plasticizers in consumer products. As other flame retardants such as polybrominated diphenyl ethers (PBDEs) have been phased out, certain OPFRs are increasing in production. TBEP is currently used as a plasticizer, lubricant and flame retardant, and has been detected in several abiotic compartments, as well as in fish and birds. Despite the widespread use of OPFRs and their detection in wildlife, very little is known of their metabolic processes or toxicological effects. The objectives of the present study were to assess the embryonic metabolism of TBEP, and the effects of *in ovo* exposure to TBEP on survival, development and reproduction in a model songbird species, the zebra finch (*Taeniopygia guttata*). To assess embryonic metabolism of TBEP, zebra finch embryos were exposed to TBEP via egg injection. Either a vehicle control (DMSO) or 50 µg TBEP/g egg was injected into the albumin the day the egg was laid. A subset of eggs was collected at several time points over the 14 day incubation period (3 eggs per dose group on each of day 0, 5, 10 and 14 of incubation), and the whole contents of the eggs were measured for TBEP concentrations. There were no significant differences between concentrations in day 0 (unincubated) eggs compared to day 5 and day 10 eggs, however day 14 eggs had significantly lower concentrations of TBEP, which suggests that TBEP metabolism in zebra finches embryos occurs primarily during late incubation. We are conducting further TBEP egg injection studies in zebra finches to assess effects of early exposure to TBEP on the survival, growth, reproduction and physiology of songbirds.

TP020 The effects of organophosphate flame retardants on endocrine signaling pathways in human cancer cell lines

A. Reers, Simon Fraser University; J.E. Elliott, Environment Canada; T.V. Beischlag, Simon Fraser University / Faculty of Health Sciences

Since the ban of polybrominated diphenylethers (PBDEs) as flame-retardants in many countries, they have been widely substituted by organophosphate flame retardants (OPFRs). However, available toxicological data and the information concerning the potential adverse effects of OPFRs are limited. This study examines the ability of six OPFRs to disrupt estrogen, androgen and arylhydrocarbon receptor signaling pathways. The compounds of interest were: triethylphosphate (TEP), tricresyl phosphate (TCP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP), tris(2-carboxyethyl)phosphine (TCEP), tris(2-chloroisopropyl) phosphate (TCPP) and tris(2-butoxyethyl) phosphate (TBEP). Endogenous target gene expression was measured by quantitative real-time PCR using the well-established prostate cancer, LNCaP, and endometrial cancer, ECC-1 cell lines. In addition, concomitant alterations in protein expression were assessed. Both cell lines were exposed to an OPFR individually with concentrations ranging from 10 nM – 20 µM and in combination with a synthetic androgen R1881 and β-estradiol (E2), respectively. TBEP and TCP had no transcriptional effects on AR, or ER signaling in LNCaP and ECC-1 cells. At the mRNA level TEP in combination with R1881 decreased the level of prostate-specific antigen expression in LNCaP and increased the level of pS2 ECC-1 cells in combination with E2. Initial results for the AhR signaling pathway suggested an increase of cytochrome P450 1A1 mRNA expression in ECC-1 cells when exposed to TBEP, TCP or TEP at concentrations of 10 µM and 20 µM. This is of significance for the estrogen signaling pathway as it has been shown that dioxin-dependent AhR activation can repress ER signaling. This data is critical as the interference of these compounds with the aforementioned signaling pathways is essential in the developmental and reproductive processes of wildlife and humans and ultimately can lead to hormonal imbalances and irregularities.

Predicting and Ensuring Models and Test Data

TP021 Influence of diet and starting age on the performance of the amphipod Hyalella azteca or the midge Chironomus dilutus in water or sediment exposures

N.E. Kemble, USGS – Biological Resources Division / Columbia Environmental Research Center; D.K. Hardesty, J.P. Hughes, USGS / Columbia Environmental Research Center; C.G. Ingersoll, US Geological Survey / Columbia Environmental Research Center; C.D. Ivey, USGS – CERC / Columbia Environmental Research Center, USGS / Toxicology; J.L. Kunz, US Geological Survey / Columbia Environmental Reserach Center

The current USEPA and ASTM chronic sediment testing methods start with < 8-h old midge and with about 8-day old amphipods. The primary objectives of this project are to: (1) determine if older life stages of midge or younger life stages of amphipods can be used to improve performance of test organisms in long-term water or sediment exposures; (2) evaluate the influence of improved diet on amphipods or midge in chronic water-only or whole-sediment toxicity tests; and (3) evaluate the relative sensitivity of different ages of midge in copper exposures or in exposures to dilutions of contaminated sediments. Methods will be described for culturing known-age midge or amphipods to start exposures. Younger midge larvae (1st instar) have been shown to be much more sensitive to contaminants in acute exposures compared to older larvae. However, inconsistent control performance has been observed in water or sediment exposures started with younger midge. Studies have shown improved survival and emergence in water or sediment exposures started with 4- to 7-d-old midge larvae (typically >80% survival in exposures conducted for up to about 16 days) compared to exposures started with < 8-h old midge larvae (frequently < 80% survival in exposures conducted for up to 20 days). Less-than-8-h-old larvae would be at the 1st instar, 4-d-old larvae would be about 2nd instar, and 7-d-old larvae would be about 3rd instar. Various improved diets (either quantity or quality) in amphipod exposures have resulted in amphipods reproducing by about Day 24 in water-only exposures when studies are started with 8-d old amphipods compared to after Day 28 in exposures with the addition of standard food of 1 ml of YCT fed daily. If amphipods begin to reproduce while in sediment before Day 28, it will be difficult to quantitatively isolate these second generation amphipods from sediment. The two diets that are currently being evaluated include either (1) a ramped amount of YCT or (2) a ramped amount of diatoms (*Thalassiosira weissflogii* (ReedMariculture) and Tetramin. Starting water or sediment exposures with 4-d-old rather than 8-d-old amphipods resulted in delaying the onset of reproduction to Day 28 with >5 young/female produced by Day 42.

TP022 Optimizing the Performance of Hyalella azteca in Chronic Toxicity Tests: Results of Feeding Studies with Various Foods and Feeding Regimes

T.J. Norberg-King, USEPA / ORD, NHEERL, Mid-Continent Ecology Division; T. Highland, USEPA; R.J. Hockett, USEPA / Mid-Continent Ecology Division; D.J. Hoff, Ecotoxicology Analysis Research / Mid-Continent Ecology Division; D.R. Mount, USEPA / ORD

The freshwater amphipod *Hyalella azteca* is a common organism used for sediment toxicity testing in the United States and elsewhere. Standard methods for 10-d and 42-d sediment toxicity tests with *H. azteca* were last revised and published by USEPA/ASTM in 2000. Under the methods in the manual, a single fixed ration of the food (yeast-wheatgrass-trout chow or YCT) is recommended for the entire 10-day or 42-day test. Recently, we began a series of studies evaluating different foods, combinations of foods, and feeding rates. Clean quartz sand was used as the substrate in these studies to insure that all nutrition came from the food added rather than from sediment. For the 42-day chronic amphipod tests, we compared various foods, including diatoms, wheatgrass, TetraMin, YCT and several of these foods in combination. We monitored survival, growth, and reproduction at intervals of 7, 10, 14, 21, 28, 35, and 42 days of exposure. It was immediately clear that the standard fixed ration of 1 ml YCT/chamber-day limited amphipod growth and reproduction in the latter portions of the 42-d exposure. Higher growth and reproduction was achieved with a variety of alternate foods or feeding schedules, with 42-d weights of *Hyalella* in excess of 0.8 mg dwt/

individual and reproduction over 10 young per female. Results of several experiments will be presented along with their implications for future revisions of the EPA/ASTM test method. This abstract does not necessarily reflect EPA policy.

TP023 Assessment of six indicators for algal cell viability

A. Calomeni, Clemson University; J.H. Rodgers, Clemson University / Shool of Agricultural, Forest, and Environmental Sciences

Reliable accurate and unambiguous response parameters are needed to discern responses of algae to exposures of phytotoxic compounds or elements. The focus of this research was to analyze the utility of six bioindicators using two prokaryotic algae (*Planktothrix agardhii* and *Microcystis aeruginosa*) and one eukaryotic alga (*Pseudokirchinerialla subcapitata*). Chlorophyll *a,* pheophytin *a* and 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) can be useful for determining responses of an assemblage to a particular stimulus. Cell density, percent staining due to neutral red and erythrosin b can be used to discern differences in an algal population after an exposure. To prepare known mixtures of live and dead cells, algae cultures were boiled for five minutes and mixed with viable algae in the logarithmic growth phase to create cell suspensions containing 0, 25, 50, 75 and 100% viable cells. The six parameters were used to discern differences in the viability of the cell suspensions. For all algae studied, measurable differences could be discerned using chlorophyll *a*, INT absorbance, cell density, and percent erythrosin b stained cells. Chlorophyll *a* was highly positively correlated with viability while staining due to erythrosin b was highly negatively correlated with algal viability ($R^2 > 0.92$). INT absorbance as well as erythrosin b staining may be added to algal viability determinations in order to more accurately discern responses of either an assemblage or an algal population after a phytotoxic exposure.

TP024 Effects of Feeding and Organism Loading Rate on PCB Accumulation by Lumbriculus variegatus in Sediment Bioaccumulation Testing

L.P. Burkhard, D. Hubin-Barrows, USEPA; N. Billa, Wildlife International; T.L. Highland, USEPA / ORD, NHEERL, Mid-Continent Ecology Division; R.J. Hockett, USEPA / Mid-Continent Ecology Division; D.J. Hoff, Ecotoxicology Analysis Research / Mid-Continent Ecology Division; D.R. Mount, USEPA / ORD; T.J. Norberg-King, USEPA / ORD, NHEERL, Mid-Continent Ecology Division

Sediment bioaccumulation test methods published by USEPA and ASTM in 2000 specify that the *Lumbriculus variegatus,* a freshwater oligochaete, should not be fed during the 28-day exposure and recommends an organism loading rate of total organic carbon in sediment to organism dry weight of no less than 50:1. It is commonly observed with sediments from Superfund sites that the *L. variegatus* weights decrease over the 28-day exposure period and that many tests are performed with ratios of total organic carbon in sediment to organism dry weight of less than 50:1, particularly when sediment organic carbon is low (e.g., < 0.5%). A series of bioaccumulation tests were performed to evaluate the influence of feeding and loading rate upon the growth and contaminant bioaccumulation by the organisms. With sediments contaminated with polychlorinated biphenyls (PCBs), accumulations of PCBs in *L. variegatus* were similar between loading rates of approximately 50:1 and 25:1. For loading rates greater than 50:1, accumulation of PCBs tended to be less than those at the 50:1 loading rate. In contrast, for loading rates less than 25:1, accumulation of PCBs tended to be greater than those at the 50:1 loading rate. In most but not all sediments tested, PCB accumulation patterns were similar at all levels of chlorination. Weight change during the exposures varied considerably among sediments, but organisms receiving supplemental food (TetraMin®) had higher ending weights compared to unfed organisms tested in the same sediment. Organisms provided flaked fish food in the form of fine flakes had higher ending weights than those fed the same food in the form of a blended suspension of the same food. Results from both the loading rate and feeding exposures will be presented and recommendations discussed. This abstract does not necessarily reflect USEPA policy.

TP025 Single Particle-ICP-MS "The" Metrology Tool for Engineered Nanoparticles in Environmental Matrices

C. Stephan

Nanomaterials containing metals are increasingly being used in consumer, industrial, and medical products. These materials are subsequently being released into the environment. Methods for detecting, quantifying, and characterizing these materials in complex matrices are critical for the eventual understanding of their implications to environmental quality and human health. This work describes Single Particle Inductively Coupled Plasma Mass Spectrometry (SP-ICP-MS) as a metrology tool in assessing the fate of engineered Nanoparticles in complex aqueous samples. Single Particle (SP-ICP-MS) measures Both Ionic (ng/L) and Particle Concentration (part/mL), measures Particles Size and Size distribution, measures nanoparticles composition and tack particles agglomeration and dissolutions. Two case studies will be discussed; 1) Silver nanoparticle transformation in surface water, the effects of salinity, particle concentration, particle size, and particle surface chemistry. 2) Nanoscale Zero Valent Iron (NZVI) in environmental matrices employing the use of *Dynamic Reaction Cell with Ammonia* in order to reduce spectral background levels improving particle size detection.

TP026 Impact and Importance of Time Factor in Toxicogenomic-based High-throughput bioassays

C. Gao, Northeastern University / Civil and Environmental Engineering; A. Gu, Northeastern University / Department of Civil & Env Engineering, Biotechnology Initiative Program; D. Weisman, University of Massachusetts, Boston / Department of Biology

The advances of toxicogenomics have brought forth a range of high-throughput approaches for toxicity tests based on the molecular-level responses to toxicants of organisms. A typical assay monitors the expression levels of a large amount of genes/proteins in parallel, which greatly reduce the time and cost compared to traditional animal tests. However, the control of data quality and appropriate interpretation of data have been challenging. In this study, we investigate the influences of time factor on the toxicogenomic assay results by carrying out an experiment that includes a continuous experiment with real time reading. We applied our gfp-fused *E coli.* whole-cell array containing 108 stress-response (i.e., oxidative stresses, DNA damages etc.) related genes to 12 chemicals of varying toxicological characteristics, and obtained the real time gene expression levels involved in different stress response pathways simultaneously for 2 hours. By examining the resulting time series data, we found several possible sources of systematic errors that may impact the results. For example, the slight differences in growing stages of cells due to small inoculation deviation across plates, may consequently lead to different interpretation and comparison of results because of the different statuses of cells. Furthermore, considering the inherent correlation between data at adjacent time points can help confirm whether an abnormal value is due to significant biological response or irrelevant experimental errors. Our result shows that incorporation of time factor into the data dimensions can help identify and eliminate experimental anomaly. On the other hand, the high-dimensional data produced by such experiments are hard to analyze, and calling for innovative application of bioinformatics data analysis methods. In our study, by implementing data classification approaches including hidden Markov models and random forest, we demonstrated that the time series data contained rich information regarding dynamic biological processes that is different from static single or isolated data points. As a result, the chemical prediction accuracy using data with fewer genes but longer time range is shown to be superior to those with more genes but shorter time range. This finding may indicate a new way of finding relevant genes when looking for the toxicological mechanisms of a certain chemical by considering the dynamic other than "snap shot" of the behaviors of genes.

TP027 Reconsidering the use of ethinylestradiol and testosterone as positive controls in mollusk toxicity tests for endocrine disrupting chemicals

L.L. Lagadic, INRA / Ecotox & Quality of Aquatic Environ; A. Barsi, INRA / UMR ESE; V. Ducrot, INRA / Ecotoxicology and Quality of Aquatic Ecosystems; A. Giusti, University of Liege / Laboratory of Animal Ecology et Ecotoxicology

An OECD guideline is currently under development for testing endocrine disrupting chemicals (EDCs) with the freshwater mollusk *Lymnaea stagnalis*. This guideline aims at conducting *in vivo* tests to assess effects of chemicals on apical endpoints relevant to endocrine disruption. Literature studies suggest that classical reproductive endpoints (*i.e.*, numbers of clutches and eggs produced per snail) are sensitive enough to allow the detection of different modes of action of EDCs in *L. stagnalis* since fecundity and oviposition are under different hormonal controls in this species. Nevertheless, establishing relationships between changes in snail reproduction and EDC modes of action, as described in vertebrates, is not straightforward due to the lack of knowledge on steroid metabolic pathways and steroid-receptor-mediated

effects. Estradiol and testosterone (or their synthetic equivalents ethinylestradiol and methyltestosterone) are often used as 'reference' compounds (*i.e.*, positive controls) in most studies on chemical-induced endocrine disruption in mollusks. However, this is under debate since several recent reviews questioned the role of vertebrate-type estrogens in controlling mollusk reproduction. n the present study, we measured classical reproductive endpoints as described in the draft OECD guideline and also some additional endpoints (egg abnormalities) expected to be particularly responsive to endocrine disruptors in *L. stagnalis* chronically exposed to ethinylestradiol and testosterone. We showed that uptake of testosterone from the exposure medium occurs in *L. stagnalis* and resulted in increased esterified testosterone tissular levels. Internal concentrations of ethinylestradiol are currently quantified. In contrast to what we expected, no apical endpoint related to the fecundity or to the egg quality was significantly modified in the tested concentration ranges of ethinylestradiol and testosterone. This result is discussed with respect to the presence of estrogenic (ER) and androgenic (AR) receptors in *L. stagnalis* and other possible interactions (e.g., non ER-mediated) between chemicals and snail reproductive physiology. Finally, we recommend not using vertebrate-type steroids like estradiol and testosterone (or their synthetic equivalents) as reference controls in mollusks reproductive toxicity tests, in order to avoid false negatives or positives when assessing the effects of suspected EDCs.

TP028 Chemical biodegradation: evaluating model selection techniques to identify believable models

A.C. Lee, Simulations Plus, Inc. / Life Sciences; M.S. Lawless, M. Waldman, R. Fraczkiewicz, R.D. Clark, W.S. Woltosz, Simulations Plus, Inc.

Chemicals that degrade slowly persist longer in our environment and therefore are potentially more hazardous than those that readily biodegrade. However, measurement of a chemical's biodegradation half-life can be time-consuming and costly. Thus, *in silico* methods to predict whether a chemical is likely to be readily biodegradable are valuable. We have created artificial neural network ensemble models for chemical biodegradability based on different architectures, i.e., they each contain different numbers of neurons and molecular and atomic-level descriptors. We then examined several model selection methods to determine which method picked the best model as judged by their performance on an external chemical biodegradation data set. The model selection techniques rely on the performance statistics (accuracy, Matthews correlation coefficient, Youden index, etc.) of the training and test sets. We examined several model selection methods including Pareto optimization, Borda voting, overlapping polygons, and dot product. The "Pareto optimization" method is based on statistics computed for the training and test sets. "Borda voting" generates a rank for each performance statistic and selects the model with the lowest rank sum. The "Overlapping polygons" method considers normalized performance statistics represented as radar plots (polygons) for both the training and test set. We then superimpose the polygons and calculate the overlapping area. The model with the largest overlapping area is selected. The "Dot product" selection method computes the dot product of the training and test statistics from which the model with the highest dot product is selected. We then compare and contrast these model selection techniques in order to identify which one selects the best biodegradation model from a pool of candidate models applied to external data.

TP029 Comparison of Model Estimated and Measured Concentrations of Polychlorinated Biphenyl (PCB) Congeners in Sediment Porewater

M.M. Grover, J.M. Conder, ENVIRON International Corp; J. Arblaster, ENVIRON International Corp; V.J. Kirtay, SPAWAR Systems Center San Diego; B. Chadwick, SPAWAR Systems Center San Diego; V.S. Magar, ENVIRON International Corp / Ecology & Sediment Management

The biologically available portion of polychlorinated biphenyls (PCBs) in sediment is related to the level of freely dissolved molecules in the sediment porewater. This bioavailable portion can be either measured directly or estimated with equilibrium partitioning models using synoptic measurements of concentrations of PCBs in bulk sediment and the content of total organic carbon (TOC) and/or black carbon (BC). In this analysis, concentrations of freely dissolved PCB congeners in surface sediment porewater were measured via *in situ* solid phase microextraction (SPME) at ten sampling stations in PCB-impacted sediments at the Puget Sound Naval Shipyard, Bremerton, Washington, USA. Measured concentrations of PCB homologs in porewater were compared to concentrations estimated by one- (TOC) and two-carbon (amorphous OC and BC) models. It was hypothesized the two-carbon model would be more accurate than the one-carbon model. In the one-carbon model, geometric mean concentrations of total PCBs (sum of the tri- to heptachlorinated biphenyls) in porewater were 90 (95% confidence interval is 38-210) times greater than measured concentrations. One-carbon model-predicted concentrations of total PCBs and sums of each homolog group were significantly greater than observed values ($\alpha = 0.05$). Conversely, concentrations of total PCBs in porewater were more accurately estimated by the two-carbon model, with estimates 1.8 (0.49-6.5) times greater than measured concentrations. No significant difference ($\alpha = 0.05$) was found between modeled and observed concentrations of total PCBs and four of the five homolog group summations. Although the two-carbon model appeared to be a more accurate estimate than the one-carbon model, both model predictions were imprecise on a sample-by-sample basis. For example, individual sample two-carbon model-predicted concentrations of total PCBs deviated from measured values by over three orders of magnitude (two-carbon model predictions ranged from 0.2 to 55 times the measured concentrations). In conclusion, measurement of concentrations of PCBs in porewater is recommended as the most accurate and precise approach to estimate bioavailable portions of freely dissolved PCBs in sediment porewater.

TP030 Radioisotope Dating of Sediments Using Cs-137 Time Markers: Where Exactly is the 1954 Horizon?

S.L. Huntley, Cardno Entrix / Sediments and Watershed Integrated Management (SWiM)

The radioisotope cesium-137 (^{137}Cs) is one of the most common radioactive tracers used for assessing recent rates of sediment accumulation in aquatic systems. Primarily released to the environment as fallout from the atmospheric testing of nuclear weapons during the cold war era, the year 1954 has typically been assigned as the year in which ^{137}Cs was first detectable in environmental media (e.g., the 1954 horizon). Similarly, the year 1963 has typically been assigned as the year of greatest ^{137}Cs fallout and the highest detectable^{137}Cs activities in environmental media (e.g., the 1963 peak), particularly in the northern hemisphere. Average sediment accumulation rates can be estimated from the depths of the1954 horizon and the 1963 peak relative to the year in which a sediment core was collected. With a half-life of about 30 years, typical peak sediment ^{137}Cs activities of 2 to 4 pCi/g in 1963 will still be detectable 60 years later in 2023 with estimated residual peak activities ranging from 0.5 to 1 pCi/g, slightly greater than typical detection limits which range from about 0.1 to 0.5 pCi/g. In contrast, the 1954 horizon was potentially lost relatively soon after its first appearance in 1954, with lowest detected ^{137}Cs activities creeping slowly upwards in sediments towards the depth of the 1963 peak. To address this problem, a method was developed to reconstruct the approximate original depth of the 1954 horizon using detectable ^{137}Cs activities at and below the 1963 peak and information on the escalation of atmospheric nuclear weapons testing in the 1950s and early 1960s. Uncertainties associated with the approach are discussed relative to other uncertainties associated with radioisotope dating of sediments as well as those associated with the absence of any information on the historical depth of the ^{137}Cs 1954 horizon.

Aquatic Toxicology

TP031 A long-term effect assessment of tertiary pesticide mixtures on aquatic invertebrate communities using mesocosms

S. Hasenbein, Technische Universität München / Chair of Aquatic Systems Biology; R.E. Connon, University of California, Davis / School of Veterinary Medicine, Aquatic Toxicology Laboratory; J. Geist, Technische Universität München / Aquatic Systems Biology Unit, Department of Ecology and Ecosystem Management; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy, Physiology and Cell Biology; K. Miles, US Geological Survey; S. Lawler, University of California, Davis

Aquatic communities are often adjacent to areas of intense pesticide use that discharge complex mixtures of contaminants into surface waters. Current environmental regulation does not provide water quality criteria for contaminant mixtures and their effects on aquatic communities. The deleterious effects of exposure to contaminants are often more severe when organisms are exposed during early life stages and development – impacts that may sometimes manifest themselves much later in life in the form of reduced ecological fitness, including impaired predator avoidance, growth, and reproductive success. These effects can only be monitored by a

long-term study that takes into account food web systems. The effects of a spring application of tertiary contaminant mixtures of type I (permethrin) and type II (lambda-cyhalothrin) pyrethroid pesticides, along with chlorpyrifos (organophosphate) on the food chain of aquatic invertebrates were investigated. Natural conditions were simulated as closely as possible by using a controlled outdoor mesocosm-system. Subsamples of zooplankton and macroinvertebrates were identified and counted in order to evaluate the effects of pesticide application on the community on a weekly basis. Identified functional groups of invertebrates included primary producers and the various levels of consumers. Fate of all three pesticides was monitored by determining pesticide concentrations in water and sediment samples. Pesticide mixture effects on survival, community structure, and colonization will be presented.

TP032 Arachidonic acid increases reproduction and alters pyriproxyfen-induced sex ratios in Daphnia magna

G.K. Ginjupalli, Clemson University / PHD student / Department of Biological Sciences

The nuclear receptor HR97g is preferentially expressed in reproductive adults in the ovaries. In vitro testing demonstrated that HR97g is activated by pyriproxyfen at pharmacological concentrations and inhibited arachidonic acid (AA) at physiological concentrations. AA is one of three unsaturated fatty acids, along with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) that are preferentially retained in the daphnid ovaries. We hypothesized that AA might be associated with reproduction and environmental sex determination in *Daphnia*. Reproduction assays with the three fatty acids determined that only AA alters female/male sex ratios in the presence of pyriproxyfen. However, the primary effect was not decreased male production, but increased female production. Next, we tested whether enriching an algal diet with AA (*P.subcapitata* has moderate AA levels and *C. vulgaris* has poor AA levels) enhances overall reproduction and sex ratios. AA enrichment of a *C. vulgaris* diet significantly enhances overall fecundity at 1.0 and 4.0 µM by 30% to 40% respectively in the presence and absence of pyriproxyfen. This indicates that AA is crucial in reproduction regardless of environmental sex determination. Overall, dietary supplementation with AA increases reproduction; however, AA is metabolized to a number of eicosanoids. Therefore, we recently investigated the effect of PGE2, a metabolite of AA known to influence egg laying behavior in insects, on daphnid reproduction. Similar to AA, PGE2 enhances overall reproduction in *C.vulgaris* fed animals, but unlike AA there were no effects on sex ratios. In summary, AA supplementation increases reproduction and represses pyriproxyfen-induced environmental sex determination. A diet rich in AA may be protective from some reproductive toxicants and supplementation beneficial for commercially important decapod crustaceans.

TP033 Assessing legacy lead contamination in a unique urban watershed

T. Cyphers, University of Wisconsin – La Crosse / Department of Biology and River Studies Center; S. Ryan, R. Perroy, C. Belby, G. Gerrish, University of Wisconsin La Crosse; T. King Heiden, University of Wisconsin – La Crosse / Department of Biology

The La Crosse River Marsh in La Crosse, WI is a unique urban watershed recognized for its high biodiversity within an urban setting. A local gun club utilized the marsh as a trap shooting range between 1932-1963, resulting in significant quantities of lead (Pb) shot being discharged into the marsh. Despite one salvage operation in 1952, Pb-contamination remains high. We have found Pb shot densities of >43,000 pellets/m^2 and surface sediments contain as high as 23,000 ppb Pb in some areas of the marsh. While reports of malformed invertebrates have been noted, it is not clear whether the Pb is bioavailable, posing ecotoxicological and human health risks. Using a combination of field and laboratory studies, we have begun a multi-disciplinary approach to assess the impacts these contaminated sediments have on the marsh ecosystem. We have measured Pb levels in plants (duckweed), invertebrates (Tricoptera larvae) and fish from a variety of trophic levels at reference sites (0-10 ppm), and areas with low (200-1000 ppm), medium (1,000 – 4,000 ppm) and high (4,000 – 8,000 ppm) Pb-contaminated surface sediments. The Pb from contaminated sediments is bioavailable, and initial findings suggest that Pb levels within duckweed and invertebrate samples correlate with the Pb found within sediments. Standard sediment toxicity assays with zebrafish were used to determine sublethal and acute toxicity caused by exposure to Pb-contaminated sediments. While the sediments were found to attenuate the C-start response and caused subtle developmental toxicity that resembles Pb-toxicity, toxicity was not dose-dependent. Further, since toxicity from Pb-contaminated sediments was similar to reference sediments, we cannot attribute toxicity solely to Pb. We are currently measuring expression of ALA-D in zebrafish larvae as an indicator for Pb-induced toxicity. Our on-going field and laboratory assessments over several seasons will be correlated with various marsh physiochemical properties such as pH, DO, and temperature. Assessments of invertebrate species compositions in conjunction with these field and laboratory bioassays should give us additional insight into the impact that this legacy lead contamination has on water quality and the biotic community that inhabit the marsh.

TP034 Assessment of the sensitivity of sturgeons to dioxin-like compounds through molecular investigation into the aryl hydrocarbon receptor pathway

J.A. Doering, University of Saskatchewan; S.B. Wiseman, University of Saskatchewan / Toxicology Centre; S. Beitel, University of Saskatchewan – Toxicology Centre; J.P. Giesy, University of Saskatchewan / Toxicology Centre and Department of Veterinary Biomedical Sciences; M. Hecker, University of Saskatchewan / School of the Environment & Sustainability

Sturgeons are ancient species of fish and due to their endangered status are of interest in context with ecological risk assessment of anthropogenic stressors. However, current risk assessment attempts are hampered by a lack of knowledge about the sensitivity of sturgeons to toxicants of concern. One class of pollutants of concern is dioxin-like compounds (DLCs) which are known to cause a variety of adverse effects in fishes. All adverse effects of DLCs are believed to be mediated by activation of the aryl hydrocarbon receptor (AhR). However, nothing is known about this pathway in ancient fishes including sturgeons. To characterize the AhR pathway of sturgeons and its responses to exposure with DLCs, juvenile white sturgeon (*Acipenser transmontanus*) were exposed to a model AhR agonist, Beta-naphthoflavone (BNF), via intraperitoneal injection. Whole transcriptome responses were investigated in exposed and unexposed livers of sturgeons using *Illumina* sequencing and RNAseq. Abundances of transcripts of genes known to be regulated by the AhR pathway, including those encoding proteins that catalyse Phase I and II metabolism of xenobiotics, were greater in livers from exposed sturgeon (as great as 21-fold). In addition, abundances of transcripts of genes from pathways not known to be involved in activation of the AhR were different. Furthermore, sequences for 3 AhRs (AhR1, AhR2, AhR3), 2 aryl hydrocarbon receptor nuclear translocators (ARNT1, ARNT2), and one aryl hydrocarbon receptor repressor (AhRR) were identified. It was found that the AhR1 shares greatest amino acid identity to piscine AhR1s, the AhR2 shares greatest amino acid identity with piscine AhR2s, and the AhR3 shares greatest amino acid identity with tetrapod AhR1s. All three AhRs had greatest expression in livers and hearts of sturgeons. Following exposure to BNF, transcript abundance of all 3 AhRs were up-regulated over basal (as great as 5-fold). Sturgeons were found to be highly responsive to exposure to DLCs with numerous critical pathways being affected. Because subtle differences in the amino acid sequence of the AhR result in significant differences in sensitivity between avian species, we further tested the AhRs in sturgeon by investigating the function of each AhR in mediating dioxin-like responses *in vitro*. The goal of these studies is to develop novel adverse outcome pathways for sturgeons and to delineate the toxicological significance of the AhRs in sturgeons.

TP035 Chronic copper toxicity to white sturgeon (Acipenser transmontanus): An evaluation of the influence of control survival on effect concentrations

R.A. Consbrock, US Geological Survey, CERC / Toxicology, Columbia Environmental Research Center; N. Wang, USGS / Columbia Environmental Research Center; C.G. Ingersoll, B.G. Brumbaugh, US Geological Survey / Columbia Environmental Research Center

White sturgeon (*Acipenser transmontanus*) are experiencing poor recruitment in the trans-boundary reach of the upper Columbia River (UCR) in eastern Washington State, USA. Environmental contamination has been identified as one of several contributing factors to the decline of white sturgeon. A previous study, conducted in 2010, indicated newly-hatched white sturgeon were sensitive to copper in a 53-day exposure (EC20 of 1.55 µg Cu/L based on dry weight). However, the control survival in this study was low (71% on test day 25; 68% on test day 53). Water quality characteristics were: hardness 100 mg/L as CaCO3, pH 8.1, DOC 0.4 mg C/L. The mortality

was primarily observed from about 17 to 22 days post hatch during the transition to exogenous feeding. A repeated 24-day copper toxicity test was conducted in 2012 again starting with newly-hatched sturgeon and under similar test conditions as the 2010 study. Mean control survival was 93% at the end of the 24-d exposure, which met the test acceptability requirement of greater than 80% control survival. The EC20 was 1.44 µg Cu/L based on dry weight and 1.72 µg Cu/L based on biomass, which were similar to those obtained in the 2010 53-d exposure. Importantly, these EC20s were below the USEPA chronic water quality criterion adjusted to our test water characteristics. Hence, the higher control mortality observed in 2010 during the transition to exogenous feeding, likely did not influence the sensitivity of white sturgeon to the toxic effects of copper.

TP036 Developmental Toxicity of Louisiana Crude Oil Spiked Sediment to Fish Embryos

B. Hemmer, USEPA / Gulf Ecology Division; C.R. Jackson, USEPA; J. krzykwa, Student Contractor to USEPA; M.G. Barron, USEPA / Gulf Ecology Division; S. Raimondo, USEPA / Gulf Ecology Division, National Health and Environmental Effects Laboratory

Polycyclic aromatic hydrocarbons (PAHs) cause a number of developmental abnormalities in developing fish embryos, which has been primarily demonstrated through water-accommodated fractions. PAH-bound sediment is a more ecologically relevant route of exposure to many developing fish embryos. We conducted a series of assays to assess the extent of developmental abnormalities in fish embryos exposed to sediment spiked with weathered Louisiana Sweet Crude (LSC) oil. Reference sediment (Choctawhatchee Bay, FL) was spiked at nominal concentrations of 1900, 3800, 7500, 15000, 30000 mg/kg. Three separate assays were conducted using the zebrafish (*Danio rerio*), each of which consisted of the five nominal treatments, one sediment control, and one water control. For each assay, one embryo was loaded into each well of a sterile 12-well plate, with treatments randomly placed throughout for a total of 27 replicate embryos per treatment. Observations for development, abnormalities, mortalities, and hatch were made daily for 4 days. At 48 hours, the most commonly observed abnormality was developmental delays, whereas by 96 hr edemas (pericardial, yolk sac) and musculoskeletal (scoliosis) deformities were most common. A reproducible dose-response was observed for all three tests and lowest observable effects concentrations for all developmental abnormalities are reported. Concentrations causing effects and lethality to 20% and 50% of individuals (EC20, EC50, LC20, and LC50) are reported for 48 and 96 hour time periods of each test. Additional methods were developed to test the phototoxicity of LSC-spiked sediment to fish larvae, as well as extending the sediment embryo development assay to the estuarine species, the sheepshead minnow (*Cyprinodon variegatus*).

TP037 Direct and Indirect Effects of the Pesticide Vertimec® 18 EC in a Tropical Freshwater Mesocosm

J. Resende, Department of Hydraulic and Sanitary Engineering; A. Vasconcelos, M. Pereira, E. Espindola, University of São Paulo

A large number of single species laboratory tests were conducted to understand the consequences of pesticides to biological communities, but these studies focused on direct effects. The integration of basic ecological principles into the design and implementation of ecotoxicological research is essential for assessing direct and indirect effects on the aquatic food chains. We performed an outdoor mesocosm experiment (20 days) to evaluate the effects of pesticide Vertimec® 18 EC over the phytoplankton community structure with the presence of tadpoles of the species *Lithobates catesbeianus* (bullfrog) to simulate complex biological relationships. Different treatments were analyzed, including one case of no contamination (control) and two cases of contamination: 0.002 $mL.L^{-1}$ (the concentration expected to reach the water body after application of the pesticide) and 0.008 $mL.L^{-1}$ (the LC_{50} for tadpoles of bullfrogs). Such conditions were considered both with absence and presence of tadpoles. Conductivity, pH, dissolved oxygen, temperature, turbidity, chlorophyll *a* and nutrients were also monitored. The results indicated temporal variability and among treatments, with differences when considering the absence/presence of tadpoles or addition of the contaminant. It was observed a total of 94 phytoplankton species in eight classes, Cyanobacteria (19.1%), Chlamydophyceae (4.3%), Chlorophyceae (45.7%), Zygnemaphyceae (6.4%), Euglenophyceae (10.6%), Cryptophyceae (4.3%), Chrysophyceae (1.1%) and Bacillariophyceae (8.5%). In the treatments with the presence of the pesticide, as an indirect effect of the increase in the concentration of nutrients and mortality of zooplankton, the density of some phytoplankton groups tended to increase (e.g., Cyanobacteria, Cryptophyceae and Euglenophyceae). The richness of phytoplankton decreased in the treatment with tadpoles. In the treatment with the highest concentration of the pesticide, all tadpoles died 24 hours after the beginning of the experiment, indicating that Vertimec® may be more harmful to tadpoles in natural conditions. As a result of the death of the tadpoles, there was an even more significant increase in the concentration of nutrients in the water, with an associated increase in the phytoplankton density. These results indicated that direct and indirect effects occur through the addition of the contaminant and highlighted the suitability of experiments in semi-natural systems to understand these effects.

TP038 Effects of organic and inorganic fungicides on the food choice of a key shredder

J.P. Zubrod, A. Feckler, D. Englert, N. Koksharova, N. Schnetzer, M. Konschak, K. Englert, R. Schulz, Institute for Environmental Sciences; University of Koblenz-Landau; M. Bundschuh, Department of Aquatic Sciences and Assessment; Swedish University of Agricultural Sciences

Decomposer-detritivore-systems are considered responsible for the fundamental stream ecosystem function leaf litter breakdown. Microbial decomposers (especially fungi) are of great importance in this process as they modify the leaf material chemically and physically (i.e., conditioning) resulting in a higher food quality for shredders (i.e., detritivorous macroinvertebrates). Besides other possible pathways, fungicides may affect these systems by altering the leaf-associated microbial communities, causing indirect, i.e. food-quality related, effects on shredders. Thus, the present study investigates this pathway using food choice experiments with the key shredder *Gammarus fossarum* and a set of organic (azoxystrobin, carbendazim, cyprodinil, quinoxyfen and tebuconazole) as well as inorganic (three copper-based substances and sulfur) model fungicides. For this purpose, gammarids were given the choice between leaf discs conditioned in absence (=control) and presence of fungicides. *Gammarus* generally preferred leaves from the control over those conditioned in the presence of organic fungicides. In contrast, leaves conditioned in the presence of inorganic fungicides were preferred over control discs. This indicates that organic and inorganic fungicides act via two distinct mechanisms: Food quality of leaves seems to be generally reduced by organic fungicides probably due to their intended specificity to fungi. On the other hand, inorganic fungicides have a broad-spectrum activity. Consequently, these substances may also affect leaf-associated bacteria and thus lower the competitive pressure for fungi, resulting in promoted fungal growth. This assumption is supported by an increasing leaf-associated fungal biomass with increasing concentration of copper-based fungicides. However, further analyses indicate that an altered fungal community composition may be even more important for gammarids' food choice than fungal biomass. Our results clearly show that organic and inorganic fungicides have the potential to alter the food quality of leaf material for shredders. Moreover, due to their potentially far-reaching consequences on the energy processing within stream ecosystems, such indirect, food quality related effects need to be considered during the environmental risk assessment of fungicides.

TP039 Frequent contamination by neonicoinoid insecticides in Prairie Pothole wetlands: Effects on decomposition and biological function

A. Main, School of Environment and Sustainability; J.V. Headley, K.M. Peru, Environment Canada / Aquatic Ecosystem Protection Research Division, Water Science and Technology Directorate; P. Mineau, Carleton University Campus / National Wildlife Research Centre; A.J. Cessna, Environment Canada / Aquatic Ecosystem Protection Research Division, Water Science and Technology Directorate; C.A. Morrissey, University of Saskatchewan / Dept. of Biology / School of Environment and Sustainability

Canada's Prairie Pothole Region encompasses a plethora of ecologically significant wetlands, providing critical stopover and breeding habitat to migratory waterfowl, among other organisms. Yet these wetlands, embedded in a matrix of industrialized agriculture, are under threat from heavy pesticide use. Neonicotinoid insecticides, specifically designed as seed treatments on major prairie crops (e.g., canola), frequently drain into agricultural surface waters. The potential impact to aquatic ecosystems is largely unknown, and the frequency of use, presence, and contamination level of neonicotinoids in wetlands is currently being evaluated. We measured Prairie Pothole Region neonicotinoid prevalence by detecting 4 registered active

ingredients—clothianidin, thiamethoxam, imidacloprid, and acetamiprid. Insecticide concentration measurements were coupled with rapid assessments of multiple wetland and landscape features that affect neonicotinoid concentrations and wetland susceptibility to insecticides. As a means of quantifying biological function, we deployed leaf litter bags to measure the effects of neonicotinoid concentration levels and persistence on litter decomposition rates and, consequently, bottom-up limitation of aquatic food chains. Neonicotinoids may reduce decomposition rates, thus altering the potential health and function of wetland systems. Field validation of estimated distribution maps indicates that neonicotinoid applications are widespread and increasing in frequency, and Prairie Pothole Region wetlands frequently contain detectable neonicotinoid concentrations at levels which may be toxic to aquatic life. We aim to improve and inform policy decisions regarding neonicotinoid use in Canada and promote ecologically sound wetland conservation practices.

TP040 Investigating the Molecular and Pathological Effects of Chronic Dietary Selenomethionine in Juvenile White Sturgeon (Acipenser transmontanus)

S. Patterson, University of Saskatchewan / Toxicology Centre; J. Zee, University of Saskatchewan / School of the Environment and Sustainability; M. Hecker, University of Saskatchewan / School of the Environment & Sustainability

Sturgeon are an ancient family of fishes that have evolved little over the past 200 million years. Of the 26 species worldwide many are endangered. White sturgeon (*Acipenser transmontanus*) are endemic to western North America where they are prized by Aboriginal peoples and sport fisheries. Unfortunately, their populations have been steadily declining since the late 1800's. Factors that have been hypothesized to contribute to these declines include overharvesting, habitat alteration and pollution. Due to their longevity and benthic lifestyle white sturgeon might be particularly susceptible to bioaccumulative toxicants. Selenium (Se) is an essential micronutrient, but can be toxic to aquatic organisms at relatively low concentrations and is prevalent in surface waters due to natural and anthropogenic sources, such as leaching from natural weathering, agricultural runoff, discharge from mining and milling operations and fossil fuel combustion. The organic form of Se, Selenomethionine (SeM), is the major dietary form to which aquatic organisms are exposed and has become a particular environmental concern as it persists, bioaccumulates and biomagnifies through the food chain. The goal of this study was to characterize the sensitivity of white sturgeon to dietary SeM exposure, and to characterize the molecular mechanism of toxicity. Juvenile sturgeon were administered food spiked with 0, 5, 25, or 100 ug/g bw of SeM for 90 days. After 10 days a sub-sample was collected to investigate transcriptional responses by use of *Illumina* sequence-by-synthesis technology in order to identify physiological processes that might be adversely impacted by exposure to SeM. In addition, effects of SeM on the cortisol response to stress were evaluated after 90 days of exposure by quantifying concentrations of cortisol in blood plasma using liquid chromatography mass spectrometry (LC-MS). Furthermore, abundances of transcripts of genes along the hypothalamus-pituitary-interrenal (HPI) axis using Q-RT-PCR, and histopathological changes in interrenal tissues were investigated. Ultimately, the goal of this research is to increase understanding of sensitivity of juvenile white sturgeon to SeM and to aid in the risk assessment of this unique and endangered species.

TP041 Metamorphosis in insects alters contaminant transfer, diet tracers and risk to wildlife

J.M. Kraus, USGS; D.M. Walters, USGS / Fort Collins Science Center; J. Wesner, Colorado State University; C. Stricker, USGS; T.S. Schmidt, USGS / Water Resouces Div; R. Zuellig, USGS

During metamorphosis, insects undergo large chemical changes altering both contaminant burden and stable isotope signatures commonly used to trace diet. Such changes, although understudied, raise important questions about predator exposure to contaminants, contaminant flux across ecosystem boundaries and our understanding of food webs. Specifically, 1) which factors predict contaminants transfer and changes in diet tracers from larvae and adult in insects?; 2) How do these changes impact wildlife and our understanding of food web structure, especially across ecosystem boundaries? To address these questions, we conducted a meta-analysis of all relevant studies (n = 47), focusing how different classes of contaminants and light stable isotopes changed during metamorphosis from larval to adult insect life stages. Other considerations included taxonomic grouping, type of metamorphosis and study design (laboratory vs. field). Contaminants differed greatly in their propensity to remain in insect bodies across metamorphosis. For example, organic PCBs become more concentrated in adults, while most metals and PAHs become less concentrated, apparently as a result of being excreted during or immediately after metamorphosis. This pattern suggests that risk to wildlife of exposure to PCBs increases when adult insects are the prey, while exposure risks for most metals and PAHs are higher when of larvae are consumed. In a riparian setting where insects form an energetic link between aquatic and terrestrial ecosystems, these findings suggest different management concerns regarding polluted waters depending on the nature of the contaminant. Stable isotope signatures also were changed by metamorphosis, dependent on which isotope was considered. Nitrogen isotope ratios did change significantly with metamorphosis (on average 1‰) while carbon did not. Although slight, this change could alter conclusions about trophic dynamics within a system. The majority of all species on earth are metamorphosis insects, which are integral to nearly every non-marine food web on earth. Our findings highlight in the importance of understanding effects of metamorphosis on insect chemistry when managing and studying these food webs.

TP042 Monitoring Sediment Contamination: A Case Study of the Ravensbourne River

N. Mokwe Ozonzeadi, University of Westminster / Life Sciences

Sediment is a major transporter and sink for heavy metals in rivers, and poses significant risks to aquatic and benthic organisms. At present in the UK, there are no mandatory sediment quality standards. This is partly due to insufficient toxicity data but also due to a debate over appropriate sediment monitoring and analytical techniques. The aim of this research was to examine the problems associated with sampling river sediment in order to monitor compliance with any future sediment environmental quality standards (EQS). The Ravensbourne River is a tributary of the River Thames located in the highly urbanised South Eastern area of London. Sediment was collected from the bed (Van Veen grab), bank (hand trowel) and suspended (integrated tube sampler) sediment of the Ravensbourne between July 2010 and December, 2011. The sediments were analysed for the total concentration of selected heavy metals – Cd, Cu, Ni, Pb and Zn and by sequential extraction to determine metal binding to different sediment phases using the Maiz *et al.* (1997) and Tessier *et al.* (1979) methods. Metal binding to different sediment particle sizes was also determined. In general, the average metal concentrations in all the sediment compartments followed the order: Zn>Pb>Cu>Ni>Cd. The sediment concentrations ranged from 0.34mg/kg – 6.87mg/kg, 19.34mg/kg – 1673mg/kg, 7.68mg/kg – 60.72mg/kg, 36.87mg/kg – 820mg/kg and 84.74mg/kg – 994.56mg/kg for Cd, Cu, Ni, Pb and Zn respectively. There were no significant differences in the metal concentrations retained in the different compartments by the < 63µm sediment fraction but there were differences between the 63µm to 2mm fractions of the bed and bank. The distribution of metals between sediment phases varied with the sequential extraction technique. The importance of these differences in monitoring sediment environmental quality is discussed.

TP043 Polyhalogenated natural products in fish and mussels from the German North Sea and other marine regions

C. Hauler, University of Hohenheim; G. Rimkus, Landeslabor Schleswig-Holstein; W. Vetter, University of Hohenheim

Recent analyses of marine environmental samples not only revealed the presence of man-made polyhalogenated contaminants but also of halogenated natural products (HNPs). Some of these up to date more than 5,000 described HNPs show bioaccumulation throughout the food chain. The structural similarity to anthropogenic contaminants suggested that some HNPs may also have adverse effects on marine biota. For instance, sediments polluted with HNPs showed teratogenic activity in the zebrafish (*Danio rerio*) embryo test. Therefore, thorough analyses on polyhalogenated compounds should also cover HNPs. In addition, most of the data on HNPs in the marine environment was obtained from marine mammals. The aim of the present study was the detection and quantification of HNPs in species located on a lower trophic level, i.e., fish and mussels. These samples were screened by GC/ECNI-MS on the presence of polyhalogenated compounds. The majority of brominated and mixed halogenated natural products were mainly quantified by acquisition of the characteristic fragment ions $[Br]^-$ (m/z 79, 81), $[BrCl]^-$ (m/z 114, 116, 118) and $[HBr_2]^-$ (m/z 159, 161). To our surprise, many samples contained considerably higher concentrations of

HNPs than PCB 153. HNPs frequently detected covered polyhalogenated alkaloids (e. g. 2,3,3',4,4',5,5'-heptachloro-1'-methyl-1,2'-bipyrrole (Q1), polyhalogenated 1,1'-dimethyl-2,2'-bipyrroles (PDBPs) and the recently discovered 2,3,4,5-tetrabromo-1-methylpyrrole [1, 4]), the monoterpene MHC-1, and two phenoxyanisoles (2'-MeO-BDE 68 (BC-2) and 6-MeO-BDE 47 (BC-3)). In addition, the HNP patterns of fish fillet differed from those observed in mussels.

TP044 Sensitivity of white sturgeon (Acipenser transmontanus) to copper, cadmium, and zinc in acute exposures

R.D. Calfee, US Geological Survey / Columbia Environmental Research Center; E.E. Little, USGS – Biological Resources Division, US Geological Survey / Columbia Environmental Research Center; H.J. Puglis, US Geological Survey / Columbia Environmental Research Center; E.L. Beahan, US Geological Survey; B.G. Brumbaugh, US Geological Survey / Columbia Environmental Research Center; C. Mebane, US Geological Survey / NOAA Fisheries Liaison

The acute toxicity of cadmium (Cd), copper (Cu), and zinc (Zn) to white sturgeon (*Acipenser transmontanus*) were determined for seven developmental life stages in flow-through water-only exposures. Test waters consisted of five concentrations of each metal and a control. Concentrations ranged from 0.01–600 µg/L for cadmium, 0.1- 300 µg/L for copper, and 0.4-10,000 µg/L for zinc with exposure concentrations increasing with older life stages. Sturgeon were tested at 2-, 16-, 30-, 44-, 61-, 72-, and 89- days post hatch (dph). Metal toxicity varied by life stage. Lethal concentrations to 50 percent of the test population (LC50) were based on mortality, while EC50 (effective concentration to 50 percent of the test population) were based on mortality, loss of equilibrium and immobilization. Control survival in these 4-d toxicity tests across all life stages was 100 percent with the exception of the 16 dph tests where survival ranged from 65 to 80 percent (the test acceptability requirement was set at 90 percent). Sturgeon EC50s ranged from 3.02 to >104.6 µg Cd/L (based on test water hardness) with sensitivity increasing during later stages of development. The acute data indicate that sturgeon sensitivity to copper decreases markedly during development with the most sensitive stages occurring early at 2-, 16-, and 30 dph with EC50s (based on test water) ranging from 1.51 – 2.59 µg Cu/L which is below the Nationally recommended water quality criteria. Sturgeon were less sensitive to zinc with EC50 values well above environmental concentrations, however sublethal effects were evident with changes in behavior. The most sensitive life stage was at 2 dph with an EC50 of 146.7 µg Zn/L. Our results indicate that exposure to metals may be a factor in early lifestage survival of white sturgeon.

TP045 Sublethal effects on behavior of white sturgeon (Acipenser transmontanus) exposed to copper

H.J. Puglis, R.D. Calfee, US Geological Survey / Columbia Environmental Research Center; E.E. Little, USGS – Biological Resources Division, US Geological Survey / Columbia Environmental Research Center; E.L. Beahan, E.L. Brothers, US Geological Survey

White sturgeon (*Acipenser transmontanus*) in the trans-boundary reach of the Upper Columbia River (UCR) have experienced poor recruitment for decades. Although sturgeon spawn and eggs and early life stages of white sturgeon are observed in the river, limited numbers of young-of-the-year have been found in habitats considered to be suitable for this life stage. Previous studies have indicated that copper, commonly associated with slag contamination, was lethal to young white sturgeon at low concentrations. In addition, adverse behavioral responses were evident with early onset during the first few days of acute and chronic exposures and became progressively more severe over exposure duration and concentration of copper. Further studies were conducted with copper in order to accurately identify and interpret the behavioral changes seen in previous studies. Larval sturgeon (1-2 days post hatch) were exposed to sublethal concentrations of copper for 14 days. Abnormal behavioral changes were observed within the first several days of exposure including loss of equilibrium and immobilization. Digital video tracking software was used to analyze the swimming behavior and analyses showed swimming velocity, distance moved, and time swimming decreased with increasing copper concentration. We conducted a four day exposure with slightly older sturgeon (24 dph) and observed similar abnormal behavioral changes. The onset of these changes was very rapid, within the first 24 hours of exposure. Our results indicate that copper may play a role in the recruitment failure observed in the UCR as larval white sturgeon swimming behavior was impaired to the extent that survival in the field would be jeopardized as fish would be swept downstream or readily captured by predators. Future studies will focus specifically on swimming performance after exposure to zinc and endurance as well as the vulnerability of white sturgeon to predation after exposure to copper or zinc.

TP046 The increasing impact of road salt: Long-term chloride trends in streams of the northern United States

S.R. Corsi, US Geological Survey / Water Resources Division; L. De Cicco, US Geological Survey-WI; M. Lutz, US Geological Survey-WRD

Road salt applications in the United States have increased each decade since the 1940's. Chloride concentrations in many northern US streams are also on the rise with some concentrations exceeding chronic toxicity benchmarks for much of the year. Long-term trends of chloride concentrations were examined for 31 monitoring sites on 20 streams that had chloride concentration and flow records of 18 to 47 years. Trends were determined using weighted regression on time, discharge, and season (WRTDS). This technique provides the capability to examine long-term trends and represent variability based on seasonal and discharge-related influence. Over time, all but two of the streams examined had increasing chloride concentrations. Eight of these streams had mean winter concentrations approaching or exceeding the USEPA chronic water quality criteria of 230 mg/L at the end of the period of record. Concentrations of chloride during the winter were approximately equal to or higher than summer concentrations at all sites. In addition, summer concentrations at most sites had an increasing long-term trend suggesting that chloride is stored in hydrologic reservoirs, such as the shallow groundwater system, during the winter and slowly released throughout the entire year. Chloride concentrations increased with decreasing flow, indicating a dilution effect with increased water volumes from snowmelt and rainfall. The severity of impact from these multi-month and multi-year chloride exposures to aquatic organisms is not well understood given that most chronic toxicity assessments are based on 7 to 28-day exposures. The continued increase in urbanization and impervious areas that require winter deicing suggest that increasing chloride trends are likely to continue.

TP047 Toxicity of Selenium to White and Green Sturgeon

W. Beckon, US Fish and Wildlife Services / Division of Environmental Contaminants

Fish of the genus *Acipenser* (sturgeon) are likely to be among the most vulnerable to selenium exposure in the San Francisco Estuary because these fish feed predominantly on benthic invertebrates, including the Asian clam, *Corbula amurensis*. This clam is an efficient bioaccumulator of selenium. The best data available for the most sensitive endpoint for sturgeon come from studies in which the survival of larvae was monitored following micro-injection of organic selenium (L-selenomethionine) into the yolk sacs of newly hatched larvae. Benchmark larval selenium concentrations from these studies were translated, by means of regressions, to selenium concentrations in the tissue and diet of adult white and green sturgeon. This analysis indicates that white and green sturgeon are among the most sensitive of fish to adverse effects of selenium, with the listed green sturgeon being the more sensitive of these two species. These levels of sensitivity evidently put sturgeon at substantial risk at current levels of exposure in the San Francisco Bay area. Selenium concentrations in food items of sturgeon in the San Francisco Bay area are almost always high enough that they may cause at least 10 percent mortality in hatchling green sturgeon (≥3.58 µg/g), and they are frequently high enough that they may cause at least 10 percent mortality among hatchling white sturgeon (≥10.8 µg/g) as well.

TP048 Toxicity of Smelter Slag-contaminated Sediments and Associated Metals from Lake Roosevelt to White Sturgeon

E.E. Little, USGS – Biological Resources Division / Columbia Environmental Research Center; R.D. Calfee, H.J. Puglis, US Geological Survey / Columbia Environmental Research Center

White sturgeon (*Acipenser transmontanus*) have experienced poor recruitment in the trans-boundary reach of the Upper Columbia River. Among potential causes, the presence of smelter slag contamination in spawning and rearingareas is a possible concern and the focus of our research. Fish maintained consistent and prolonged contact with slag-contaminated sediments and did not avoid contaminated sediments when provided a choice between contaminated and uncontaminated sediments, nor did they avoid aqueous mixtures of metals. Leachates resulting from aging water with slag-contaminated sediments for 96 hours were lethal to 30 day post-hatch sturgeon with

aqueous copper concentrations as great as 11.8 ug/L. Similar toxicity was observed during aqueous exposure to copper alone and in combination with cadmium and zinc. The latter metals were not lethal, but induced adverse behavioral changes including immobility and loss of equilibrium. These behavioral changes often occurred within the first 24 hours of exposure and were similar to those observed when fish were exposed to slag-contaminated sediments from UCR sites. Fish were impaired to the extent that survival in the field would be unlikely. The results suggest that metals associated with smelter slags may pose harm to early lifestage sturgeon if they occur in spawning and rearing areas and support the need for further investigation of the toxicological vulnerability of sturgeon.

TP049 Lake Sturgeon Health Assessment: Sensitivity of early life stages to polychlorinated biphenyls and dioxin

D.E. Tillitt, US Geological Survey / Columbia Environmental Research Center; E.E. Little, USGS – Biological Resources Division / Columbia Environmental Research Center; R. Gale, US Geological Survey / Columbia Environmental Research Center; D. Nicks, US Geological Survey; J. Candrl, H.J. Puglis, US Geological Survey / Columbia Environmental Research Center; J. Buckler, Five Rivers Services, Inc; M. Baker, NOAA

The aquatic foodweb of the Great Lakes has been contaminated with polychlorinated biphenyls (PCBs) since the mid-20th Century. Although concentrations of PCBs in fish and birds have declined in the past four decades, distinct areas of elevated contamination still exist, including designated Areas of Concern (AOC). Lake sturgeon (*Acipenser fulvescens*) occur throughout all of the Great Lakes; however, populations have been on the decline for two centuries due in part to overfishing, pollution, and dams that limit access to spawning areas. Though contaminant dynamics and effects have been relatively well investigated in salmonids, much less is known about contaminant thresholds in sturgeon that are associated with adverse effects on development, growth, behavior, and survival. The objectives of this study were to evaluate the risk of PCB and dioxin exposure on early life stages of lake sturgeon. Morphological pathologies, mortality, and swimming performance were used as endpoints to assess the effects of PCB-126 and TCDD exposure. Lake sturgeon eggs dosed with 3,3',4,4',5-pentachlorobiphenyl (PCB 126) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were reared to 60 days post fertilization. Pericardial and yolk sac edema, tubular heart, yolk sac hemorrhaging, and small size were the most commonly observed pathologies in both TCDD and PCB-126 exposures, beginning as early as 4 day post-fertilization. Many of these pathologies responded in a dose-dependent manner and effective doses (ED_{50}) and threshold doses (ED_{15}) were calculated. In most cases, the TCDD ED_{50} values were similar to TCDD LD_{50} values. In contrast, PCB-126 ED_{50} values for the same pathologies were generally higher than PCB-126 LD_{50} values. Threshold doses indicate certain lake sturgeon populations in the Great Lakes are under an elevated risk from PCB or TCDD-related toxicity.

TP050 Sub-lethal Concentrations of Antibiotics Stimulate Colony Formation and Alter Substrate Utilization by Cave Bacteria

P.K. Byl, Hume-Fogg Academic High School / Vanderbilt Research Experience for High School Students; D. Solomon, Tennessee State University / Civil Engr; S. Trimboli, WKU – Mammoth Cave International Center for Science & Learning; J. Oster, Vanderbilt University / Earth and Environmental Science; R. Toomey, Mammoth Cave National Park Service / Science & Resources Managment; J. Byl, Vanderbilt University / Program in Law & Economics; T.D. Byl, US Geological Survey / Department of Interior

The karst terrain of central Kentucky and middle Tennessee is home to many caves, including Tennessee's longest cave, Blue Spring Cave, and the world's longest known cave, Mammoth Cave, Kentucky. These caves have unique ecosystems, including bacteria that produce biologically active molecules. Storm runoff carries chemicals from the surface into the caves which can have positive or adverse effects on the microbes and other organisms that live in the cave waters. The US Geological Survey, in partnership with the Mammoth Cave National Park, Tennessee State University, and Vanderbilt University is conducting a study to determine substrate utilization patterns and antibiotic resistance of aquatic microbial communities from Mammoth and Blue Spring Caves. The field sampling sites represent an assortment of cave levels and hydro-geochemical conditions, such as cave streams and dripping speleothem formations. Surface sampling was limited to storm events, but cave sampling was not restricted to storms since all the subsurface sites had perennial flow. Bacterial sensitivity to antibiotics was quantified using agar plates with 10% trypticase soy media augmented with 0.00, 0.01, 0.1, 1.0, and 10.0 mg/L antibiotic (erythromycin, kanamycin, gentamicin, tetracycline or quaternary ammonia compounds). Biolog Ecolog™ plates with 31 different substrates were used to evaluate community substrate utilization patterns. Bacteria resistant to antibiotics existed in all levels of Mammoth Cave. However, the number of colony forming units (cfu) generally decreased as the antibiotic dosage increased. One exception to this pattern was erythromycin, which significantly ($p < 0.05$) increased the number of cfu when the media was supplemented with sublethal [0.1 and 1.0 mg/L] doses. Addition of 0.1 mg/L erythromycin to the Biolog inoculum increased the initial Shannon diversity index values, indicating erythromycin enhanced evenness of substrate use in the first 48 hours. These preliminary results suggest that erythromycin may have evolved as a signal for cave bacteria, possibly stimulating growth by inducing a change in substrate utilization patterns at sublethal concentrations.

PAH in the Environment: Advances in Assessment of Occurrence, Sources, and Human and Ecological Risks

TP051 Polycyclic Aromatic Hydrocarbons (PAHs) in Surface Water, Sediment and Fish (Clarias gariepinus) in Ovia River, Nigeria

L.I. Ezemonye, I. Tongo, K. Akpeh, B. Aighobahi, A. Onokpasa, University of Benin / Animal and Environmental Biology

The levels and distribution of polycyclic aromatic hydrocarbons (PAHs) in surface water, sediment and fish (*Clarias gariepinus*) samples from Ovia River, Nigeria was investigated. The concentrations of PAHs were assessed to reflect matrices, seasonal and spatial variations. Total PAH concentrations in the matrices (Σ16PAHs) ranged from 0 – 0.056 mg/L, surface water, 0 – 2.96 mg/kg-dry weight (dw), sediments and 0 -2.28 mg/kg-dry weight (dw), fish (*Clarias gariepinus).* The mean concentration of PAHs in sediment samples were significantly higher ($p< 0.05$) than water and fish samples. However a prevalence of low molecular weight PAHs was observed with a considerable predominance of 3-rings PAH in all the matrices. Naphthalene (2-ring PAH) was the most dominant congener in surface water, Acenaphathalene (3-ring PAH) in sediment and Fish samples had Florene (3-ring PAH) dominating. The dominant PAHs comprised about 19% (Naphthalene), 22% (Acenaphathalene), and 39%(Florene) respectively, of the seventeen PAHs detected. Seasonal variation showed higher concentrations in the dry season for all the matrices while spatially, Ekenhuan, the downstream station had the highest concentration of PAHs in all the matrices. The sources of contamination were closely related to anthropogenic activities such as domestic discharge, automobile emissions and runoffs. Estimated mean values in the matrices were generally above the recommended Ecotoxicological benchmarks (0.01µg/L) indicative of possible environmental and health risk and hence the need for constant monitoring of the water bodies.

TP052 Assessment of PAH bioavailability at restoration sites

A.A. Brennan, University of Minnesota Duluth / Water Resources Science; N.W. Johnson, University of Minnesota Duluth / Civil Engineering

The St. Louis River Estuary (SLRE) in Duluth, MN is designated as a Great Lakes Area of Concern in part due to widespread sediment contamination from PAHs as a result of ongoing and past industrial practices. Many of the heavily contaminated (Superfund) sites have been remediated or are in the process of remediation; therefore, the focus of management efforts is now shifting towards restoration of moderately contaminated areas. An ongoing pilot scale project in a moderately contaminated, high-priority restoration embayment with urban stream sources will utilize navigational dredged materials from the working Duluth-Superior harbor by placing a relatively thin layer cap on areas in the embayment affected by legacy contamination. Although current practices in the state of Minnesota for assessing sediment contamination at moderately contaminated areas involve only bulk sediment analysis, this project is assessing the effectiveness of capped layers in reducing contaminant migration to surface waters, sediment, and biota utilizing bioavailability based techniques which predict organism exposure by quantifying the fraction of dissolved contaminants in the porewater or the bioavailable phase. Passive porewater sampling methods and bioaccumulation assays are used to monitor contaminant availability in post-remediated sites in the SLRE as well as pre- and post-remediation at 21st Avenue West. Preliminary measurements at post-remediated areas show minimal PAH availability in surficial sediments suggesting minimal movement of PAHs through the capped layers or from other surficial sources. Locally, the results

of this project will determine the contaminant-related implications of using dredged sediments for restoration activities in the St Louis River Estuary as well as the importance of ongoing sources including urban streams and remobilized sediment. On a broader scale, this project seeks to serve as a template for the integration of data from bioavailability based techniques in assessments of site restoration in Minnesota and the Great Lakes.

TP053 Derivation of Alternate Dermal Absorption Factors for Benzo(a) pyrene and other Carcinogenic Polycyclic Aromatic Hydrocarbons in Aged Site Soils

B.H. Magee, ARCADIS; D. Pfeiffer, Arcadis US, Inc. / Senior Scientist

Dermal absorption factors (ABSd) are used in the dose equation used to estimate exposure from dermal contact with soil. The ABSd is used to reflect the desorption of the chemical from soil and the absorption of the chemical across the skin and into the bloodstream. In 2001, USEPA listed a ABSd for benzo(a)pyrene (BaP) and other polycyclic aromatic hydrocarbons (PAHs) of 13% in Exhibit 3-4, Recommended Dermal Absorption Fraction From Soil. This default ABSd value is based on a study by Wester et al. in which the dermal penetration of BaP was tested in solvent freshly added to a low carbon, sandy soil matrix and used immediately without ageing. A critical review of Wester et al. indicates that the study is not relevant for estimating the dermal absorption potential of BaP and other cPAHs from aged site soils. Other studies of dermal absorption of PAHs from soil and other complex matrices show lower BaP dermal penetration than does Wester et al. This poster presents a critical review of the Wester et al. study. In addition, this poster summarizes dermal absorption data available from peer-reviewed cPAH studies and estimates an alternate ABSd value relevant to BaP and other cPAHs in aged site soils.

TP054 Evaluations of Toxicity in Java medaka (Oryzias javanicus) embryo microinjected with PAHs

K. Ito, S. Uno, Education and Research Center for Marine Resources and Environment, Faculty of Fisheries; J. Koyama, Kagoshima University / Faculty of Fisheries

Effects of naphthalene, phenanthrene and benzo(a)anthracene to embryo of estuarine fish, Java medaka (*Oryzias javanicus*) were examined using the microinjection technique. These polycyclic aromatic hydrocarbons (PAHs) are common and widely distributed in rivers, lakes, and coastal areas. Fish embryo is sensitive to pollutants like PAHs, resulting to induction of mortality, reduction of hatching rate, and development of abnormalities. However, it's not known which PAHs with same concentration could cause to most severe effects to embryo. Microinjection technique has been a powerful tool to inject and control the desired amount of contaminants in embryo. In this study, 0.18, 1.8, 18 and 180 ng/egg of naphthalene or phenanthrene, and 0.018, 0.18, 1.8 and 18 ng/egg of benzo(a)anthracene were injected into the oil globule of embryos within 8 hr after fertilization and incubated for 20 days. Hatching and mortality rates, and abnormalities including heart and others were observed. Mortalities of exposed embryo were 0-12.5%, 0-29.4% and 28.6-61.9%, while hatching rates were 87.5-100%, 70.6-100% and 38.1-71.4% for naphthalene, phenanthrene and benzo(a) anthracene, respectively. In addition, mortalities in all exposed group to benzo(a)anthracene were high at day 13 to 15 (the number of mortalities from 2 to 8). Furthermore, a concentration of 18 ng/egg benzo(a)anthracene induced blood stasis on yolk membrane (day 4), low circulation of blood and irregular form of yolk (day 5), and hypertrophy of atrium and ventricle (day 10), however, there were no abnormalities of embryo injected with naphthalene and phenanthrene. Finally, the toxicity of PAHs tends to be strongly dependent on increase of the number of aromatic rings. Naphthalene and phenanthrene with low molecular weight could be lower toxicities than benzo(a)anthracene, although these are generally much accumulated in embryo. Benzo(a)anthracene could affect to embryo, because embryo exhibited some abnormalities and a relatively high mortality rate at day 13-15, although the highest exposure concentration were ten times lower compared with that of other PAHs.

TP055 Structure toxicity of alkyl-substituted polycyclic aromatic hydrocarbons (PAHs) to Japanese medaka embryos (Oryzias latipes)

H. Lin, Queens University; G. Morandi, Queen's University; P.V. Hodson, Queen's University / School of Environmental Studies

Alkylated polycyclic aromatic hydrocarbons (PAHs) are a suite of compounds predominating in crude oils. The alkylation at different locations on the benzene rings of the PAH changes the size and shape, which could result in different interactions with tissue receptors and different severities of toxicity. This project assessed the toxicity of chrysene, benzo[α]anthracene, and several alkylated derivatives to the embryos of Japanese medaka (*Oryzias latipes*) using the partition controlled delivery (PCD) method of exposure. With PCD, a desired exposure concentration is maintained by equilibrium partitioning of alkyl-PAHs from polydimethylsiloxane (PDMS) films. The occurrence of blue sac disease (BSD) as a classic sign of embryo toxicity was recorded and median effective concentrations (EC50) were calculated to represent the severity of toxicity of each alkyl-PAH. Toxicities will be compared to the octanol water partition coefficient (K_{ow}) as a representative of physical and chemical property that describes the relative lipid solubility of compounds and their propensity for bioaccumulation. The ultimate goal is to determine if structure-activity relationships can be extended to alkylated chrysenes and benzo[α]anthracenes. Preliminary results demonstrate that chrysene was not embryo toxic within the limits of its solubility, whereas benzo[α]anthracene caused sublethal signs of BSD, but not mortality.

TP056 In a green world, shouldn't all analyses be micro?

D. Mauro; J. Roush, Accutest Laboratories, Inc.

Most methods for the analysis of organic compounds in water, soil or other solids rely on an extraction step where the sample is treated with solvents to collect and concentrate trace constituents. This step can be labor intensive and costly, as well as generate substantial volumes of potentially hazardous solvents as wastes. In addition, large quantities of soil and water, which may contain hazardous chemicals, are collected but never analyzed, and must also be handled as waste. For several years, microextraction methods have been promoted by a relatively small group of advocates for reducing the labor needed and minimizing the amount of waste generated in the laboratory over existing methods. One such approach was developed at META Environmental, Inc. in the early 1990s with support of EPRI and its member utilities. Two microscale solvent extraction (MSE) methods for the simultaneous extraction and analysis of volatile and semivolatile organic compounds in soil or water were developed. Validation reports were sent to EPA's Office of Solid Waste on September 27, 1996 and on April 11, 1997. The methods were reviewed by the SW-846 Organic Methods Workgroup and, after several rounds of review and comment, EPA published Methods 3511 and 3570 on their web site as part of the SW-846 Update IVB in November 2002. MSE methods require smaller sample volumes, use up to 90% less solvent and generate up to 90% less waste, and can result in faster and lower cost sample preparation overall. MSE methods have been used successfully by META under both field and fixed laboratory conditions for over 20 years and have been shown to produce data comparable to other EPA methods. This paper will review META's experience with EPA Methods 3511 and 3570, discuss some improvements to the methods that we have made, and highlight the advantages and disadvantages of the methods. Method performance will be demonstrated with a focus on sediment samples and inter-method comparisons.

TP057 Transcriptomic analysis of AHR-dependent and AHR-independent genes involved in the synergistic cardiac developmental toxicity of PAHs

N. Jayasundara, Duke University / Nicholas School of the Environment; L.V. Garner, Cardno Entrix; J.N. Meyer, Duke University / Nicholas School of the Environment; H. Tsai, National Taiwan University; K. Erwin, Duke University; M. Kirby, Duke University / Department of Pediatrics; R.T. Di Giulio, Duke University / Nicholas School of the Environment

Polycyclic aromatic hydrocarbons (PAHs) are known to induce a wide range of toxic effects including developmental defects in vertebrates. Exposures of fish embryos to PAHs have been shown to cause cardiac deformities. However, the exact mechanisms of cardiac developmental toxicity remain unknown. The aryl hydrocarbon receptor (AHR), a transcription factor that regulates the transcription of a battery of genes, is activated by some PAHs and plays a role in mediating toxicity. Studies show that exposure to a simple PAH mixture consisting of an AHR agonist (benzo(a)pyrene-BaP) with fluoranthene (FL), an inhibitor of cytochrome p450 1(CYP1) – a

gene induced by via activation of the AHR – results in pericardial effusion and an elongated heart (the "stringy heart" phenotype). Knockdown of AHR2 is shown to prevent the toxicity of BaP+FL. In this study, we utilized a transcriptomic approach to identify novel genes involved in the cardiac toxicity caused by a simple PAH mixture and to determine if these genes are controlled in some part by the AHR. We injected zebrafish embryos with an AHR2 morpholino or control morpholino to knockdown AHR2 gene. We then exposed embryos to BaP and FL at 36 hpf; embryos were dosed with DMSO, 100 mg/L BaP, 500 mg/L FL, or 100 mg/L BaP + 500 mg/L FL. From exposed embryos, we extracted heart tissues to identify cardiac-specific gene expression changes due to PAH exposure at various time points (2h, 6h, 12h, and 18h). Overall data showed that gene expression differences between the AHR knockdown and control embryos decreased over time, suggesting that the AHR mediated gene expression due to PAH ligand-binding decreases within less than 54 hpf. Furthermore, despite exposure to BaP+FL resulting in a deformed heart, this mixture resulted in smaller effect on overall gene expression compared to BaP and FL alone. Genes induced by BaP+FL in AHR knockdown and control embryos were primarily involved in Ca^{2+} cycling, cardiac muscle contraction, and cytoskeletal development and reorganization. Further analysis on transcriptomic data identified a number of candidate genes such as Pdlim, a gene involved in cardiac looping, that might be directly underlying PAH induced cardiac deformity in fish. Ingenuity pathway analysis on transcriptomic data identified several pathways might be playing a key role in mediating AHR-dependent cardiac toxicity.

Amphibian and Reptile Ecotoxicology: Progress and Challenges in Understanding Chemical Effects, Exposure, and Risk

TP058 Improving reptile risk assessment: working towards a standardized toxicity test with reptiles using the western fence lizard (Sceloporus occidentalis)

S.M. Weir, Texas Tech University / Environmental Toxicology; L.G. Talent, Oklahoma State University; S. Yu, Texas Tech University / Department of Environmental Toxicology; C.J. Salice, Texas Tech University / Environmental Toxicology

Reptile ecological risk assessment is currently limited by a lack of toxicity data for reptiles, and little knowledge of contaminant exposure. Recent advances in the use of the Western fence lizard (*Sceloporus occidentalis*) as a model organism creates opportunities for filling important data gaps. One of the main reasons reptiles continue to remain underutilized is based, in part, on the lack of standardized test protocols for reptiles stemming from nonexistent regulatory requirements for reptile toxicity testing. We present methodological advances and data focused on building and refining a standardized toxicity test (oral and dermal exposure) for the Western fence lizard. We tested the potential toxicity of propylene glycol, acetone, and porcine gelatin capsules as carrier solvents for generating dietary toxicity estimates. We used a maximum of 100 µL of each solvent and compared results to a 100 µL dose of moderately hard water. We also fed standard #5 gelatin capsules to lizards to observe any dosing effects and to ensure the capsules completely dissolved by 48 hours post exposure. We observed lizards for up to 14 days post exposure to the solvents and water. We also performed a behavioral assay at 48 hours post exposure to test for sublethal effects of the solvents. We found no significant effects of either organic solvent on lizard behavioral assays or signs of toxicity following dosing. Exposure to acetone resulted in a marginally significant decrease in sprint speed, which may suggest that large volumes of acetone (e.g., 100 µL) could be causing undesirable additional sublethal effects when used for toxicity experiments. Capsules appeared to have no effect on lizards, and in all cases were completely absent from the gastrointestinal tract by 48 hours post exposure. As further proof of concept, we also present preliminary LD50 data for reptiles exposed to pesticides using an "up and down" method for dietary and dermal LD50 estimation. Preliminary analysis suggests that dietary exposure likely results in greater toxicity, but compared to dermal toxicity, there are no significant differences. Future efforts are aimed at further refining and vetting these toxicity testing methods for a range of chemicals including rodenticides, insecticides and herbicides.

TP059 Endosulfan and α-cypermethrin inhibit repair of DNA photo-adducts and decrease transcription of nucleotide excision repair genes in Xenopus laevis

S. Yu, Texas Tech University / Department of Environmental Toxicology; S. Tang, Texas Tech University / The Institute for Environmental and Human Health; G.D. Mayer, Texas Tech University; G.P. Cobb, Baylor University / Department of Environmental Science; J.D. Maul, Texas Tech University / The Institute of Environmental and Human Health / Department of Environmental Toxicology

Pesticide use and UVB radiation have been suggested to adversely affect amphibians; however, little is known about their interactive effects and potential mechanisms. Studies have shown that greater toxicity associated with pesticide photo-degradation products contributes to the synergistic effect of pesticides and UVB. However, other mechanisms may exist. One possible mechanism is that genotoxic pesticides may inhibit repair of UVB-induced DNA adducts by interfering with DNA repair pathways. In the present study, we investigated the combined effects of UVB and each of the two common insecticides (endosulfan and α-cypermethrin) on induction of DNA photo-adducts and transcription of nucleotide excision repair (NER) genes in African clawed frog (*Xenopus laevis*) embryos. We exposed embryos to a low and high concentration for each pesticide (25 and 50 µg/L for endosulfan, 2.5 and 5.0 µg/L for α-cypermethrin) for 96 h, and added environmentally relevant levels of UVB during the last 7 h. Levels of cyclobutane pyrimidine dimers (CPDs) and transcription of DNA repair genes were measured immediately after the exposure. Both pesticides at high concentrations, when combined with UVB, induced higher levels of CPD compared to control or UVB only. Both pesticides alone reduced transcription of XPA and endosulfan also reduced transcription of HR23B, indicating direct effects of pesticides on key NER enzymes. Our results indicate that UVB and pesticides can act synergistically on DNA photo adduct formation and a potential mechanism is the alteration of transcription of critical NER genes.

TP060 Effects of maternal dietary selenium exposure on early life stages of the amphibian Xenopus laevis

A.J. Masse, Toxicology Centre / Toxicology Centre; J.R. Muscatello, Stantec / Department of Toxicology; D.M. Janz, University of Saskatchewan / Toxicology Centre

Selenium (Se) is a contaminant of potential concern in aquatic systems due to its efficient incorporation into food webs, and its role as a developmental toxicant in oviparous vertebrates such as fish and birds. Currently, there is a lack of Se toxicity data for amphibians, particularly regarding tissue-based thresholds in early life stages. The objective of this study was to determine dose-response relationships for early life stage toxicities in the model amphibian Xenopus laevis. Following a 60-day dietary exposure to food augmented with L-selenomethionine (Se-Met) at nominal concentrations of 0, 10, 30 and 90 µg Se/g dry mass, adult female X. laevis were bred with untreated males. Evaluated endpoints included egg Se concentration, fertilization success, hatchability, survivability, developmental rates and frequency of malformations in developing larvae. Preliminary results indicate that Se had no effect on fertilization success or adult survival in X. laevis, but is incorporated into eggs at concentrations that reflect adult dietary exposures. Further research will determine the frequency, severity, and type of malformations in tadpoles at 5 days post-fertilization and after completion of metamorphosis. Overall, this study will contribute to a better understanding of the sensitivity of amphibians to Se exposure.

TP061 How Six Ambystoma Species' Blood Metal Content, Blood Cell Morphology, Leukocyte Profiles and Parasite Loads are Affected by Metals in the Environment

H.M. DeMali, Arkansas State University / Biological Sciences; J.L. Bouldin, Arkansas State University / Department of Biological Sciences / Environmental Sciences Graduate Program; P. Sikkel, S. Trauth, Arkansas State University / Biological Sciences

This study investigates how six *Ambystoma* species' blood cell morphology, leukocyte profiles, and parasite loads are affected by metal content in the environment. Overall, the study serves as a thorough comparative parasitology study identifying the parasite assemblages in *Ambystoma* salamanders to metals. One of the primary objectives, identifying hemoparasite, endoparasite, and ectoparasite assemblages in the six *Ambystoma* species in Arkansas, will help determine the species' fitness. The relationship between environmental factors, including metal content, and physiological responses is also included in the study. Specifically, examining stress levels via leukocyte profiles, blood

metal content, in addition to examining the relationship of parasites with their salamander hosts with respect to metal content. The environmental metal content for each sample will be determined. Soil and water metal concentrations are compared to metal content within each individual's blood to help determine the bioavailability and persistence of the metals present in their environment.

TP062 Preliminary developmental effects of cryopreservation procedures on embryos of Xenopus laevis

K.T. Carlisle; J.R. Rayburn, Jacksonville State University / Department of Biology

In an effort to evaluate the feasibility of cryopreservation of *Xenopus laevis* embryos for education and research purposes, this project evaluated the effects of cryoprotectants on embryos of *X. laevis*. Cryoprotectants, chemicals that are used to prevent damage due to ice crystal formation within the cells during the freezing and thawing process, are used in cryopreservation of bovine, fish, mouse, and human embryos to allow for optimal survival rates. Even though cryoprotectants are beneficial to the process, the extended exposure to these chemicals can cause developmental effects on the embryos, effecting survival, malformation, and growth. As part of a series of cryopreservation experiments, the effects of low temperature (4°C), PVP, Galactose, DMSO, and a cryoprotectant cocktail on the development of *X. laevis* embryos were studied using the Frog Embryo Teratogenesis Assay-Xenopus (FETAX) protocol. *X. laevis*, the South African clawed frog, was used for this experiment as it has historically been an important model organism used in developmental biology and molecular biology research. *X. laevis* embryos are easily manipulated and their developmental stages well established. FETAX is a rapid test for identifying potential developmental toxicity of chemicals/mixtures. For this experiment, early stage (

TP063 Pesticides, nutrients and disease: Do restored wetlands provide quality habitat for amphibians in an agricultural landscape?

K.L. Smalling, US Geological Survey; R. Reeves, Iowa State University; W.A. Battaglin, E.L. Muths, M. Vandever, C.L. Pierce, M.L. Hladik, US Geological Survey

The potential threats to amphibian species in an agricultural landscape include habitat loss and degradation due to agricultural practices, climate change, and disease. Despite the dramatic landscape transformations across much of the United States, populations of native amphibians continue to persist. Legislation through the Conservation Reserve Enhancement Program (CREP) restores wetlands lost through landscape modifications and aims to reduce contaminant concentrations and loads in surface waters. However, the benefits of this increased wetland area as amphibian habitat may be negated if the quality of the wetlands is insufficient to support viable amphibian populations. The current study was designed to assess the relative quality of the habitat for amphibians by examining amphibian population and population health metrics, and major habitat indicators including water and sediment quality, pesticides, and disease. Surface water, sediment and frogs collected throughout the spring and summer of 2012 and 2013 from six wetlands (3 CREP and 3 reference) were analyzed for over 90 current-use pesticides using gas chromatography mass spectrometry. Capture-recapture sampling of leopard frogs (*Lithobates pipiens*) was also conducted in 2012 and 2013 and population sizes were estimated. In 2012, 14 compounds were detected in water samples, 8 compounds were detected in sediment, and 16 compounds were detected in liver tissue samples. In surface water samples, fungicides were detected frequently, atrazine was detected at the highest concentrations and a greater number of compounds were observed in the CREP wetlands. A similar number of compounds were detected in livers and sediments from the two wetland types and the contaminant profile in the frogs were similar to what was observed in the sediment. The chytrid fungus, Bd, was detected in water from all sites with generally higher densities observed in the reference wetlands. In drought conditions CREP wetlands with a longer hydroperiod may provide essential habitats for amphibians. However, the benefits of increased habitat area from these CREP wetlands could be negated by the risk of increased chemical and pathogen exposure. The results of the study will provide critical information on the impacts of stressors on amphibians and will aid in the assessment of subsequent risks to their fitness and long-term population persistence.

TP064 Life stage and life history influences on amphibian sensitivity to chloride salts

B.K. Williams, USGS / Columbia Environmental Research Center; E.E. Little, USGS – Biological Resources Division / Columbia Environmental Research Center; H.J. Puglis, US Geological Survey / Columbia Environmental Research Center; E.L. Beahan, E.L. Brothers, US Geological Survey

Chloride salts are widespread contaminants in aquatic ecosystems, entering wetlands and streams through runoff and infiltration of road products (deicers and dust suppressants), acid mine drainage, and domestic and industrial effluents. Although amphibian larvae are commonly found in surface water habitats with elevated chloride concentrations, the degree of amphibian exposure is driven by the breeding phenology and larval period length of individual species. Any chloride toxicity associated with this exposure will depend on the sensitivity of the species and life stages exposed, as well as the major cations present, which vary with chloride source. We performed 96-hour acute tests with wood frog (*Lithobates sylvaticus*) and gray treefrog (*Hyla versicolor*) embryos and early tadpoles exposed to three chloride salts to address three objectives: 1) evaluate differences in sensitivity to chloride exposure among life stages, 2) compare chloride sensitivity between two anurans with differing life histories, and 3) compare the toxicity of NaCl, $CaCl_2$, and KCl. Results thus far indicate greater chloride sensitivity of anuran embryos relative to later aquatic life stages in both species. Wood frogs appeared to be less sensitive to chloride exposure overall than gray treefrogs, possibly because the 96-hour exposures covered a relatively smaller portion of the wood frogs' longer larval period. At all life stages, chloride toxicity varied with major cations present, with a relative toxicity ranking of $K^+ > Ca^{2+} > Na^+$. In addition, embryos of both species frequently demonstrated sublethal effects (axial malformations and behavioral abnormalities) at concentrations up to 50% lower than lethal levels. Ongoing tests are evaluating the chloride sensitivity of stream-associated amphibian larvae (the southern two-lined salamander *Eurycea cirrigera* and the mudpuppy *Necturus maculosus*) for comparison with the current work on pond-breeding species. These results highlight the importance of considering the influence of life history traits on chloride exposure and toxicity to amphibian species, which would improve the accuracy of estimating the risk of elevated chloride levels to amphibians.

Molecular, Genetic, Multi-Generational and Evolutionary Ecotoxicology

TP065 Exposure to G-1, a selective agonist for GPER, results in changes in gene expression in adult fathead minnow liver and brain tissues

S. Jayasinghe, University of Florida / Department of Physiological Sciences, Center for Environmental and Human Toxicology, Department of Environmental and Global Health; K.J. Kroll, University of Florida / Physiological Sciences; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology; T. Sabo-Attwood, University of Florida / Department of Environmental & Global Health, Center for Environmental and Human Toxicology

Estrogen (17β-estradiol / E2) regulates a variety of biological processes, including reproduction and development in vertebrates. In fish, E2 controls molecular events that are vital to successful reproduction such as the hepatic synthesis of vitellogenin (Vtg), a precursor egg yolk protein, and regulation of genes in the brain that influence behavior. Classically, E2 exerts these effects via nuclear estrogen receptors (ESR); however, several research groups have shown that G protein-coupled estrogen receptor-1 (GPER, formerly known as GPR30) mediates E2 activation of signal transduction through non-genomic membrane initiated pathways. Both ESRs and GPER are also targets of endocrine disrupting compounds. The precise role of GPER in the liver and brain has been minimally explored. As the GPER is not well characterized in fish the aims of our study are to: (1) assess the tissue-specific expression of GPER in adult fathead minnows (FHM, *Pimephales promelas*); (2) determine the effect of GPER activation on hepatic vitellogenin-1 (Vtg1) synthesis in FHM males and females using a selective GPER agonist (G-1) and antagonist (G-15) and (3) conduct microarray analysis to study the changes in gene expression in different parts of the FHM brain as a result of GPER activation. Using qRT-PCR we found that GPER mRNA shows a strong spatial distribution in adult fathead minnows and is detectable in many vital organs with the highest levels measured in brain followed by gallbladder, trunk kidney, heart, gonads, liver, muscles and intestine. Interestingly, GPER mRNA revealed different spatial patterns within defined regions of the adult brain and expression levels are distinct by gender. We

are currently generating global transcriptomics profiles on select areas of the brain from fathead minnows exposed to G-1 to begin to identify downstream targets of GPER in this tissue. To assess whether GPER is involved in regulating hepatic Vtg1 synthesis, we exposed fathead minnows aqueously to G-1 (5, 30 and 100 ng/L) for 48 hours and measured Vtg1 expression levels by qRT-PCR. Results show a dose dependent increase of Vtg1 expression compared to vehicle control in both males and females. These findings suggest that control of hepatic Vtg synthesis likely involves both nuclear and membrane receptors that are sensitive to E2 activation in adult FHM. Collectively, these studies reveal a role for GPER in liver and brain tissues of fish by controlling genes relevant to reproduction.

TP066 How does exposure of yellow perch (Perca flavescens) to nickel and cadmium affect the transcriptome – results from a 1000 candidate-gene microarray

B. Bougas, INRS – Centre Eau Terre Environnement; E. Normandeau, Universite Laval; F. Pierron, Université de Bordeaux; P.G. Campbell, INRS – Centre Eau Terre Environnement; L. Bernatchez, Universite Laval; P. Couture, INRS – Centre Eau Terre Environnement

The molecular mechanisms underlying nickel (Ni) and cadmium (Cd) toxicity and their specific effects in fish are poorly understood. Documenting gene transcription profiles offers a promising approach towards identifying the molecular mechanisms affected by these metals and to discover biomarkers of their toxicity. However, confounding environmental factors can complicate the interpretation of the results and the detection of biomarker genes for fish captured in their natural environment. In this study, the transcription levels of 1000 previously identified candidate genes included on a custom developed yellow perch oligo DNA microarray were used to investigate the response of yellow perch (*Perca flavescens*) individuals to both metal (Ni and Cd) and non metal (temperature) stressors. Specifically, we aimed at 1) identifying transcriptional signatures specific to Ni and Cd exposure; 2) investigating the mechanisms of Ni and Cd toxicity; and 3) developing a predictive tool to identify the effects of Ni and Cd contaminants in wild fish. A total of 479, 287 and 177 genes were found to be differentially expressed when the temperature varied and at different concentrations of Ni and Cd, respectively. These two metals were found to mainly impact genes involved in iron metabolism, transcriptional and translational processes, vitamin metabolism, blood coagulation, and calcium transport. This study demonstrates the usefulness of our developed microarray tool to detect and characterize the impact of different stressors, such as metal contamination and changes in temperature.

TP067 Gene expression rhythms in rainbow darter male (Etheostoma caeruleum) across an annual reproductive cycle

P.A. Bahamonde, University of New Brunswick / Biology department; M.R. Servos, University of Waterloo / Department of Biology; C.J. Martyniuk, University of New Brunswick; K.R. Munkittrick, Canadian Rivers Institute, Biology

We have been studying intersex in male rainbow darter (*Etheostoma caeruleum*) associated with exposure to sewage effluents. To understand changes in the gene transcriptome associated with intersex it was necessary to have a better understanding of normal annual changes in the transcriptome. The goal of this research is to identify patterns of gene expression associated with the different stages of gonad development during the annual cycle. Molecular pathways involved in ovarian or testis development have been poorly studied. While most studies focus on female ovarian changes, there is a gap in understanding testis development. A customized second generation microarray for rainbow darter (8x15k) was used to identify patterns of gene expression – in terms of mRNA abundance – in male rainbow darter gonads during an annual cycle. Rainbow darter males were collected infield work surveys in May (spawning), August (post-spawning), and October (recrudescence) 2011, and January (developing) and March (pre-spawning) 2012, using a back pack electrofisher from a clean area at the Grand River, ON, Canada. Gonads were flash frozen in liquid nitrogen, and mRNA was extracted with TRIzol according to the protocol. Histology was performed on one lobe of the testis to establish the different proportion of spermatogenic cell types during the different seasons. A decomposition of the gene profile with a k-means algorithm showed that the 9,854 genes had 21 unique patterns of gene expression. Further analyses of gene ontology terms and molecular pathway changes will be presented. This is the first report on the seasonal change on gene expression in a multiple spring spawner teleost fish.

TP068 The application of toxicogenomic biomarkers in juvenile tilapia to investigate potential endocrine disruption in a major South African river

C.C. Truter, J.H. van Wyk, Stellenbosch University / Botany and Zoology; P. Oberholster, CSIR / Natural Resources and the Environment; A. Botha-Oberholster, Stellenbosch University / Genetics

The Olifants River is an important South African river, providing water for human consumption and agriculture. The upper catchment of the Olifants River is severely impacted by anthropogenic activity including mining, agriculture, informal settlements and poorly maintained waste water treatment plants (WWTPs). The aim of this investigation was to assess the potential impacts of organic contaminants in the upper Olifants River on the endocrine system, through the monitoring of the expression of selected genes associated with the gonadal, thyroid and adrenal endocrine axes in juvenile tilapia exposed *in vivo*. Surface water samples were collected from six locations representing different land cover regions (*i.e.* agriculture, mining, WWTP and natural lands) during summer. The non-polar and slightly polar organic compounds were extracted from water samples using C18 solid phase extraction columns, and reconstituted in iodated buffered deionized water. Juvenile 22 days post fertilization Mozambique tilapia, *Oreochromis mossambicus*, were subsequently exposed for 48 hours to the organic compounds as well as a solvent control. The expression of the vitellogenin-1 (*vtg-1*), androgen receptor-1 (*ar-1*), aromatase (*cyp19a1b*), thyroid receptor-1 (*tr*-α), *tr-β*, glucocorticoid receptor-1 (*gr-1*) and mineralocorticoid receptor (*mr*) genes was quantified in fish whole body homogenates using RT-qPCR with β-actin as normalizer. The expression of *cyp19a1b* was significantly lower in fish exposed to the organic compounds from one of the localities representing agriculture land cover, than fish from the solvent control exposure group. Certain triazole and imadozole fungicides are known aromatase inhibitors in fish, and the lowered *cyp19a1b* expression may therefore have been associated with fungicides present in the river. Interestingly, there was no significant difference in the expression of *vtg-1*, *ar-1*, tr-α, *tr-β*, *gr-1* or *mr* in fish exposed to contaminants representing the six localities and those representing the solvent control exposure group. The current results therefore suggest low estrogenic, androgenic, thyroid and corticosteroid potency associated with organic contaminants in the upper Olifants River.

TP069 Whole spectrum of cytochrome P450 genes and molecular responses to PAH exposures in the hermaphroditic fish Kryptolebias marmoratus

B. Kim, Hanyang University; J. Rhee, Research Institute for Natural Sciences, Hanyang University; J. Lee, Hanyang University / Dept of Chemistry, Hanyang University Graduate School / Dept of Chemistry

PAHs are known to act as strong mutagens and/or carcinogens but the available information is limited on their molecular mechanism for gene modulation prior to the phenotypic appearance of physiological toxic effects. Previous studies with fish show modulation of expression of key genes in relation to stress response against PAHs exposure, but there is still a lack of studies on responses of cytochrome P450 (*CYP*) genes and changes in biotransformation upon PAHs exposure. In this study, we used the full spectrum of *CYP* genes of the hermaphroditic fish, *Kryptolebias marmoratus* to understand their potential mode of action on PAHs-triggered molecular mechanisms. We also analyzed further CYP-involved detoxification and endogenous steroidogenic metabolism after exposure to different concentrations of PAHs over different time courses in *K. marmoratus*. Also, detoxification- and antioxidant-related enzymes' activities were analyzed with different concentrations of PAHs. These data suggest that whole *CYP* profiling can be a way of understanding and uncovering the mode of action particularly with respect to emerging chemicals such as PAHs exposure with the new finding that PAHs have dual functions on CYP-involved metabolisms.

TP070 Adverse outcomes in zebrafish following a parental benzo[a] pyrene exposure

C.E. Thornton, University of Mississippi / Department of Pharmacology and Environmental Toxicology Research Program; J. Corrales, University of Mississippi; X. Fang, Yale University; M.B. White, University of Mississippi; L. Ballard, T. Clerk, USDA ARS / Genomics and Bioinformatics; B.E. Scheffler, USDA ARS; K.L. Willett, University of Mississippi / Environmental Toxicology Research Program

An adverse outcome pathway is a conceptual model that links a direct molecular initiating event such as a molecular interaction between a xenobiotic and a specific biomolecule and an adverse outcome at a biological level of

organization. Benzo[a]pyrene (BaP) is a xenobiotic with carcinogenic effects that also acts as an endocrine disruptor. We hypothesized that BaP exposure would cause: 1) phenotypic malformations in the gonad that would affect reproductive success; 2) developmental deformities in the offspring that would lead to decreased survival and ability to reproduce without additional BaP exposure; and 3) changes in gene expression that would be conserved across generations and related to the adverse phenotypes observed. Adult zebrafish (2 females x 2 males, N=10 replicate tanks per treatment) were fed 2% body weight/day flake food treated with 0, 11.6, 110, 1086 µg BaP/g flake (equivalent to 0, 0.23, 2.2, and 22 µg BaP/g fish/day) for 22 days. Parental gonads were sectioned and evaluated for pre-vitellogenic, vitellogenic, mature, and corpus atreticum oocytes in females and spermatogonia, spermatocytes, and spermatids in males. Ovarian atresia was significantly decreased following high dose BaP exposure. The number of fertilized eggs was significantly decreased in P0 fish exposed to 22 µg BaP/g fish. Total egg production was not significantly affected by dietary exposure to BaP in the P0, F1, and F2 generations. F1 (but not F2 or F3) mortality was significantly increased in larvae whose parents were exposed to 2.2 and 22 µg BaP/g fish by 48 and 56 hpf, respectively. At 96 hours post fertilization (hpf), F1 body length, body and tail shape, pericardial edema and swim bladder were negatively impacted by parental exposure to 2.2 and 22 µg BaP/g fish. Adult P0 and F1 gonad, liver and heart samples were analyzed by RNASeq to identify the cellular responses correlated with the observed adverse outcomes (*n*=1-3/treatment/generation/tissue). Finally, Ingenuity Pathway Analysis was used to link BaP-affected genes to a biological function such as cardiac and growth disorders. Based on these results, dietary BaP exposure negatively impacted zebrafish parental reproduction and F1 development, while trans-generational effects on zebrafish development continue to be assessed. Supported by NIEHS R21ES019940.

TP071 Dietary exposure to EE2 altered genes involved in key signaling receptors pathways in female largemouth bass (Micropterus salmoides)

R.C. Colli-Dula, University of Florida / Physiological Sciences; K.J. Kroll, D. Barber, Univeristy of Florida / CEHT; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology

17alpha-ethinylestradiol (EE2), used for birth control in humans, is a potent estrogen that is found in wastewater and has the ability to interfere with the endocrine system in fish. We used a transcriptomic approach to investigate mechanisms of action caused by dietary exposure of EE2 (0.07 mg EE2/kg feed) in the livers of female largemouth bass during the reproductive season. In addition to changes normally ascribed to soluble estrogen receptors, we identified in our data a large group of genes involved in key signaling pathways including protein-kinase cascades (MAPK) as well as phosphoinositol 3-kinase. We also observed changes in signaling receptors involved in the immune system with the majority of the transcripts up regulated. The data suggests that EE2 exposure also activates signaling pathways via non-genomic mechanisms within cell cycle regulation. The non-genomic signatures included genes involved in protein kinase, protein tyrosine, phosphoinositide, transcription factors, G protein, cell differentiation/regulation of transcription and tumorigenesis. This result suggests that EE2 might act via nongenomic action on target genes implicated in tumorigenesis or liver cancer in female LMB.

TP072 Metagenomic techniques for monitoring invasive species in the Great Lakes

E. Pilgrim, USEPA / Ecological Exposure Research Division

The effectiveness of any invasive species monitoring network is determined by its ability to detect rare, non-native individuals against the natural aquatic community. Standard field collection methods often require very large sample volumes to detect individuals of rare species, and these methods are often labor intensive, time consuming, and require significant expertise for taxonomic identification of specimens. Ongoing advances in molecular taxonomy and DNA sequencing technology (such as eDNA for Asian carp) present new opportunities for developing sensitive invasive species detection methods which could also reduce some of the time, cost, and effort involved with processing these samples. Next-generation DNA sequencing allows for DNA extraction, PCR amplification, and DNA sequencing from bulk, unsorted samples. When compared against genetic databases, this DNA sequence data can be used to generate community profiles from each sample which in turn provide a novel means for detecting rare individuals from these bulk samples. While eDNA approaches target specific taxa of concern, we have chosen to investigate community-level data sets that have the potential to detect any non-native individuals, targeted or otherwise, thereby supplying a molecular genetic method for detecting new invasions from new, unknown species. We will present results from our 2012 fish/benthos sampling efforts in Lakes Superior and Huron, and compare the molecular detection sensitivity to standard methods. We will discuss the strengths and weaknesses of such a community molecular genetic approach to invasive species detection, some of the requirements for its use (e.g., building DNA sequence databases), and its potential utility in a Great Lakes-wide detection network.

TP073 The role of histone modification in phenotypic plasticity of Daphnia pulex

S. Saleh, University of Saskatchewan / Department of Biology; J. Andres, University of Saskatchewan; S. Niyogi, C. Todd, University of Saskatchewan / Department of Biology

Epigenetics is the study of mitotically and/or meiotically heritable changes in phenotype that can't be explained by changes in DNA sequence. Different abiotic and biotic stresses can potentially alter the epigenetic state. Identical clones of the water flea (*Daphnia spp.)* can develop heavy-metal resistance, and produce a wide range of inducible defenses in response to the chemical cues released by predators (i.e., kairomones). Though it has been recently shown that the epigenetic repertoire of *Daphnia* includes different histone alterations, the role of chromatin modification in phenotypic plasticity is still unknown. *Daphnia pulex* embryos exposed to 100mcg/L of Ni had a higher survival rate (70% Vs. 50%) when compared to their mothers. Similarly, embryos exposed to fathead minnow (*Pimephales promelas*) kairomones developed neckteeth. The presence of histone deacetylation inhibitors (HDACis: Butyrate and Tritchostatin A) at very low concentrations (0.1mcg/L) seems to suppress these phenotypic responses. Our results strongly suggest that the remarkable adaptability observed in *D.pulex* clones has an epigenetic basis and relates to complex changes in the chromatin accessibility landscape.

TP074 Toxicity and sublethal effects of phthalates in Silurana tropicalis

J. Mathieu-Denoncourt, Royal Military College of Canada / Chemistry and Chemical Engineering Department; S.R. de Solla, Environment Canada / Wildlife Toxicology Research Section; V.S. Langlois, Royal Military College of Canada / Chemistry & Chemical Engineering

Due to their versatility, robustness and low production costs, plastics are used in a wide variety of applications. Polymers that are not easily processable or useful in their natural form are mixed with plasticizers, chemicals that render polymer flexible and elastic. However these chemicals are often not covalently bound to plastics, and thus leach from products into the environment. Furthermore, several studies have shown that plasticizers such as phthalates bind to the thyroid hormone receptor, disrupt the balance between androgens and estrogens, down-regulate the gene expression of enzymes and transport proteins involved in testosterone synthesis and induce oxidative stress. As few studies have focussed on the effects of phthalates in aquatic species, this project aims at elucidating their effects on amphibians as well as determining their mechanisms of action. A series of acute (72 h) and chronic (metamorphosis climax) exposures were conducted using the frog *Silurana tropicalis*. Larvae were exposed to geometric concentrations of monomethyl (MMP; 0.24 – 2400 ppm), dimethyl (0.115 – 1150 ppm) and dicyclohexyl (0.6 – 600 ppm) phthalates. Toxicity and cellular stress were assessed by larval mortality and the expression of oxidative stress-related genes. Sublethal endpoints such as malformations and expression of reproductive and thyroid hormone-related genes were also evaluated. Acute exposures to 2400 ppm MMP significantly increased mortality and gut abnormalities. Chronic exposure to MMP showed a reduction in survivability and accelerated time of metamorphosis. Gene expression, morphologic measurements and sex ratios will be presented. This study provides valuable data that will help better assess and manage the risk of the production or use of phthalates to the Canadian environment.

TP075 Evolved resistance to PCB126 and PAH induced cardiac teratogenesis in Gulf killifish (Fundulus grandis) from the Houston Ship Channel

E. Oziolor, E. Bigorgne, C.W. Matson, Baylor University / Environmental Science

The Houston Ship Channel, connecting Houston, Texas to Galveston Bay and ultimately the Gulf of Mexico, is heavily industrialized and includes several areas that have historically been identified as containing significant levels of mercury, dioxins, furans, PCBs, and PAHs. Gulf killifish, *Fundulus grandis*, inhabit this entire estuarine system, including the most contaminated areas. *F. grandis* is the sister species of the well-established estuarine model organism *F. heteroclitus*, for which heritable resistance to both PCB and PAH toxicity has been documented in several populations. Populations of *F. grandis* collected from contaminated and non-contaminated sites were collected and manually spawned in the laboratory. The F1 embryos were exposed to various concentrations of PCB126 and coal tar and then screened for cardiac deformities. Embryos from populations collected from contaminated sites show a >1000 fold increase in resistance to PCB126 induced teratogenesis and a >10 fold increase in resistance to coal tar. Reciprocal crosses between reference and contaminated populations exhibit an intermediate level of resistance, which suggests that observed protection is genetic and biparentally inherited. EROD data confirm a reduction in CYP1A induction in resistant populations of *F. grandis*, consistent with responses previously described for resistant populations of *F. heteroclitus*, specifically a recalcitrant AHR pathway. The decreased levels of cardiovascular teratogenesis, and decrease in CYP1A inducibility in response to PCB126 and a PAH mixture suggest that *F. grandis* populations in the Houston Ship channel have adapted to chronic contaminants exposures via a similar mechanism previously described for *F. heteroclitus*. To the best of our knowledge, this is the first documentation of a pollution tolerant population of *F. grandis*. Further investigations of DNA damage differences through flow cytometry suggest a more complex adaptation to chronically contaminated populations. Additionally, the mechanistic similarities between population adaptation observed in this study with previous work in *F. heteroclitus* suggest that genetic variation predating the evolutionary divergence of these two species may be the best explanation of observed similarities and the seemingly independent parallel evolution of pollution tolerance in genetically distinct populations.

TP076 Does multi-generation exposure to copper contamination influence metallothioneins production, ATP and respiration rate: study on Tribolium castaneum

S. Zmudzki, M. Zurek, Jagiellonian University / Institute of Environmental Sciences; P. Gibas, Uniwersytet Jagielloński / Institute of Environmental Sciences

In the last two centuries human activity has significantly increased and has massively impacted natural environment, not only in term of agriculture but also in rapid population growth. New forms of industrial activity appeared and heavily influenced wildlife and natural environment. Thus dozens of new substances appeared in environment, as well as some essential elements increased concentration becoming toxicants. Nowadays huge areas are affected by pollution and totally changed. Environmental pollution can influence not only abiotic conditions in ecosystem but also all trophic and spatial levels. Strongest visible output for these phenomenon is decreased population size, advancing inbreeding depression and influence many traits important for population abundance, physiological traits and individuals' fitness. In this study we explored how multi-generation exposure of flour beetle *Tribolium castaneum* to elevated level of copper concentration influences metallothioneins and ATP level. In order to determine the influence of copper contamination on the metabolic activity of the organism, we measured the respiration rate. For this purpose laboratory inbred (20 individuals) populations of *Tribolium castaneum*, selected for 30 generations for copper resistance (medium contaminated with copper at 1000 mg/kg) were used as well as populations maintained in parallel in uncontaminated medium. To answer the research question in stake and investigate the problem we used 2-generation experiment setup to separate effect of genetic change (adaptation) from other effects (direct factor effect and maternal effect) which can occur in term of multi-generation exposure to certain factor. Preliminary results prevailed that metellothioneins' production after multi-generationally exposed populations (for females) shows the pattern of adaptation. Significantly elevated level of proteins production was present in directly affected populations, as well as in populations when direct effect and maternal effect were removed in experimental setup. This indicates that in adapted populations elevated level of metallotioneins' is present constantly and is not only induced directly by factor. Analysis on ATP and respiration rate are in progress and results will be shown.

TP077 Migrations between small isolated copper-adapted and non-adapted populations: case studies on the flour beetle Tribolium castaneum

S. Zmudzki, Jagiellonian University / Institute of Environmental Sciences; P. Gibas, Jagiellonian University

The research aimed at gaining knowledge on the functioning of small isolated populations exposed to chronic pollution with toxic chemicals and the impact of migrations' on these populations. In particular we investigated populations exposed to copper pollution, which can be a strong selective factor influencing functioning of populations in the environment. Research in population size and its importance for population abundance have a long history especially in conservation biology. Despite this in case of populations affected by long-term toxic pollution especially in invertebrates these phenomena are almost unknown. There are well-worn views on the impact of migrants on the fitness of small populations. Long-term isolation, lack of gene flow leads to fitness reduction and degeneration of population. Usually under natural conditions without anthropogenic impact of additional factors, immigrants significantly increase the genetic diversity and adaptation by increasing heterozygosity (heterosis). Studies on migration between populations exposed and adapted to contamination are very rare. In presented research we designed and conducted migrations within selected lines of *Tribolium castaneum* obtained from 25-generation exposure to copper contamination experiment. Migrations were carried out between nonadapted and adapted populations, as well as between the same type of populations. Further analyses were performed on progeny of the first generation, and after five generations. This will help us to assess the direct impact of migrations, as well as the possibility of return to the baseline. To indicate these differences standard individual level indices like reproductive success, the EC50 for reproduction and copper level in the medium were estimated. The level of metallothioneins, total copper content in the body, the level of energy and metabolic rate (respiration) will also be measured and determined. In the aim of expoloring the influence of multi-generation exposure on elimination and excretion rate a toxicokinetics study will also be conducted. Toxicokinetics assays for populations affected by migrations will let to find whereas this type adaptations (if occur) can be lost because of migrations. Results to be obtained from these studies can help to fill a gap in contemporary knowledge of basic ecotoxicology, and can be used in conservation biology. Project is in progress and results will be shown on the conference.

TP078 Micro-evolutionary response in a natural Daphnia magna population under Cu and Zn stress

J.D. Hochmuth, Ghent University; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology; K. De Schamphelaere, Ghent University / Environmental Toxicology and Aquatic Ecology

A 10 week experimental evolution study was carried out under semi-field conditions to test for micro-evolutionary effects in a natural *Daphnia magna* population exposed to a control, 2 Cu (45µgCu/L and 180µg Cu/L), and 3 Zn (240µg Zn/L, 428µg Zn/L, and 760µg Zn/L) concentrations. We investigated if the long-term exposures to Cu or Zn resulted in higher organism fitness compared to that in the control evolution treatment and in the original (= start) population. At the end of the natural selection experiment After 4 months of culturing under control conditions, thus eliminating any acclimation history, a life-table experiment was conducted to determine any changes in fitness solely due to adaptation, measured as an increase in mean population fitness. We observed a significantly higher total reproduction at 760µg Zn /L and at 180µg Cu/L in the respective metal evolved populations compared to the long-term control evolved population and the start population. In the population evolved at 760µg Zn/L acclimation and adaptation to the metal had enabled the population density to recover, matching that of the control, despite an initial reduction of 75% of the clones. Under long-term exposure to 180µg Cu/L, however, despite lower initial mortality (50%), acclimation and adaptation effects were not sufficient to lead to a full recovery of the population density. Our results confirm that micro-evolution can occur after only a few generations but that adaptation in itself is not a guarantee for a complete recovery of the population density.

TP080 Genotoxicity of Cotton Herbicide Mixtures in Southern High Plains Playa Lakes

W.H. Mimbs, West Texas A&M University / Department of Life, Earth, and Environmental Science, Oklahoma State University; W.J. Rogers, Wt A&M University; G.C. Barbee, West Texas A&M University / Life, Earth & Environmental Sciences

The ecological ramifications of heavy pesticide use in the Southern High Plains have yet to be extensively studied. Concentrations seen in previous studies are in the low parts per billion (ppm) and are not high enough to directly cause mortality in non-target organisms. Genotoxicity, which may be induced at very low concentrations, has not been studied using pesticides and pesticide mixtures at environmentally relevant concentrations. The freshwater crustacean *Ceriodaphnia dubia* (*C. dubia*) and the piscine species *Gambusia affinis* (*G. affinis*) were used in an acute exposure comet assays and micronucleus assays to assess the genotoxicity of two cotton herbicides, diuron and metolachlor. Both organisms were exposed to each herbicide separately and in mixtures at environmentally relevant concentrations detected in playa lakes of the Southern High Plains. Tissue samples from both species were used in the Comet assay and the Micronucleus test. In both species using the comet assay, the genotoxicity of the individual herbicides was negligible (ranging from 0.5-2 µg/L metolachlor and 1.5-6 µg/L diuron) compared to the negative control. However, the herbicide mixtures (primarily using a 3:1 environmentally relevant ratio) exhibited appreciable genotoxicity and had body/tail ratios similar to methyl methanesulfonate (MMS; 10 mg/L), the positive control. The highest concentrations (3 and 6 µg/L) of diuron may also be genotoxic considering results from the G. affinis studies. This suggests a possible potentiative effect that might not be observed if only individual chemicals are assayed, or if only chemical analysis (e.g., GC-MS) alone is relied upon to assess ecological health risks. The micronucleus assay, however, demonstrated no genotoxicity in any instance. Thus, non-target organisms inhabiting playa lakes adjacent to agricultural activities requiring pesticide use may be subject to DNA damage as a result of these, and other, herbicide mixtures. The possibility exists for this DNA damage to manifest eventually as reproductive, growth, or mortality effects, potentially impacting population levels of species inhabiting playa lake areas.

Sustainability and Human Health

TP081 Size-Resolved Indoor Air Particle Analysis of PFAs by GC-MS

R.A. Di Lorenzo, Memorial University / Chemsitry; C.J. Young, Memorial University / Department of Chemistry

Perfluorinated acids (PFAs), specifically perfluorinated carboxylic acids (PFCAs) and perfluorinated sulfonic acids (PFSAs) are common analytes for environmental study. Although oceanic transport of PFAs and gas phase transport of volatile PFA precursors has been investigated, the role of atmospheric particulate matter on the global dissemination of PFAs is unknown. Particle size plays a significant role in their atmospheric lifetime and thus their potential for atmospheric transport. Furthermore, the role of indoor air particles as a route of biological uptake in humans has yet to be assessed. Particles below 2.5 µm in diameter (PM2.5) have the ability to breach the alveolar membrane in the lungs and make their way into the bloodstream. For these reasons, it is necessary to investigate size-resolved data to determine the potential bioavailability of compounds sorbed to these particles, as well as their potential for particle-mediated, atmospheric transport. Traditionally, PFAs are quantified in various matrices by liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) but these analyses usually require extensive sample preparation and clean-up. We will describe recent efforts to develop a simple yet rigorous analytical method for the part-per-trillion level quantification of PFAs in atmospheric particulate matter by gas chromatography–mass spectrometry (GC-MS). This is achieved through the optimization of a simple liquid-liquid extraction followed by benzyl esterification of the native PFAs before injection onto the GC column. The esterification is achieved through the formation of the corresponding acid chloride species via reaction with thionyl chloride, followed by esterification with benzyl hydroxide. This method not only allows for rigorous and sensitive quantification of PFAs, but allows for the simultaneous analyses of the relevant volatile PFA precursors. We will highlight this method's potential by quantifying PFAs in indoor air particles across fourteen size bins from 18 – 0.010 µm collected using a nanoMOUDI-II particle sampler.

TP082 High Molecular Weight PAHs in Cigarette Smoke and Pine needle

S. Bhandari, Texas Southern University / Environmental and Interdisciplinary Science; H. Hwang, Texas Southern University

Cigarette smoke and traffic emission are two major sources of persisted carcinogenic contaminants, especially polyaromatic hydrocarbons (PAHs). PAHs with molecular weight of 278 and 302 have been gaining more attention due to their higher cancer potency. This study analyzed high molecular weight PAHs in Pine needle and Cigarette samples that have not been measured commonly. Pine needle samples collected from Houston (total 15 sites), and 6 cigarette samples collected from indirect smoking were analyzed for high molecular weight PAHs using a GC-MS. Some of them were found more carcinogenic than benzo[a]pyrene, which had been used as a reference PAH. Total concentrations of these PAHs (more than 25 PAHs) in pine needles and cigarette samples varied from 30 to 573 ng/g (wet wt.) and 0.29 to 476 ng/cigarette respectively. Benzo[b + j + k]fluoranthenes were most abundant and followed by indeno[1,2,3-cd]pyrene and benzo[ghi]perylene. Though concentration of high molecular weight PAHs were found at lower concentration compared to low molecular weight PAHs, Dibenzo[ah] anthracene and dibenzo[al]pyrene (high molecular weight PAHs) accounted more than 50% of the total cancer potency because of their higher cancer potency factors, which are 5 and 10 times, respectively, of benzo[a]pyrene. Concentrations of benzo[a]pyrene in some samples of pine needles and cigarette were found above USEPA screening guideline. PAH concentrations will be compared to sources (distance from highways and/or busy traffic ways) to see impacts of vehicle emission, and in between direct and indirect smoking to see the impact of cigarette smoke on indoor contamination.

TP083 What might be in our dust? – Modeling emissions of organics from indoor surfaces

S. Molander, Chalmers University of Technology / Department of Energy and Environment – Environmental Systems Analysis; P. Andersson, Umea University; K. Fransson, Chalmers University of Technology / Environmental Systems Analysis, Energy & Environment; P. Haglund, Umeå University / Department of Chemistry; T. Holmgren, Swedish Armed Forces / National CBRN Defence Centre; T.V. Rydberg, IVL Swedish Environmental Research Institute; J. Tivander, Chalmers University of Technology / Environmental Systems Analysis, Energy and Environment; J. Westerdahl, IVL Swedish Environmental Research Institute

Organic substances from a variety of different indoor sources are emitted to the indoor environment, and finding their ways to dust. Examples of such sources are paint on walls and ceilings, textiles and consumer goods like plastic articles from which unintended emissions occur during the use-phase. The modeling approach employed in this research aims at a quantification of emissions from a large set of products occurring in a typical developed country – Sweden. The approach is "bottom-up" combining models providing information on aggregated product surface areas, together with their material composition, and a diffusive mass-transfer emission model, covering the emission process from product surface to air. Here we will present results related to emissions of a high number of specific organic chemicals from indoor walls, PVC floors, furniture and clothes. To estimate the surface areas an approach relying on trade statistics, longevity data and products descriptions was used. The emissions modeling were made using a generic model (partly experimentally validated) to predict emissions of organic chemicals diffusion within the material, equilibrium partitioning at the material surface, and convective mass transfer through a boundary layer to bulk air. Chemical characteristics like diffusion coefficients and initial concentrations within the materials, together with Abraham solvation parameters and material characteristics like polymer composition and thickness together with surrounding conditions (e.g., temperature, ventilation rate, etc.) served as input for emission calculations. A simple model aiming at average content of additives, provided chemical compositions of materials together with initial concentrations for a number of different materials based on, for instance, declarations of contents and material handbooks. The combination of the diffusion model with the models of surfaces and their composition gives a unique emission model providing the possibility to feed in results to environmental fate modeling, and environmental chemical analysis opening for empirical validation of the modeling approach. The modeling also illustrates the possibility to, in a stepwise manner, cover the emissions of substances from products, and doing this in a way permitting countrywide emission calculations and ranking of chemicals as inputs to prioritization.

TP084 Legacy and current used flame retardants in house dust from Vancouver, Canada

M.E. Shoeib, Environment Canada / Atmospheric Science and Technology Directorate; L.M. Jantunen, Environment Canada; G.M. Webster, Simon Fraser University

The phase out of Penta and Octa formulations of the polybrominated diphenyl ethers (PBDEs) in North America and Europe has led to the increased use of alternate FRs to meet flammability standards for different products. Organophosphate esters (OPs), in particular triesters, are high-production-volume chemicals used as plasticizers to enhance the properties of plastics and as flame retardants to protect textiles, furniture and many other materials. The indoor environment holds a large inventory of products containing these materials which can be released to the surrounding air and dust. Therefore, the indoor environment, where people spend 90% of their time, is an important exposure route for humans especially childrens. In this study eight OPs (tri-phenyl phosphate (TPP), tris(2-chloroethyl) phosphate (TCEP), tris(2-chloroisopropyl) phosphate (TCPP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP) and tris(2-ethylhexyl) phosphate (TEHP), tris (2,3-dibromopropyl) phosphate (TDBPP), tris(p-cresyl) phosphate (TPCP) and tris(2-butoxyethyl) phosphate (TBEP)) were measured in more than 100 dust samples collected from homes in Vancouver, Canada during 2007 – 2008 as part of the Chemicals Health and Pregnancy (CHirP) study. The high levels and detection frequencies of the OPs in indoor dusts reflects their ubiquitous presence in indoor environment. The OPs concentrations were compared with concentrations of fifteen PBDEs and eighteen non-PBDEs FRs previously analysed in the same samples. The total amounts of FRs and other chemicals of health concern also (poly and perfluorinated chemicals) were in the range of hundreds of mg per one gram of dust. Adults and toddlers exposure will be estimated based on these data. A Spearman correlations were also perform for PBDEs congeners in the dust and maternal serum indicates indicating that Penta BDE-related congeners in dust (i.e., BDEs 47, 66, 85, 99, 100, 153 and 154) were moderately correlated with BDE 47, 99 and 100 in maternal serum (Spearman's rho = 0.25 to 0.44, $p < 0.01$) suggesting, as expected, that dust may be an important route of exposure for these chemicals. The octa-related congener BDE 85 in dust was only weakly correlated with BDE 85 in serum (Spearman's rho = 0.18, $p=0.02$). There was no correlation between BDE 209, the main congener in the Deca BDE formulation, in dust and serum.

TP085 Atmospheric Particulate Matter Components, Oxidative Stress and Inflammatory Markers in a Macrophage Model: a Case Study in the Los Angeles Basin

D.S. Antkiewicz, Wisconsin State / Wisconsin State Laboratory of Hygiene / Environmental Toxicology, University of Wisconsin – Madison / Wisconsin State Lab of Hygiene; D.A. Perkins, University of Wisconsin – Madison / Wisconsin State Laboratory of Hygiene; J.D. Hemming, Wisconsin State Lab. of Hygiene / Environmental Toxicology; Z. Sijan, University of Wisconsin-Madison; M.M. Shafer, University of Wisconsin-Madison / Environmental Chemistry & Technology Dept.

Air pollution in the form of atmospheric particulate matter (PM) is a major concern world-wide. It is estimated that over 6 million people die early annually due to exposure to PM. Adverse human health effects include asthma, bronchitis, respiratory infections, cardiovascular disease and cancer. Oxidative stress is an important toxicity pathway for PM exposures and the formation of excess Reactive Oxygen Species (ROS) can elicit an inflammatory reaction in tissues. PM species implicated in ROS formation include transition metals, elemental carbon and specific organic compounds, e.g., PAHs and quinones – however the role and specific activity of these and other PM components in mediating PM toxicity is poorly understood.
To advance our understanding of the mechanisms leading to toxicity and to identify its specific drivers, we applied to water extracts of urban PM multiple chemical and physical speciation tools, including filtration, metal chelation, ion exchangers, and a dissolved organic matter sorbent. This sample speciation was coupled with (1) an *in vitro* rat alveolar macrophage respiratory burst assay measuring ROS via the DFCH-DA fluorescent probe, (2) a dithiothreitol (DTT) assay measuring the sample's redox potential, (3) an ELISA assessing rat tumor necrosis factor-alpha, and (4) a PCR-Array screen of relative expression levels of 84 inflammatory cytokines and cytokine receptors as markers of inflammation-inducing potential of PM. Detailed chemical characterization (including 48 elements by SF-ICPMS) was performed on the fractionated extracts. Air samples used in the study were collected from the locations in LA Basin, CA that receive aerosol inputs from contrasting sources. Our results indicate that soluble (< 0.22 µm) species contributed to the majority of cellular ROS activity, which varied substantially between different LA Basin urban environments. No significant decrease in respiratory burst was observed with soluble organic matter removal, while chelation treatments were effective in reducing ROS activity, suggesting a major role of soluble transition metals. Moreover, the fingerprint of gene expression associated with each type of PM was quite different. The gene expression data coupled with the ROS measurements and the chemical composition of PM will help us better understand the relationship between PM toxicity and inflammatory response, as well as potentially provide insights into cell signaling involved in the processes.

TP086 Where did the flame retardants in my dust come from?

A. Golnoush, University of Toronto / Geography; E. Goosey, University of Toronto / Earth Sciences; A. Saini, University of Toronto / Department of Physical and Environmental Sciences; M.L. Diamond, University of Toronto / Department of Earth Sciences

What is in my dust? From Allen et al. we know that North American pentaBDE is related to PBDEs in foam furniture and decaBDE is related to TVs. We asked two questions: (1) which products are most closely associated with halogenated flame retardant (HFR) in dust, and (2) how has this product-dust relationship shifted with newer products containing PBDE replacements? To answer these questions we collected floor dust (vacuum clean + nylon sock, sieved to < 150 µm) from the most used room (usually TV room) in 35 homes in Toronto and also took surface wipes of products in those rooms. The products wiped had elevated Br levels as determined using XRF. XRF screening indicated the highest Br content of 4-13% in TVs. Surface wipes from electronic products were dominated by BDE-209, 206 and 207 and numerous PBDE replacements, namely DBDPE, TDCPP, TBB, TBPH, HBB, PBEB, OBIND and syn-dechlorane plus. House dust was highest in BDE-99, 47, 209, TDCPP, TBB, TBPH, DBDPE and OBIND. PBDE concentrations in dust were generally 10 times higher than "novel" flame retardants. Using Principle Components Analysis, we found that the chemical profiles in dust most closely resembled that of products with lower molecular weight HFRs, in particular TDCPP and TBB, which is indicative of flexible polyurethane foam found in foam furnishings rather than electronics. Chemical profiles of only two house dust samples were similar to electronic products in those rooms. We conclude that HFRs in furniture foam is the greatest source of HFRs in house dust from the most used room rather than electronics.

TP087 Towards understanding the role of fabrics in the sorption of flame retardants and phthalates

A. Saini, University of Toronto / Department of Physical and Environmental Sciences; E. Goosey, University of Toronto / Earth Sciences; J. Okeme, University of Toronto / Department of Physical and Environmental Sciences; M.L. Diamond, University of Toronto / Department of Earth Sciences

Flame retardants have been added to different materials including upholstery fabrics and clothing to increase their resistance to fire and meet flammability standards. Phthalate esters are one of the highest production chemical groups in the world that are primarily used as plasticizers. The indoor environment, with its limited air exchange rate and degradation pathways, holds a large inventory of products and materials that contain these compounds. Clothing acts as a platform for the deposition and re-suspension of the particle-phase chemicals and as a sorbent phase for the gas phase chemicals. As such, fabrics can release chemicals even after the removal of the source, thus making them available for human exposure. While considering the tendency of different types of fabrics to absorb chemicals from surroundings, they behave differently according to their physical-chemical properties. Cotton and wool, being hydrophilic, do not absorb hydrophobic organic chemicals readily whereas synthetic fabrics like rayon and acrylics have hydrophilic/ hydrophobic characteristics, therefore they show higher affinity to both hydrophilic and hydrophobic organic chemicals. We report on the sorption of a range of flame retardants and phthalates by cotton and polyester fabrics that were deployed in an indoor environment for up to 40 days along with a low volume pump. Sampling media from the low volume pump was exchanged at weekly intervals to obtain time weighted air concentrations of the target compounds. To determine the sorption rates of each compound, the equivalent air volumes sampled by the cotton and polyester fabrics were calculated over a given exposure period using mass of the chemical sequestered by the sampling media within the deployment

period and concentration of the target analytes. Preliminary results confirm the expectation of higher sorption rates of the target analytes to polyester than those for cotton.

TP088 Improving the Multimedia Indoor Model for SVOCs with a Multi-layered Foam Furniture Submodel

A. Krol, University of Toronto; M.L. Diamond, University of Toronto / Department of Earth Sciences; X. Zhang, University of Toronto Scarborough / Chemistry / Environmental Sciences; D. Serodio, University of Toronto / Department of Earth Sciences

Some semi-volatile organic compounds (SVOCs) which undergo multimedia partitioning are ubiquitous in the indoor environment, where North Americans receive their greatest environmental exposure. The Multimedia Indoor Model is a fugacity based model that quantifies the fate of SVOCs in a defined indoor environment. The model compartments are indoor air, polyurethane foam (PUF) furniture, carpet, floors, and thin surface films on walls oriented upwards, downwards, and vertically. PUF is widely recognized as a sink for SVOCs due to its strong sorptive capacity, as well as a source of additive flame retardants. The previous steady-state version of the model assumed that a chemical emitted indoors was uniformly distributed throughout the PUF in accordance with the chemical's K_{OA}. However, studies have found that PUF (as a passive sampler) poses a significant kinetic resistance to uptake and hence the assumption of uniform uptake and distribution in the relatively huge mass of a foam cushion is unreasonable. To address the effect of kinetic resistance, a dynamic version of the Multimedia Indoor Model was developed with a coupled multi-layered PUF compartment. The model was run with a single emission and constant emission for suite of halogenated flame retardants and phthalates that vary in log K_{OA} from 7.6 (Diethyl phthalate) to 19.2 (Decabromodiphenyl ethane). The previous assumption that the PUF furniture compartment reaches steady-state uniform distribution was not met within a 10 year time period and influenced the distribution of chemical in all compartments.

TP089 Heterogeneity of flame retardants within the home

E. Goosey, University of Toronto / Earth Sciences; M.L. Diamond, University of Toronto / Department of Earth Sciences; S. Chaudhuri, University of Toronto / Department of Chemical Engineering and Applied Chemistry; A. Saini, University of Toronto / Department of Physical and Environmental Sciences

In the last decade polybrominated diphenyl ethers (PBDEs) have been the focus of numerous environmental & health related studies because of their persistence, bioaccumlation & toxicity (PBT). Stringent flammability codes, coupled with the recent polybrominated diphenyl ethers (PBDE) voluntary & legislative bans (in N.America & Europe, respectively) have resulted in a broad range of FR compounds available to manufactuerers. Replacement flame retardants (FRs) consist of other halogenated compounds & organophosphates (halogenated & non-halogenated). Currently in North America, common replacements for penta-BDEs are ethylhexyl-tetrabromobenzoate (EH-TBB), Bis(2-ethylhexyl) tetrabromophthalate (BEH-TEBP) (constituents of Firemaster 550) & tris(1,3-dichloropropyl) phosphate (TDCPP). Here we assess the presence of 12 alternate FRs & 12 common PBDE congeners in Canadian homes, & identify partitioning behaviour between settled dust & organic films (window wipes), assessment of FR prevalence in dermal wipes from occupants, as well as addressing the variability between homes, rooms & within-rooms. The partitioning of compounds were investigated to identify the factors influencing the partitioning of FRs within indoor compartments, variability of partitioning between different compounds, and the influence of this partitioning on human exposure. PBDEs & alternative FRs concentrations varied between rooms and between homes. Their presence is much greater in bedrooms & living rooms, relative to kitchens which correlates well with the number of potential sources in each of those rooms as well as the cleaning frequency. Also, concentrations in washrooms were lower than other rooms, & this is correlated to the lack of potential sources (e.g., few electronics in washrooms). The partitioning of alternative FRs was found to be similar to that of PBDEs. Low volatility compounds replacing decaBDE were particularly prevalent to the dust, but were also identified in the window wipes. The concentrations of these low volatility FR compounds in window films were not as high as anticipated. This is likely to be because of potential photolytic debromination (as noted with BDE209) occuring in the window films or the slow sorption rate in the film and equilibrium not being met. It was concluded that dust is a major pathway within the indoor environment to human exposure for alternative FRs.

TP090 Screening organic chemicals for human exposure potential in the indoor environment: The roles of chemical properties and biotransformation rates

X. Zhang, University of Toronto Scarborough / Chemistry / Environmental Sciences; J.A. Arnot, ARC Arnot Research & Consulting / University of Toronto Scarborough / Department of Physical and Environmental Sciences; F. Wania, University of Toronto at Scarborough / Department of Physical & Environmental Sciences

Chemical management programs require the assessment of thousands of new and existing chemicals for hazard, exposure and potential risk to humans and the environment. Measured exposure data are unavailable for the vast majority of chemicals requiring evaluation and screening-level exposure models are needed for exposure- and risk-based prioritization efforts. Mass balance fate and bioaccumulation models have been combined to simulate human exposures from "far-field" environmental sources (i.e., food, water, outdoor air) for exposure-based screening using metrics such as chemical intake fractions and internal concentrations. Indoor exposure pathways (e.g., dust ingestion and dermal absorption) also need to be considered when estimating total exposure and body burden. Mass balance models that link indoor chemical sources, indoor fate and internal human concentrations can be used to interpret and support measurements of chemicals in indoor media and in human biomonitoring programs. In this study, we develop, parameterize and apply a steady-state screening-level human exposure model that combines an indoor chemical fate mass balance model and a mechanistic human bioaccumulation model. The model includes processes for chemical fate in the indoor environment and uptake and elimination in a human. We parameterize the model for representative conditions and conduct exposure simulations for humans in different age groups. We use a set of hypothetical neutral organic chemicals comprising a range of properties (partition coefficients, molar mass, hydroxyl radical reaction half-life) to examine the role of these properties on the calculated human exposure potential. Given the spatial scale of human interaction with the indoor environment humans may directly influence the chemical mass balance and indoor residence time for certain chemicals.. Therefore, we use the linked mass balance model to examine the influence of human biotransformation of organic chemicals on the overall fate of chemicals in the indoor environment. A case study of 40 volatile and semi-volatile organic chemicals compares exposure-based screening results using the human intake fraction and internal concentration as exposure assessment endpoints. The differences in the case study calculations and the priority ranking are highlighted and discussed.

TP091 Planetary boundary analysis suitable basis for chemical footprinting?

M. Zijp, L. Posthuma, J. Struijs, RIVM; A. Hollander, Radboud University Nijmegen; D. van de Meent, RIVM / Institute of Wetland and Water Research

The footprint concept has been coined to express whether gross chemical emissions (separate compounds or mixtures) are larger than the capacity of the environment to accommodate emissions, with too big a footprint implying undesired impacts. The (planetary) boundary concept was coined to define the aforementioned capacity, but is unknown for chemical mixtures. Both concepts can be of great use in sustainability assessment and communication, while they enable summarizing complex information. Triggered by these needs and the *Nature*-publication of Rockström et al. on this, we developed a stepwise approach to derive a chemical footprint, for products, processes or for regional emissions of multiple chemicals. The major part of the footprint derivation is science-driven, from emission modeling up till derivation of toxic pressures (so-called msPAF: multi-substance Potentially Affected Fraction). Example results for pesticide emissions suggest a clear decline. Comparing raw toxic pressure output versus policy-defined protection endpoints allow an absolute expression of chemical footprints. Example results show in how far chemical emissions occupy the 'environmental space' defined by the protection criterion. Results appear very sensitive to that criterion, so that further research into natural tipping points towards chemical exposures is warranted. In adopting the footprint and planetary boundary concepts as potentially useful idea for presenting cumulative chemical impact of large groups of compounds, we developed a methodology for chemical footprint quantification, we tested it in various case studies, and we explored relative and absolute use of the concepts. We present the outcomes, and discuss relative and absolute interpretations of the results, and show that the steps provide robust results for chemical management priorities, despite the possibility that absolute prediction of global biodiversity impacts of all chemicals are beyond reach as yet.

TP092 Increased green remediation for soil and groundwater cleanup: Driving force, sustainable elements and assessment

C.C. Zhang, University of Houston-Clear Lake / Environmental Sciences

The environmental remediation sector in the US accounts for a small fraction (< 0.05%) of the GDP, but it represents an annual total of approximately $5 billion in the remediation cost. By achieving the clean-up goal for contaminant removal, the cleanup itself also adds a significant environmental footprint to the contaminated sites at the local to global scale. For example, the estimated CO_2 emissions from the use of five common cleanup technologies (pump and treat, thermal desorption, multiphase extraction, air sparging, and soil vapor extraction) in Superfund sites over an approximately 10-year period are equivalent to the annual operation of a typical coal-fired power plant. Therefore, the incorporation of green and sustainable elements for the cleanup of contaminated soil and groundwater has been increasingly recognized in the last 10 years. This paper critically analyzes the current state of green remediation in the context of driving forces as well as the potential impediments, the well-recognized best management practices as well as some promising opportunities to an increased sustainability at various stages of site cleanups (site characterization, remedial design, construction, operation and monitoring), and the methodological issues associated with currently employed quantitative assessment tools to measure the greenness (relative sustainability) of soil and groundwater remediation technologies. Lessons learned from limited case studies on the increased use and improved assessment of green remediation will be provided. Like other sectors, traditional cleanup technologies are facing a paradigm shift from mere cost-effectiveness of a selected remedial strategy to a holistic approach considering economic, social and environmental impacts. Some future perspectives and challenges will also be discussed in the context of increased use of green remediation.

TP093 Screening water samples for human health risks

S. Kools, EcoTox; M. Schriks, A. van Wezel, L. Puijker, KWR Watercycle Research Institute

Highly sensitive *in vitro* CALUX bioassays are available which allow detection of estrogenic (ERα), androgenic (AR), progestagenic (PR), and glucocorticoid (GR) activities. We have previously derived trigger values for the ERα-, AR-, PR-, and GR CALUX bioassays for hormonal activities in (drinking) water samples. These trigger values define a level above which human health risk cannot be waived *a priori* and additional examination of specific endocrine activity may be warranted. These trigger values were based on 1) acceptable or tolerable daily intake (ADI/TDI) values of specific compounds, 2) pharmacokinetic factors defining their bioavailability, 3) estimations of the bioavailability of unknown compounds with equivalent activity, 4) relative endocrine potencies, and 5) physiological, and drinking water allocation factors. They are expressed as an activity equivalent to a potent reference compound, i.e., ng 17β-estradiol (E2)-equivalents (eq)/L, dihydrotestosterone (DHT)-eq/L, dexamethasone (DEX)-eq/L, and ng Org2058-eq/L for the ERα-,AR-, PR-, and GR CALUX bioassays, respectively. No hormonal activity has been detected in Dutch drinking water (with detection limits a factor 5 to 979 below the trigger values), indicating that at present removal of the responsible compounds during drinking water production is effective, and therefore no human health risks are to be expected from hormonal activity in Dutch drinking water. In this presentation, new developments and future challenges in human health based risk assessment for compounds in drinking water are discussed.

TP094 A Pressurized Liquid Extraction Technique for the Analysis of PAHs, Hopanes, Pesticides, PCBs and PBDEs from Air Filter Samples

A. Clark, Baylor University / Department of Chemistry and Biochemistry; P. Nallathamby, Baylor University / Waco; T. Barrett, R.J. Sheesley, S. Usenko, Baylor University

An analytical method has been developed for the pressurized liquid extraction (PLE) of a wide range of semi-volatile organic compounds (SVOCs) from quartz fiber filters (QFFs). This method was developed to improve the analysis of valuable and rare samples, such as those collected in an air sampling campaign and/or those from pristine remote environments. Approximately 50 SVOCs were selected as molecular markers of incomplete combustion (including, specifically, motor vehicle use), agricultural, industrial, and urban areas from five different compound classes including polycyclic aromatic hydrocarbons (PAHs), hopanes, organochlorine pesticides, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs). Currently, there is no analytical method capable of the extraction of all five compound classes in a single automated technique. Building on the work of Primbs et al. (2008) and Sheesley et al. (2010), we were able to utilize PLE as well as extraction solvents methylene chloride and methanol. Utilizing a Dionex Accelerated Solvent Extractor (ASE 350), we examined the extraction efficiencies of varying ratios of methylene chloride to methanol (from 1:1 to 5:1) at high temperatures (100˚C) and pressures (1500 psi). Prior to extraction, QFF samples were spiked with target analyte standards and isotopically-labeled surrogate standards. Extracts were concentrated, spiked with isotopically-labeled internal standards, and analyzed by gas chromatography coupled with mass spectrometry utilizing electron ionization and electron capture negative ionization. Target analytes were surrogate recovery corrected to account for analyte loss during sample preparation. Triplicate spike and recovery and method detection limit experiments were compared to previous studies. The developed method was applied for further validation to samples collected downwind of Fort Worth, TX in June 2011.

TP095 Urinary Phthalate Metabolites as Biomarkers to Assess a Nail Salon Workplace Exposure Intervention Program

J. Varshavsky, University of California, Berkeley / Environmental Health Sciences Division, School of Public Health; S. Harwani, California Enviromental Protection Agency / Department of Toxic Substances Control / Environmental Chemistry Laboratory; M. Snider, S. Petropoulou, J. Park, California Environmental Protection Agency / Department of Toxic Substances Control, Environmental Chemistry Laboratory; M. Petreas, California Department of Toxic Substances Control, California Environmental Protection Agency / Environmental Chemistry Laboratory; P. Reynolds, Cancer Prevention Institute of California and Stanford University / Department of Health Research and Policy and Stanford Cancer Center; T. Quach, Stanford University and Cancer Prevention Institute of California / Department of Health Research and Policy and Stanford Cancer Center

Phthalates are hormonally active chemicals produced in over 470 million pounds per year with numerous commercial applications, including in plastics, paints and personal care products (nail polishes, lotions, etc.). These chemicals have been linked to an array of reproductive and developmental health problems. A predominant number of nail salon workers in California are women of reproductive age, and 50-80% of these workers are low-income Vietnamese immigrants who have limited access to safety information and health care services. To address this concern, a method was modified from literature to measure the primary metabolites of diethyl-, diisobutyl-, dibutyl-, di(2-ethylhexyl)-phthalates in urine using tandem LC-MS/MS equipped with a Gemini C6-Phenyl column (Phenomenex, 100 mm x 2.00 mm, 3 µm). After SPE clean-up using Agilent Nexus ABS Bond Elut SPE cartridges, the recoveries of monoethyl- (MEP), monoisobutyl- (MIBP), monobutyl- (MBP), and mono(2-ethylhexyl)-phthalate (MEHP) monoesters ranged between 85-95%. We detected no significant laboratory background for these compounds, and sample extracts were tested for short term stability stored at 4˚C and results indicated that they were stable over 7 days. We measured these four metabolites in the urine of 17 San Francisco Bay Area nail salon workers before and after an educational intervention that promoted strategies expected to reduce chemical exposures. The results of this study provide a way to evaluate whether educational intervention translates into reduced chemical exposures by measuring levels of phthalate metabolites in urine. Further studies are needed to examine specific factors influencing phthalate workplace exposures in nail salons and to optimize strategies for reducing phthalate and other chemical exposures in this occupation. Disclaimer: The views expressed herein are those of the authors and do not necessarily reflect those of the Department of Toxic Substances Control, California Environmental Protection Agency.

TP097 Electrochemical removal of trichloroethylene from groundwater: Polarity reversal for sustainable treatment

L. Rajic, Northeastern University / Civil and Environmental Engineering; N. Fallahpour, Northeastern University / Civil and Environmental Engineering; A. Ciblak, Northeastern University; A.N. Alshawabkeh, Northeastern University, College of Engineering / Department of Civil and Environmental Engineering

Solar-powered electrochemical technologies for groundwater treatment use solar panels to generate a low-level direct current (DC) through electrodes in wells which enables manipulation of groundwater chemistry through electrolysis to create conditions favorable for either reduction or oxidation of the contaminants. There are several advantages to this approach: 1) it is

sustainable and driven by a renewable energy source, 2) it does not require the addition of solutions or chemicals into groundwater, making them environmentally friendly, and 3) the rates of redox reactions can be controlled by adjusting electric current intensity. Two transformation mechanisms are evaluated within this approach depending of electrode materials used: electrochemical reduction and electrochemical oxidation of contaminants. Solar driven electrolysis can be implemented as: *ex situ* treatment which involve groundwater pumping and treatment using electrolytic reactors and *in situ* which involve *electrolytic redox reactive barrier* where electrolytic reactors are implemented as a reactive barrier within the aquifer and *injection and/or well circulation* where electrolysis in wells generate *in situ* reducing or oxidizing conditions so that rates of release of reactive species are sustainable and can be controlled. Development of an electrochemical reactor within the aquifer for the sustained, solar-powered electrolysis is the first step in development of solar-powered electrochemical technologies. Here we present the possibility of electrode polarity reversal application for electrochemical oxidation of trichloroethylene (TCE) contaminated groundwater from karstic aquifer. TCE removal up to 69% is achieved by optimizing the polarity reversal frequency. The interval duration during polarity reversal influences the amount of charge evolved in the reaction, time for conducting the reaction and consequently the amount of target specie that interact with the electrode.

TP098 Use of a Surface Wiping Method to Predict Pesticide Risks in Residential Areas

W. Jiang, California Environmental Protection Agency / Department of Pesticide Regulation; J. Gan, University of California Riverside / Environmental Sciences

Pesticides are heavily used in residential areas and are often treated on outdoor impervious surfaces such as driveways and streets for pest controls. Previous studies have observed persistent pesticide runoff from these impervious surfaces, but no method has been developed to directly measure the runoff-transferable pesticide residues and predict their ecological risks. Surface wiping that was commonly used for assessing indoor contaminant risks to humans and was used in this study to predicted pesticide runoff potential. Strong positive correlation was observed for pesticide amounts on wiping sponges and in the runoff water (*Pearson*'s *r* at 0.92), and the regression line between predicted and measured values had a slope close to 1.0, suggesting that this method accurately measured pesticide residues that were available for runoff contamination. Most valuably, one linear regression line could predict the runoff for different pesticides and degradates, different formulations, different types of runoff, and different post-treatment periods. Significant amounts of pesticides were found attached onto loosely-residing particles, explaining the observed benthic toxicities and indicating possible transfer into indoor environment through wind or foot-tracking. Occurrence of pesticides on residential outdoor impervious surfaces was further confirmed by sampling in residential communities. Almost all sampling sites were found with pesticides and bifenthrin was the most ubiquitous one detected.

TP099 Polybrominated diphenyl ethers (PBDEs) and other flame retardants in milk from Canadian mothers

D.F. Rawn, A.R. Sadler, V.A. Casey, C. Menard, C. Corrigan, F. Breton, S. Feng, W. Sun, Health Canada / Food Research Division; T.E. Arbuckle, Health Canada / Population Studies Division; W.D. Fraser, University of Montreal / CHU Sainte-Justine Research Center

Flame retardants (FRs) have been developed and used to reduce flammability in consumer products (e.g., building materials, electronic equipment) and to improve human safety through a reduction in household fires, etc. It has been reported that, over time, many of the FRs persist in the environment and accumulate in biota. Detectable concentrations of FRs have been reported globally in wildlife and humans, with North American concentrations of some FRs (e.g., polybrominated diphenyl ethers [PBDEs]) being amongst the highest measured. FRs are often reported at elevated concentrations in indoor environments (e.g., house dust) relative to atmospheric concentrations, consistent with their use patterns. Human biomonitoring provides direct measurement of systemic exposure levels and is not dependent upon the rate of uptake or exposure route, but provides a clear measurement of how much of a given chemical an individual has retained in their body at the time of sample collection. The Maternal-Infant Research on Environmental Chemicals (MIREC) platform was developed to examine the possible impacts of environmental chemicals on the health of pregnant Canadian women and their children on a national scale. Recruitment occurred between 2008 and 2011 during the first trimester of pregnancy at ten sites across Canada. The study included the collection of human milk from participants between post-partum week 3 and 8, to ensure measurements were performed using mature milk. These samples are representative of the mothers' body burden and will provide insight into Canadian infant dietary exposure. Of the ~2000 females who participated in MIREC, approximately 850 contributed milk samples. Of these, 300 were subsampled for use in the determination of FR concentrations. Analyses of most FRs were performed using gas chromatography – high resolution mass spectrometry; although α-, β- and γ-hexabromocyclododecane (HBCD) were measured using liquid chromatography – triple quadrupole tandem mass spectrometry. PBDE concentrations observed in human milk ranged from 2.5 to 181 ng g^{-1} lipid with a median concentration of 16.6 ng g^{-1} lipid. Although FRs used as replacements for the PBDEs generally have not been well characterised in human tissues, some of the new, alternate FRs have been detected in human milk (e.g., HBCD, 2,3',4,5'-tetrabromo-2'-methoxyl-biphenyl ether and dechlorane plus). These data will support risk assessment and modelling activities for the FRs.

TP100 Effects of active volcanism in human lung function

D. Linhares, University of Azores / Department of Biology; P. Garcia, University of Azores / Department of Biology; CVARG; T. Ferreira, University of Azores / Department of Geosciences; A. Rodrigues, University of Azores / Department of Biology

Furnas volcano activity is marked mainly by highly active fumarolic fields, hot springs and soil diffuse degassing phenomena. These ground gas emissions from geothermal fields occur constantly and, villages such as Ribeira Quente exhibit very high levels of CO_2, having about 98% of the households built above geothermal fields. In this study we aimed to investigate the existence of a possible association between chronic exposure to volcanic diffuse degassing and, restrictive and obstructive airway diseases. A cross sectional diagnostic study was performed in: a study group of 150 individuals inhabiting a volcanically active environment (Ribeira Quente village) and, a reference group of 383 individuals inhabiting a village without any volcanic activity (Ponta Delgada village). For each individual, a spirometry test was performed and the forced vital capacity (FVC), volume exhaled in the end of the first second (FEV_1), maximal mid expiratory flow rate (MEF_{25-75}) and Tiffeneau-Pinelli index (FEV_1/FVC ratio) were analyzed. Information on lifestyle factors and an informed consent were obtained from each participant. Prevalence of individuals suffering from restrictive and obstructive airway disease was higher in the study group (32.6% and 12.3%, respectively) than in the reference one (10% and 2.8%, respectively). The risk of having a restrictive or obstructive airway disease was, respectively, 3.6 and 3.7-fold higher for the people living in the volcanically active area when compared to the reference group. The risk of increase in the severity of respiratory obstructions was 3.7 fold higher in the group living in the volcanically active environment. Confounding factors such as gender and alcohol consumption did not show any significant association with respiratory impairment, but age, smoking status and asthma were significantly associated with the frequency of obstructive airway disease. The prevalence rates of restrictions and obstructions in individuals inhabiting Ribeira Quente reveals a significant association between these respiratory diseases and the chronic exposure to volcanically active environment, particularly characterized by high soil diffuse degassing activity. These findings could contribute to explain the higher incidence rates of chronic bronchitis previously referred for the inhabitants of this volcanically active area and, emphasize the relevance of the implementation of preventive measures to minimize the risk of respiratory diseases.

TP101 Environmental Awareness Among Primary School Children in the Wa Municipality of the Upper West Region of Ghana

I. Nyameke, Wageningen University / Department of Environmental Toxicology

Environmental problems still kill at least 3 million children under age 15 every year globally. Observations and scientific studies conducted all over the world show that, these children are more vulnerable and heavily exposed to environmental hazards by their parents or themselves because of their behavior, mental attitude and size. These children suffer long-term effects from their early exposure to environmental hazards such as swimming in dirty rivers, playing in polluted environments, engaging in bush burning and eating unhygienic foods. Although greater environmental education programs and awareness of children's vulnerability to environmental hazards have helped

to improve children's health in a number of developed and less developed countries. However, many children, especially those living in rural and slums areas in Ghana continue to be exposed to environmental toxins and hazards. In response to the issues in Ghana, as in many parts of the world, environmental awareness program has been included as a part of the curriculum in schools and colleges on related subjects like Science, English Language, Environmental Studies, Civics and Social Science. Upon realizing the importance of environmental problems and its effects on children's health, this study was conducted to assess the level of Environmental Awareness among Primary School Children in the Wa Municipality of the Upper West Region of Ghana. The findings suggested that the primary school children in the Wa Municipality have a fair knowledge about environmental hazards and its effects on human health but the children do not actually put the knowledge they have into practice. This paper present recommend methodologies to Ghana Education Service to help the primary school children to put environmental education and awareness into practise.

Special Symposium

TP102 Animal Agriculture as a Source of Steroid Hormones: Insights and Critical Data Gaps

E.P. Kolodziej, University of Nevada-Reno / Civil and Environmental Engineering; D.L. Sedlak, University of California Berkeley / Department of Civil and Environmental Engineering; T. Harter, University of California, Davis / Land, Air, and Water Resources; D.M. Cwiertny, University of Iowa / Department of Civil and Environmental Engineering; S. Qu, UI Civil and Environmental Engineering Department

A large body of data suggests that animal agriculture, including confined animal feeding operations (CAFOs) and rangelands, is a potentially significant source of endocrine disrupting steroid hormones to receiving waters. To better characterize potential risks to aquatic organisms arising from animal agriculture, we have used a series of field and laboratory studies to investigate the occurrence, transport, and transformations of endogenous and synthetic steroid hormones from CAFOs and rangelands. Key aspects governing the potential risk steroid hormones pose to receiving waters include possible transformations of steroid hormones in animal wastes and aquatic systems and the leaching of steroid hormones from animal wastes to runoff water for subsequent transport. The data indicates a relatively small fraction of the steroid mass present in animal wastes is mobilized to runoff, although a complex mixture of steroids and associated transformation products are likely present in agricultural runoff. Characterization of steroid hormone occurrence in animal wastes and subsequent transformation processes suggests that mass balance approaches can be practically useful to guide runoff management actions to location-specific CAFO discharge scenarios. Despite these insights, a variety of critical data gaps remain regarding accurate assessment of the ecological risks of animal agriculture derived steroids. For example, recent research has indicated that product-to-parent reversion is possible for metabolites of trenbolone acetate (TBA), a potent growth promoting steroid used in most beef cattle production in the US Also, a variety of structural analogs and stereoisomers can arise from environmental transformation processes of steroid hormones, yielding a range of structures potentially capable of conserving bioactivity, while concurrent analytical approaches suggests that novel, uncharacterized steroids are present in agricultural runoff. However, most of the current research approaches used to evaluate the endocrine disruption potential of steroids do not consider these possibilities, thus limiting our understanding of the potential impacts of animal agriculture on receiving waters. These observations indicate that assessing transformation processes and using a broad array of receptor based assays across multiple endocrine endpoints is necessary to accurately understand the environmental fate of endocrine disrupting steroids derived from animal agriculture.

TP103 A Comprehensive Look at Steroid Hormones, Phytoestrogens, and Estrogenic Activity in a Commercial Sow CAFO Lagoon

E.E. Yost, North Carolina State University / Environmental and Molecular Toxicology; M.T. Meyer, US Geological Survey / Organic Geochemistry Research Laboratory; S.W. Kullman, North Carolina State University / Environmental and Molecular Toxicology

Comprehensive data is lacking on the fate of steroid hormones and phytoestrogens within the anaerobic lagoon, which is the most widely used system of waste management on swine operations in the United States. The objective of this study was to thoroughly characterize the distribution of steroid hormones, hormone conjugates, phytoestrogens, and estrogenic activity within the slurry and sludge compartments of a typical North Carolina commercial swine sow lagoon, which receives waste from pregnant and lactating sows. Sampling of lagoon liquids and solids was conducted in June 2009, April 2010, and February 2011. A suite of 42 analytes was assessed using LC/MS-MS, and estrogenic activity was determined using the yeast estrogen screen *in vitro* bioassay. Estrone and androstenedione were found to be the predominant steroidal estrogen and androgen species in the lagoon, respectively, while equol was determined to be the predominant phytoestrogen species. Hormone conjugates (estrone-3-sulfate and androsterone sulfate) were detected only at low levels, suggesting that these analytes are largely hydrolyzed to their free forms during lagoon storage. The majority of estrogenic activity in the lagoon was found to be attributable to estrone; although phytoestrogens were detected at magnitudes greater than or equal to those of steroidal estrogens, they contributed a minimal amount towards total estrogenic activity of the waste. A marked elevation of analyte concentrations was observed within suspended solids relative to the aqueous phase of the lagoon slurry, and the settling of solids effectively creates a sink for these compounds within the sludge phase of the lagoon. Results suggest that steroidal estrogens may be recalcitrant to degradation in the sludge, while androgens, progesterone, and phytoestrogens appear to be more readily degraded. Overall, findings emphasize the importance of solid phase adsorption and bacteria-mediated transformation processes in governing the fate of steroid hormones and phytoestrogens in the anaerobic lagoon, and indicate possible seasonal variations in the abundance of these compounds.

TP104 A Mass Budget for Steroid Hormones and Phytoestrogens on a Swine Farrowing CAFO

E.E. Yost, North Carolina State University / Environmental and Molecular Toxicology; M.T. Meyer, US Geological Survey / Organic Geochemistry Research Laboratory; S.W. Kullman, North Carolina State University / Environmental and Molecular Toxicology

In this USEPA STAR grant-funded study, we took a mass budget approach to track the fate of natural hormones within the waste management system of a commercial swine farrowing CAFO, which houses 5000 pregnant and lactating sows. Analysis of estrogens, androgens, progesterone, and phytoestrogens was made in relation to: 1) analyte output by individual animals; 2) analyte stability in anaerobic lagoons during waste storage; and 3) analyte mobility and attenuation following the sprayfield application of the lagoon wastewater. Through this integrated analysis, we were able to identify key operational variables that govern the fate of these compounds. Sow reproductive stage was found to be the major variable impacting the total animal output of steroid hormones. Steroidal estrogen and progesterone output increased in a defined gradient with progressing gestational stage of the sows, and up to 73% of estrogen mass output on the operation was found to be attributable to lactating sows. In the lagoon, the fate of these compounds seems to be governed primarily by biotic and/or abiotic transformation processes, as well as solid phase adsorption. Analytes common in sow urine and feces (e.g., estradiol and testosterone, as well as the phytoestrogens genistein and daidzein) were depleted or absent in the lagoon, while the primary metabolic products of these compounds (estrone, androstenedione, and equol) were abundant, suggesting the metabolic transformation of these analytes during waste storage. Likewise, conjugated estrogens and androgens were abundant in sow urine but detected only at vanishingly low levels in the lagoon, suggesting that conjugates are largely hydrolyzed during waste storage. As a result of the tendency for steroid hormones and phytoestrogens to adsorb to organic carbon, analyte levels were found to be highly elevated in lagoon wastewater suspended solids relative to the aqueous phase. The settling of these solids results in exceedingly high analyte levels in the lagoon sludge. Following the land application of lagoon wastewater to sprayfields, all analytes were attenuated to a fraction of their initial levels within the span of several days, but persisted at low levels in the topsoil for up to 2 months post-application. An end goal of this project is the prioritization of practices that may mitigate off-site transport of these compounds from swine sow CAFOs.

TP105 Effects of Manure Management on Steroid Hormones in the Feedlot and the Field

D.D. Snow, University of Nebraska; S. Bartelt-Hunt, University of Nebraska / Civil Engineering; S. Biswas, W. Kranz, University of Nebraska / Biological Systems Engineering; T. Mader, University of Nebraska / Animal Sciences; C. Shapiro, University of Nebraska / Agronomy and Horticulture; S. van Donk,

D. Shelton, University of Nebraska / Biological Systems Engineering; T. Zhang, University of Nebraska / Civil Engineering; D. Tarkalson, USDA-ARS

Effects of composting and tillage practices on steroid hormone fate and occurrence were evaluated through a series of field and laboratory experiments comparing manure and surface run-off from cattle fed with and without the use of the growth promoters trenbolone acetate, α-zearalanol, and melengestrol acetate. LC-MS/MS analysis of samples of cattle feeding pen surfaces and run-off found comparable levels of endogenous hormones, steroid transformation products, and a variety of related compounds. Synthetic steroids were detected in fresh manure and in feedlot surface soils from cattle administered synthetic steroids at concentrations up to 55 ± 22 ng/g (17α-trenbolone) and 6.5 ng/g (melengesterol acetate). Changes in steroid hormone concentrations in composted manure over time reflected significant transformation (79-87% reduction). First-order decay models fit some losses while other patterns suggest that microbial processes may also result in steroid production. Sorption experiments indicated hormone desorption is important in determining the mobility and presumably degradability in the environment. Losses of steroid hormones occur from manure fertilized soils, though low hormone concentrations in manure will produce low levels in run-off. Most samples from rain-fall simulation experiments were below detection limits, though frequency of steroid hormone detection was slightly higher in run-off several weeks after manure application. Field experiments using a synthetic estrogen surrogate applied with manure helped evaluate effects of tillage practices on potential steroid hormone losses, and indicate that incorporation can reduce losses in surface water run-off. Few soil and pore water samples contained detectable hormone levels under irrigated crops fertilized with stockpiled and composted manure. As with surface run-off, low levels in applied manure combined with dilution and degradation will result in undetectable levels of hormones below the crop rooting zone. Synthetic steroid hormones were not detected in any of the run-off or leachate from manure fertilized soils, grass, or pore water samples. Taken together, these results indicate that transport of steroid hormones from crop land treated with beef cattle manure can be minimized through manure storage, composting, and soil incorporation practices. Interception of feedlot runoff is a key factor in minimizing potential losses to surface water environments.

TP107 Effects of Singular Exposure to Androstenedione or Progesterone on Fathead Minnow (Pimephales promelas) Reproduction and Development

Z.A. DeQuattro, University of Wisconsin / Zoology; J.D. Hemming, Wisconsin State Lab. of Hygiene / Environmental Toxicology; T.P. Barry, University of Wisconsin-Madison / Department of Animal Sciences

High concentrations of progesterone (P4; 375 ng/L) and androstenedione (A4; 300 ng/L), among other steroid hormones, were found in snowmelt runoff from fields fertilized with manure from livestock feeding operations in Wisconsin. P4 is a key intermediate in the biosynthesis of several active fish steroids, including androgens (testosterone and 11-ketotestosterone), cortisol, estradiol-17β (E2), and 17α, 20β-dihydroxyprogesterone (17α, 20β-P). A4 is an active androgen in fishes and also serves as a substrate for the biosynthesis of other important steroid hormones, including androgens, estrogens and corticosteroids. Given their roles in steroid biosynthesis there is potential for them to be powerful endocrine disruptors. In separate experiments, this hypothesis was tested by exposing reproductively mature fathead minnows to nominal concentrations of 0, 10, 100, and 1000 ng/L P4 (21 day experiment) or A4 (26 day experiment), in a flow through system, and various reproductive endpoints were measured (e.g., egg number, fertilization success, secondary sexual characteristics, and hepatic vitellogenin (Vtg) mRNA expression). Additionally, fertilized fathead minnow eggs were exposed to the aforementioned nominal concentrations of P4 or A4 in static cultures to assess the direct effects of these steroids on early embryonic development and hatching success. Progesterone caused dose-dependent decreases in fecundity and fertility, and also significantly reduced GSI (gonadosomatic index) and Vtg mRNA presence in females. There were no effects of P4 on early embryonic development or hatching success. Exposure to low levels (4.45 ng/L) of A4 increased egg production by 28%, whereas exposure to high levels (697.56 ng/L) of A4 decreased egg production by 48% as compared to controls; however, these differences were not statistically significant. High levels of A4 (697.56 ng/L) induced tubercle development in females (androgenic effect), and increased Vtg mRNA presence in males (estrogenic effect). A4 significantly reduced male GSI in a dose dependent manner. A4 had no significant effects on embryonic development or hatching success. Together these data indicate that both P4 and A4, at concentrations found in the aquatic environment in association with animal feeding operations, are significant endocrine disruptors that have the potential to alter the reproductive physiology of exposed fish.

TP108 Effect of Different Tillage Techniques on the Surface Runoff of Nutrients and Fecal Steroids from Poultry Litter Amended Fields

D.J. Fisher, University of Maryland / Wye Research & Education Center / Environmental Science and Technology; K. Staver, University of Maryland, Wye Research and Education Center; L.T. Yonkos, University of Maryland / Environmental Science and Technology; S. Hutchins, USEPA National Risk Management Research Laboratory; V.S. Wilson, USEPA, ORD, NHEERL, TAD / Reproductive Toxicology Branch / Toxicity Assessment Division; D. Pote, USDA, ARS Dale Bumpers Small Farms Research Center

Effects of raw poultry litter tillage techniques on rates of surface transport of fecal steroids and nutrients to receiving waters via rain-induced runoff were investigated over multiple years using paired 20-acre research watersheds. The watersheds were located at the University of Maryland Wye Research and Education Center. Litter was applied using the standard No-till (NT) technique of direct surface litter application or by a prototype subsurface litter injection (SS) technique or a vertical tillage technique (VT). VT is a technique where the litter is lightly tilled and mixed into the top few inches of soil after surface litter application. SS tillage cuts a furrow in the soil into which the litter is dropped. The cut piece then falls back into the furrow and is rolled smooth. With this method there is little surface disruption and the litter is buried a few inches below the surface. During each annual litter application, one of the watersheds used the NT litter application technique while the other used an alternative litter tillage technique. Runoff concentrations of estrogens and estrogenicity were reduced from the VT and SS tillage techniques compared to NT. The average percentage reduction of estrogens and estrogenicity for VT compared to NT was 41% and 62%, respectively. For the SS technique the average reduction in estrogens compared to NT was 77%. Average surface runoff nitrogen losses during May were approximately fivefold lower from the VT watershed compared to the NT watershed while VT reduced phosphorous losses early in the growing season by approximately 50 percent. During the SS/NT comparisons the observed patterns of nutrient loss were similar to those measured during the VT/NT comparison with the injection of poultry litter resulting in lower nutrient runoff concentrations early in the growing season. Maryland's revised Nutrient Management Regulations require that organic nutrient sources be injected or incorporated as soon as possible, but no later than 48 hours after application. Nutrient runoff data generated during this EPA CAFO poultry litter cooperative agreement on the efficacy of alternative tillage techniques were used to help provide the scientific basis for this decision. The regulations requiring litter incorporation should result in significant reductions in nutrients and estrogens to the Chesapeake Bay.

TP109 Integrated assessment of runoff from concentrated animal feeding operations: Analytical approaches, in vitro bioassays, and in vivo fish exposures

J.E. Cavallin, USEPA, ORISE / National Health and Environmental Effects Research Laboratory; E.J. Durhan, USEPA / Mid-Continent Ecology Division; N. Evans, USEPA; K.M. Jensen, M. Kahl, USEPA / National Health and Environmental Effects Research Laboratory; D.W. Kolpin, USGS; C.A. LaLone, USEPA; E.A. Makynen, USEPA / Mid-Continent Ecology Division; S. Seidl, USEPA; L. Thomas, St. Cloud State University; D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; M. Weberg, USEPA; V.S. Wilson, USEPA, ORD, NHEERL, TAD / Reproductive Toxicology Branch / Toxicity Assessment Division; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory

While the trend toward using concentrated animal feeding operations (CAFOs) has resulted in increased efficiency in food production, this has prompted concern regarding the impact these operations have on the environment. For example, animal waste from CAFOs can contain natural and synthetic androgens and/or estrogens which can pollute surrounding waterways. To assess the potential effects on aquatic animals of exposure to mixtures of these endocrine-active chemicals, a suite of instrumental chemical analyses, in vitro bioassays, and a short-term in vivo fish test were used to assess surface stream water from six basins receiving livestock CAFO input during runoff conditions. These basins included cattle or poultry livestock, and the sites were selected for their homogenous livestock type.

In these studies, we measured concentrations of select endocrine-active chemicals using GC/MS/MS and determined estrogenic and androgenic activities using the T47D-Kbluc and MDA-kb2 in vitro bioassays, respectively. We also conducted 48 h static-renewal exposures with sexually mature male and female fathead minnows (*Pimephales promelas*) exposed to four surface water dilutions (0%, 25%, 50%, and 100% surface water), or 10 ng 17α-ethynylestradiol/L and 50 ng 17β-trenbolone/L as positive controls. Targeted gene expression and gonadal ex vivo testosterone (T) and 17β-estradiol (E2) production were measured in the fish. The T47D-Kbluc assay indicated that there was some estrogenic activity associated with all of the water samples, with the two sites eliciting the greatest response also having the highest levels of measured estrone and 17β-estradiol. The MDA-kb2 assay detected slight androgenic activity in only one of the six surface water samples. Corresponding in vivo exposures demonstrated that exposure to surface water from the livestock basins had no significant dose-dependent effect on ex vivo T or E2 production in fathead minnows, with the exception of increased male T production at one site. Significant up-regulation of hepatic vitellogenin mRNA expression in male fish (a response indicative of estrogenicity) was observed using water from one site. Overall, our study provides insights as to the utility of an integrated approach with analytical measurements, in vitro bioassays, and in vivo tests for assessing the presence and possible effects of complex mixtures of endocrine active chemicals.

TP110 High-throughput cell-free neurochemical screening assays to predict adverse outcomes in fish, mammals, and birds

P.W. Dornbos, Department of Fisheries and Wildlife; A. Arini, University of Michigan; B. Armstrong, Michigan State University; J. Head, K. Mittal, University of Michigan; C. Murphy, Michigan State University / Department of Fisheries and Wildlife; J. Rutkiewicz, N. Basu, University of Michigan

An estimated 30,000+ current commercial chemicals contain neurotoxic properties. As current animal studies are expensive, time-consuming, and overlook many at-risk organisms, development of a new, high-throughput screening method is required. *In vitro*, cell free assays have potential as relatively inexpensive, reliable screening mechanisms to flag neurotoxic compounds. Thus, compounds can be prioritized according to their toxicity for more in-depth animal dose-response studies. Given the current lack of screening methods and a limited ability to conduct whole-animal bioassays on at-risk organisms, development of such bioassays is essential for future ecological risk assessment. Here, we report on results from a series of *in vitro* screening assays assessing neurotransmitter receptors (n=10) and enzymes (n=3) associated with essential behavior and reproduction, including components of the glutamatergic, GABAergic, dopaminergic, serotonergic, cholinergic, and other neurochemical pathways. Such neurochemical receptors and enzymes were isolated from at-risk organisms (n=20) of multiple taxa, including fish (king mackerel, yellowfin tuna, goldfish, rainbow trout, and perch), birds (bald eagle, Japanese quail, chicken, and zebra finch), mammals (river otter, mink, pilot whale, common dolphin, narwhal, ringed seal, and polar bear), and biomedical species (rat, mouse, and human). The isolated neurochemical receptors and enzymes were dosed *in vitro* with a diverse set of 100 potentially neurotoxic chemicals, such as metals, rare-earth elements, pesticides, personal care products, flame-retardants, water/sediment extracts, and others. This was accomplished via development of neurochemical receptor binding and enzyme activity assays in a 96-well plate format. Specifically for receptors, total uninhibited binding of a specific radioligand was compared with radioligand binding in the presence of 50 μM of each potential toxicant. For enzymes, total product was compared with product formed in the presence of 50 μM of each potential neurotoxicant. In this presentation, we will 1) elaborate on the execution of such cell free assays and discuss 'pros and cons' of our current screening method, 2) explain initial results from our current assays and discuss potential conclusions that can be drawn from such data, and 3) discuss a strategy by which large volumes of *in vitro* data outputs may be modeled to predict individual-based adverse outcomes pathways.

TP111 Development of methods to quantify the transport of NAPL-analog hydrogel tracer beads in karst systems

H.M. Edenborn, US Department of Energy; D.J. Vesper, H. Bravo-Ruiz, West Virginia University; E.K. Herman, Bucknell University

The fate and transport of light and dense NAPL contaminants in karst systems are poorly understood due to the lack of safe and effective tracers that model the movement of these compounds. We developed novel tracer beads that are inexpensive, biodegradable, and easily-modified with regard to density to serve as analogs for NAPL contaminants. The beads are principally composed of water (>95%) present as a 2% calcium alginate hydrogel; the gel density can be modified by incorporation of either dense mineral particles or microscopic glass bubbles to create DNAPL and LNAPL analogs, respectively. Beads of a specific density are tagged with non-toxic insoluble fluorescent pigments for easy identification. Direct collection and counting of beads in an underground setting is difficult and impractical. In this study, we developed and tested the effectiveness of a system employing an inexpensive and waterproof string of LED lights as a source of UV light to illuminate the fluorescent tracer beads, and a small waterproof sports camera to record bead transport by video or automated still photography. Tests of bead transport in field and flume environments demonstrated that the battery-powered LED string provided an effective source of light for detection and recording of bead transport. Digital recording of bead transport was effective compared to the direct collection of beads, but the battery power supply to the tested camera limited the effective time of data collection to less than 30 minutes.

TP112 Testing in-vitro to in-vivo fish biotransformation extrapolation methods for very hydrophobic chemicals

J.C. Lo, Simon Fraser University / Biological Sciences; G. Allard, G. Saroya, Simon Fraser University; L. Charuaud, Universite de Nantes; C.J. Kennedy, Simon Fraser University / Department of Biological Sciences; D. Campbell, Simon Fraser University / Department of Statistics and Actuarial Science; F.A. Gobas, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment)

Metabolic biotransformation plays a key role in the elimination of very hydrophobic and potentially bioaccumulative substances in fish. The use of in-vitro biotransformation data to make predictions of in-vivo clearance in animal models has been a well-accepted procedure for pharmaceutical drugs. However, pharmaceutical drugs typically exhibit log Kow's up to approximately 4, while many environmental contaminants of concern have log Kow's greater than 5. Because of their extreme hydrophobicity, it is important to test methods for in-vitro to in-vivo extrapolation (IVIVE) before such methods are used in hazard and risk assessment. Nichols and Cowan-Ellsberry have proposed IVIVE methods based on pharmaceutical science approaches. This research reports on in-vivo and in-vitro experiments in rainbow trout with selected hydrocarbons of varying log Kow, which are designed to test the IVIVE . methodology for very hydrophobic chemicals. Dietary bioaccumulation studies in rainbow trout were conducted to measure uptake, elimination and biotransformation rates in liver and whole organisms. In-vitro bioassays in rainbow trout liver S9 homogenates were carried out to measure in-vitro biotransformation rates. Methods to extrapolate in-vitro to in-vivo biotransformation rates were then tested with the available data. We conclude that there are several experimental and computational considerations that require attention in the application of in-vitro to in-vivo fish biotransformation extrapolation methods for very hydrophobic chemicals. We further provide recommendations for the application of in-vitro bioassays in bioaccumulation assessments.

TP113 Development of the sheepshead minnow, Cyprinodon variegatus, as a model organism for immunotoxicity

L.M. Thornton, J.T. Oris, Miami University / Department of Biology; M.K. Sellin Jeffries, Texas Christian University / Department of Biology

To adequately identify and assess the risks associated with exposures to aquatic pollutants, the effects of contaminant exposures on immune function should be considered. Only a handful of fish species (e.g., *Oryzias latipes*, *Onchorynchus mykiss*) have been utilized in studies investigating the impacts of toxicant exposures on immune function; and given that these species are most typically freshwater, there is a need for the development of a marine model organism for immunotoxicity. As such, the overall goal of this project is to develop the marine/estuarine species, *Cyprinodon variegatus* (sheepshead minnow, SHM), as a model organism for immunotoxicity. To achieve this goal, normal immune function parameters in this species must first be described; therefore, the first objective of the current study was to characterize basic immune function in SHMs. This was accomplished by 1) measuring the mRNA expression of several immune function genes (interluekins, myeloperoxidase, NADPH oxidases, nuclear factor kB, toll receptors and chemokine receptors) in blood, gills, kidneys, livers, spleens and intestines and 2) evaluating resistance to the bacterial pathogen, *Vibrio*

anguillarum,in adult SHMs with no known history of pathogen or contaminant exposure. The second objective of this study was to characterize the temporal immune response of SHMs following exposure to the *V. anguillarum*. Adult SHMs were divided into two groups – one group (n =64) was subjected to an LC_{30} dose of *V. anguillarum,* while the other group (n = 48) was sham exposed. At days 1, 3 and 7 following the exposure, a subset of SHMs were sampled from each group and tissues were collected for determination of leukocyte numbers, spleen index and mRNA expression of immune function genes. The results of this study provide essential information regarding: 1) the differential tissue expression of a variety of immune function genes and 2) the normal immune response in pathogen-exposed SHMs over the course of time. By revealing the spatial and temporal changes associated with the normal immune response in SHMs, we hope to identify a suite of potential endpoints for use in immunotoxicity studies utilizing the SHM as a model organism.

TP114 DTox: A Quantitative Database of the Toxicological Effects of Dispersants and Chemically Dispersed Oil

A.C. Bejarano, University of South Carolina / Environmental Health Sciences; V. Chu, J.K. Farr, NOAA; J. Dahlin, Research Planning, Inc.

The use of dispersants during the Deepwater Horizon oil spill in 2010 revived the discussion about the use of dispersants in offshore oil spill response. One of the greatest concerns regarding the use of dispersants deals with the potential toxicological effects of exposures to chemically dispersed oil to water column organisms. Toxicity data of dispersants and chemically dispersed oil has been generated for at least four decades through laboratory and field conditions. Despite inherent limitations of dispersant use in spill response, these data are of great value to the oil spill community. However, the lack of a centralized repository has limited its use in oil spill response. As a result, the Dispersant and Chemically Dispersed Oil Toxicity Database (DTox) has been created to address that shared need by the oil spill community. DTox allows unrestricted and rapid access of users to toxicity data on dispersants and chemically dispersed oils. DTox is a quantitative database of existing toxicity data, developed through careful review and compilation of data extracted from the peer and non-peer review literature. Through a rigorously evaluation of the quality of each data source, DTox gathers quantitative information (e.g., species, exposure conditions, exposure duration, oil type and weathering, etc.), thus integrating this information into a user-friendly tool that allows for general or specific data searches. To date, over 300 papers have been evaluated for potential inclusion into the database, and it includes data for several commonly used dispersants, standard oil tests, and tens of aquatic species. Effects endpoints in DTox includes EC50 and LC50 data generated through exposure durations ranging from a few minutes to several days, and under conditions ranging from static to flow through test. These data are currently presented in the form of Species Sensitivity Distribution curves. The application of this tool has been thus far used in training exercises and drills, highlighting the usefulness of this tool. Further testing of this tool's capability will be assessed through several hypothetical spill scenarios. Although toxicity data will never be enough to answer all toxicity questions the public and the scientific community may have with regards to the use of dispersants, this centralized data repository can help make informed decisions and help identify data needs and gaps.

TP115 PCBs from pigments in children's clothing, crayons, and paper

L.A. Rodenburg, Rutgers University / Department of Environmental Sciences; J. Guo, Rutgers, the State University of New Jersey / Department of Environmental Science; P. Praipipat, S.L. Capozzi, A.J. Murphy, Rutgers, the State University of New Jersey; T.M. Kraeutler, Boston College

Polychlorinated biphenyls (PCBs) are produced inadvertently during the production of many pigments, including diarylide yellows and titanium dioxide. This inadvertent production is allowed under the Toxic Substances Control Act, and PCBs from these pigment sources make their way into consumer products. We have measured PCB 11, which is associated with diarylide yellow, along with other PCB congeners in children's clothing (from textile printing) as well as in crayons, colored pencils, plastic bags, and color printed paper samples from dozens of countries across six continents, indicating that this is a world-wide problem. As a result, schools continue to be contaminated with PCBs long after they were banned for commercial use. We have demonstrated that PCB 11 leaches out of printed paper under mild conditions, contributing to water and air pollution and possibly leading to human dermal exposure. Finally, we calculate that the amount of PCB 11 measured in the environment suggests that PCB 11 is present in pigments at or near the maximum level allowed under TSCA and virtually all of it is released from the pigment matrix to contaminate air and water, in contrast to the assertions of the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry. Alternatively, the high levels of PCB 11 measured in the environment could be due to levels in the pigment exceeding the TSCA limit, or due to breakdown of the pigment to release PCBs after the pigment is applied to the fabric, plastic, or paper.

TP116 An Adaptive Long-Term Monitoring Plan for Residual Coal Ash and Associated Selenium and Arsenic in a River Reservoir

S.J. Young, ARCADIS / Environmental; D.S. Jones, ARCADIS; N. Carriker, TVA; T.F. Baker; R.M. Sherrard, Tennessee Valley Authority / Kingston Ash Recovery Project; M.E. Stack, Jacobs Engineering / Federal Operations; M. Houck, Jacobs Engineering; M. Cagley, Tennessee Valley Authority

The December 2008 Kingston ash release discharged approximately 5.4 million cubic yards of coal ash into the adjacent river system. The initial ash release at Emory River mile (ERM) 2.5 traveled upriver as far as ERM 5.75, with some ash also being transported downstream into the Clinch River and Tennessee River. In response to this event, the TVA Kingston Ash Recovery Project was executed in three distinct phases. Phases 1 and 2 focused on mass removal of ash from the Emory River and impacted embayments, as well as closure of the failed dredge cell. Phase 3 included comprehensive human health and ecological risk assessments of the estimated 500,000 CYs of residual ash that were not removed during Phase 1 or were transported downstream during storm events. The human health risk assessment identified no unacceptable human health risks from potential exposure to residual ash. Overall, the ecological risks related to residual ash and ash-related constituents were estimated to be: 1) primarily low and, at most, moderate; 2) associated with direct exposures of benthic invertebrates to ash, arsenic, and selenium in surface sediment, and dietary exposures of birds to arsenic and selenium via consumption of benthic invertebrates; and 3) localized and diminished with increasing distance from the release area. This information was considered along with other factors in the Engineering Evaluation/Cost Analysis in order to support the risk management decision of Monitored Natural Recovery remedy for the river system. A long-term monitoring (LTM) plan was developed for biotic and abiotic media using an adaptive monitoring and management framework. These methodologies incorporated decision points at which causal effects of changed conditions are explored as an integral component of the process. Adaptive methodologies provide opportunities for effective responses to changes in monitoring results and provide objective decision points for changes in specific monitoring program components. This poster highlights the strategies employed by TVA to design a LTM plan that meets the needs of the CERCLA Removal Action Objectives and remedy selection, documents restoration of the ecological function and recreational use of the river system to pre-release conditions, and provides additional information for other purposes such as assessing Natural Resources Damages recovery, all while using practical adaptive management.

TP117 Measured Ecological Effects at Multiple Levels of Biological Organization for Biota Exposed to Residual Coal Ash and Associated Metals and Metalloids

D.S. Jones, ARCADIS; S.J. Young, ARCADIS / Environmental; A.R. Stojak, ARCADIS / Environmental Sciences, Assessment and Planning; C.B. Meyer, ARCADIS; T.F. Baker; R.M. Sherrard, Tennessee Valley Authority / Kingston Ash Recovery Project; N. Carriker, TVA

Immediately following the 2008 Tennessee Valley Authority (TVA) Kingston fly ash spill, on the basis of a few hastily-collected samples, several researchers predicted catastrophic effects on the aquatic ecology in the Emory, Clinch, and Tennessee Rivers. Selenium was the primary constituent of potential ecological concern because it is a significant contaminant of fly ash that in other studies has been shown to have adverse effects on animal reproduction. Other trace elements of potential concern in coal ash include arsenic, chromium, copper, lead, mercury, nickel, vanadium, and zinc. As part of a comprehensive environmental monitoring and assessment effort, TVA measured the responses of ash-exposed organisms at multiple levels of biological organization. These measures of effects where a critical component of the Baseline Ecological Risk Assessment (BERA) for the approximately 500,000 cubic yards of ash remaining in Watts Bar Reservoir following time-critical dredging. This poster describes the ecological effects data used in the

BERA to characterize risks and identify areas and receptors that warrant risk management. Information is presented on observations of ecological effects at the cellular, organ, organism, population, and community levels. Results are presented on species abundance and diversity, reproductive success, early life stage survival and growth, and other potential ecological effects. Particular emphasis is placed on evaluating potential reproductive effects that could have cascading effects on populations of the affected organisms and their predators.

TP118 The impact of PCB emissions from a contaminated harbor on the local atmosphere and nearby schools

A. Martinez, University of Iowa / Department of Civil & Environmental Engineering,and IIHR-Hydroscience and Engineering; N.T. Petrich, The University of Iowa / Environmental Engineering – CGRER; D. Hu, University of Iowa / Chemical and Biochemical Engineering / Department of Civil & Environmental Engineering, IIHR-Hydroscience and Engineering, Public Policy Center and School of Urban and Regional Planning; S. Spak, G. Carmichael, The University of Iowa; K.C. Hornbuckle, University of Iowa / Civil and Environmental Engineering, IIHR-Hydroscience and Engineering

In a previous study we determined that the Indiana Harbor and Ship Canal (IHSC) is a source of polychlorinated biphenyls (PCBs) to the air above it. However, we have not investigated the impact of IHSC PCB emissions into the local atmosphere, especially into two schools located less than 300 m from the canal. We hypothesized that the PCBs emitted to the air over the IHSC are continuously dispersed into the surrounding atmosphere. We addressed this hypothesis by using an atmospheric dispersion model, AERMOD. This model allowed us to predict hourly air concentration of total and individual PCB congeners (~160) for the year 2008. In addition, we employed the Weather Research and Forecasting Model (WFR) to predict local vertical as well horizontal hourly meteorological conditions at a fine grid scale (1.3 km). Performance of the model was assessed using active air samplers for nine months of 2008. One of the samplers was located in one of the school mentioned above. Results show that the IHSC is not only a source of airborne PCBs to East Chicago, but also to Lake Michigan. Annual average air concentrations of total PCBs ranged from 2.5 to 250 pg m^{-3}. In general, the model underestimated the measured values, suggesting other important sources not considered. Throughout all the year, both schools are being affected by the emissions from IHSC.

TP119 Thermal Effects Risk Assessment at Power Generating Stations

F. Cooper, N. Garisto, S. Paturi, ARCADIS SENES Canada Inc

The thermal impacts of increased water temperature discharges on sensitive biota from two power generating stations (PGS) in Lake Ontario are assessed through the risk assessment calculations. Representative fish species were selected based on site knowledge and previous studies, as well as stakeholder input. Temperatures were measured during July 2011 to July 2012, at locations within the plumes as well as reference locations (not expected to be affected by thermal plume). Various statistics such as Maximum Weekly Average Temperature were used to summarize the data. MWAT is the maximum of the 7-day rolling weekly average temperature. Risk was evaluated by calculating a Hazard Quotient (HQ) based on thermal effects criteria of the representative fish (from literature) to temperature measurements. A HQ value greater than 1 indicates the potential for thermal effects. Each of the representative fish species was assessed over different life stages (spawning, egg/incubation, larvae, growth/juvenile/young-of-year and adult) and various types of temperature exposure (acute or chronic). The HQ values were greater than 1 for some fish species during selected life stages, suggesting that potential adverse effects may occur for those species. However, whenever the plume temperatures resulted in a HQ above 1, the reference location temperatures also resulted in a HQ above 1. The presence of HQ values above 1 in the reference areas may not be indicative of potential effects, but rather that some of the thermal effects benchmarks used in the assessment are lower than background levels observed in the lake. Risk was also evaluated specifically for the egg incubation life stage for round whitefish, using the temperature difference, ΔT, between a "plume" location and a representative reference location. This criterion was established in the incubation experiments at the Wesleyville facility, Ontario. The study results suggested that for round whitefish, a periodic rise of +5°C or less over a 10-hr period/day over the incubation period will not result in adverse effects (e.g., excessive mortality) to developing eggsThe observed RWAT ΔT was at or below the ΔT criterion of 3°C at the two PGS. Overall, based on available data, no evidence of adverse impacts on fish was found to be caused by the thermal plumes from the two PGS.

TP120 Approaches to the Development of a Comprehensive Ecological Data Management System

B. Baker, V. Pellerito, URS Corporation; C. Mancini, URS Corporation / Environmental Services; J.R. Flanders, PerkinElmer, URS; C. Bartlett, DuPont Corporate Remediation Group

Ecological system studies generate complex and diverse data sets. These data include environmental quality (e.g., surface water, sediment), biological (eg., communities and/or resource inventory and monitoring), physical (eg., habitat assessments), telemetric information, and climatological data. This complexity is not limited to the type of information collected, but also to how the data are managed, analyzed, and visualized. Ecological data management depends on a data schema that best models the meanings within the field of study that spans the biological, chemical, and earth sciences. Furthermore, data representation or visualization can be complicated by the myriad of appropriate approaches that are often subfield specific. The South River Program is a comprehensive, multiyear, multidisciplinary data collection effort for a watershed in Virginia. Data collection has included a broad array of environmental data that must be managed in a way that permits complex statistical analyses and decision-making. Typically, standardized approaches to ecological data management have entailed custom developed systems that may meet the requirements of one ecological context, but may be inappropriate for another context. Some third-party software developers have designed systems that may meet ecological data management needs but are typically an add-on module for an environmental quality management system. Whether custom developed or "off-the-shelf", an ecological data management system should include certain features in order to effectively manage, analyze, and visualize data. In the case of the South River Program database, the data model: Is event oriented to associate all pertinent spatial and temporal data; Effectively stores multiple levels of data aggregations; and Is extensible to suit various ecological contexts. In addition, data retrieval and visualization requires: Extensible reporting features that allow for adjustments to the data model; Standardized but extensible reference lists such as for taxonomy; Accommodation for spatial and temporal aspects of the data; and Accommodation for data field manipulations and aggregations This presentation will describe the database being used to house the South River Program data, along with examples of approaches to data review, manipulation and evaluation.

TP121 Chesapeake Bay is our Laboratory -In Situ Sediment Toxicity and Bioaccumulation Assessment at Marine Corps Base Quantico, Virginia

C. Stransky, AMEC Earth and Environmental / Aquatic Sciences, AMEC Environment & Infrastructure; G.H. Rosen, SPAWAR Systems Center Pacific / Environmental; B. Chadwick, SPAWAR Systems Center San Diego; J.M. Guerrero, Space & Naval Warfare Systems Center; M.A. Colvin, SDSURF/SPAWAR; K. Tait, AMEC Environment & Infrastructure; G. Burton, University of Michigan / School of Natural Resources & Environment and Cooperative Institute for Limnology & Ecosystem Research

Existing tools for characterizing sediment and water quality often rely on unrealistic and disjointed independent lines of evidence potentially resulting in inaccurate management decisions. This problem is particularly acute for applications where the exposure is dynamic, sensitive to disturbance, or in general cannot be easily recreated in the laboratory. Consequently, there is a need for implementation and acceptance of more environmentally realistic, integrated tools that provide a synoptic assessment of exposure, uptake and response. For these more realistic exposure methods to gain acceptance, there is a need to improve and standardize quality controls, and to simplify field application to a level where the methods can be carried out routinely by personnel from traditional bioassay labs. A demonstration of the applicability and benefits of *in situ* monitoring was conducted at a location with elevated chlorinated pesticides (DDT and breakdown products) slated for sediment remediation using a thin layer cap at Marine Corps Base Quantico in Chesapeake Bay, VA. The latest version of the Sediment Ecotoxicity Assessment Ring (SEA-Ring) consists of a circular carousel capable of housing an array of *in-situ* bioassay chambers connected to a programmable positive flow peristaltic pump to help maintain water quality. *In situ* results using the SEA-Ring have found good agreement in results among field replicates at Quantico, but not unexpectedly, significant differences in bioaccumulation

potential *in situ* were observed relative to results derived in laboratory exposures. Relationships between accumulation of DDX in passive samplers and *in situ* exposures with the oligochaete worm *Lumbriculus variegatus* were strong; however, relationships to clam tissue concentrations using *Corbicula fluminea* were generally weak. Context of the results will be discussed from a lab versus field risk perspective. Ongoing plans to monitor the site following the placement of a thin layer cap will also be discussed.

TP122 Guidance on Alternatives Assessment and Risk Reduction

A. Stone, Washington Dept. of Ecology

Eight member states (California, Connecticut, Massachusetts, Michigan, Minnesota, New York, Oregon, and Washington) of the Interstate Chemicals Clearinghouse (IC2) worked together to create an Alternatives Assessment and Risk Reduction guidance document. Technical support was provided by EPA's Design for the Environment (DfE) Program, which has an extensive history in and experience with alternatives assessments (AAs). The IC2 formed a Guidance Team comprised of representatives from the eight participating states, DfE technical staff, and an additional technical advisor. Guidance Team members have experience in toxicology, chemistry, human health, exposure, and environmental policy, all of which are instrumental in formulating a comprehensive and complete AA Guidance document. In 2011, the Washington State Department of Ecology (Ecology) initiated the guidance development process by providing a scoping document including potential components of an alternatives assessment and solicited input from potentially interested stakeholders. The Guidance Team met regularly starting in August. The Guidance Team completed a draft Guidance document in March 2013 and a final guidance document in August 2013. The AA Guidance document is designed to meet the needs of a wide range of users. As a result, the final product is complex and comprehensive. The Guidance does not provide a single, specific methodology for conducting an AA. Instead, it presents three decision making frameworks and seven modules, each evaluating a different aspect of potential alternatives. Each module can be completed to a different level with higher levels requiring greater expertise and resources, but affording the user greater confidence in the final results. Users choose a framework, modules, and levels within modules to create an AA appropriate to the chemical, product or process under assessment. The guidance does provide some minimum expectations for an alternatives assessment. Fundamental to the guidance document is a risk reduction process that evaluates alternatives to toxic chemicals for both the lowest hazard AND exposure potential thereby providing the lowest risk and highest level of protection to human health and the environment. This presentation will discuss the specific components of an alternatives assessment and how the guidance can be used by individual assessors to identify safer alternatives to toxic chemicals in current products or processes.

Helping Contaminants Emerge: Non-targeted and Effect-directed Environmental Analysis

TP123 Data Analysis Tools for Non-Targeted Analysis of Environmental Contaminants Using Comprehensive Two-Dimensional Chromatography

J.A. Murray, National Institute of Standards and Technology / Chemical Sciences Division; B.J. Place, National Institute of Standards and Technology; J.R. Kucklick, National Institute of Standards & Technology / Chemical Sciences Division/ Hollings Marine Laboratory; N. Rosenfelder, National Institute of Standards and Technology / Chemical Sciences Division / Hollings Marine Laboratory

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCxGC-TOF/MS) is a powerful tool for non-targeted analysis. Compounds of complex samples are separated on two independent GC columns. Hence, analytes that may coelute with the sample matrix or other analytes in one-dimensional (1D) GC may be separated using GCxGC which can lead to the identification of more unknown analytes compared to 1D GC. In addition to the enhanced separation efficiency, GCxGC-TOF/MS also collects full electron impact mass spectra. Analytes can be tentatively identified by comparing spectra to the spectra found in mass spectral libraries. Peak finding, deconvolution, and mass spectra library searching is automated in many commercial software packages resulting in peak lists typically with hundreds to thousands of tentatively identified analytes. One approach to handle the daunting task of sifting through the peak tables is to eliminate unwanted search results. For example, search results found both in the sample blank and the sample can be eliminated. These search results can be found quickly by subtracting the blank chromatogram from the sample chromatogram. Scripts based on certain mass spectral information such as molecular ion, isotope patterns and ion clustering can be used to identify certain chemical classes such as organohalogen compounds, nitro compounds, phthalates, etc. Another approach to search for analytes belonging to specific classes is to examine specific regions in the three-dimensional chromatogram. By using certain column combinations, classes of analytes tend to be grouped in the same region of the chromatogram which results in structured chromatograms. By running standards of several different classes of analytes, the regions of where those classes elute can be constructed and the chromatogram from the sample can be searched for a specific group of compounds. In this presentation several of these data analysis tools will be discussed and applied to blubber samples from northern fur seals in order to identify potential emerging contaminants.

TP124 Non-Targeted Analysis of Petroleum Metabolites in Groundwater by GCxGC-TOFMS

R. Mohler, Chevron ETC; K. O'Reilly, Exponent, Exponent, Inc; D.A. Zemo, Zemo & Associates; A.K. Tiwary, Chevron; R.I. Magaw, K.A. Synowiec, Chevron Energy Technology Company

Petroleum hydrocarbons biodegrade in the environment and are converted to polar metabolites. These polar species will be extracted and quantified as Total Petroleum Hydrocarbons (TPH) unless they are removed from the extract prior to analysis on the analytical instrumentation. Silica gel cleanup (SGC) is effective at removing the polar compounds from the extract; however, one of the issues with the removal of these polar compounds from the analysis is their unknown nature and toxicity. Groundwater at fuel release sites often contains a complex mixture of hundreds to thousands of compounds including petroleum hydrocarbons, polar metabolites, and organic plant matter which makes it difficult to get a good compositional understanding of what is present. Only a limited number of these metabolites have been identified by traditional GC-MS methods because they are difficult to resolve using standard columns. Additionally, the targeted use of derivatization limits the detection of many potential metabolites of interest. The objective of this research was to develop a non-targeted GCxGC-TOFMS approach to characterize petroleum metabolites in environmental samples gathered from fuel release sites. The metabolites varied between the sites and the GCxGC method tentatively identified over 1100 unique polar compounds including acids/esters, alcohols, phenols, ketones, and aldehydes from 22 groundwater samples collected at five fuel terminals. The metabolites also varied by location within a given site, i.e., between the source zone and downgradient. The improved compositional information allowed a comparison of the toxicity of these polar compounds relative to the original petroleum hydrocarbons.

TP125 Suspect and Non-target Screening Approaches for Identification of Relevant Organic Contaminants in Sediments

A.C. Chiaia-Hernandez, Eawag / Environmental Chemistry; K. Praveen, E. Schymanski, J. Hollender, Eawag

Until now, sediments have been mainly used to characterize the contamination of compounds with highly lipophilic characteristics, whereas the knowledge of long-term contamination with more polar contaminants is not well explored. Thus, a multiresidue method was developed for the screening of more than 200 emerging contaminants with a broad range of physicochemical properties. The characterization of sediment samples was achieved after pressurized liquid extraction and liquid-liquid partitioning by liquid chromatography tandem high resolution mass spectrometry (LC-HRMS/MS) using electrospray ionization (ESI) and atmospheric pressure photoionization (APPI). The results show that biocides, musk fragrances and other personal care products were the most frequently detected compounds. Non-target screening was used as an attempt to identify unknown compounds in the extracts that were not in our target list. The non-target candidates were obtained from the chromatograms of the first layers of the sediment core (~10 years) and the most contaminated layers (around the 1970s for Lake Greifensee and 1980s for Lake Lugano, Switzerland). Background subtraction was performed using a sediment core layer from 100 years ago from each core. Automated exact mass filtering, peak detection, background subtraction, and recalibration of masses using internal standards was performed using EnviMass 1.2 (Eawag, Switzerland) in conjunction with an automated peak picking. Structure elucidation was executed with the aid of the

molecular formula generator MOLGEN-MS/MS and *in silico* fragmentation for computer assisted interpretation of mass spectra (MetFrag). HRMS in combination with our developed screening approaches enabled a retrospective analysis of the full-scan data allowing the detection of "suspected compounds", such as the tentative identification of transformation products and further identification of different biocides and disinfectants in use in Switzerland. In addition, HRMS allowed the identification of "unknown compounds" such as the mothproofing agent flucofuron and the disinfectant hexachlorophene, which were confirmed by reference standards. Our results show that sediments can be integrators in time and space for emerging contaminants, thus providing history of chemical deposition. Further, they show that suspect and non-target screening is possible even in complex matrices such as sediments.

TP126 Non-targeted screening of sponge tissues from the Caribbean for the identification of halogenated natural products (HNPs) using GC/EI-MS-SIM

N. Rosenfelder, National Institute of Standards and Technology / Chemical Sciences Division/ Hollings Marine Laboratory; J.R. Pawlik, University of North Carolina Wilmington / Center for Marine Science; W. Vetter, University of Hohenheim; J.R. Kucklick, National Institute of Standards & Technology / Chemical Sciences Division/ Hollings Marine Laboratory

The goal of the present work was to identify new halogenated natural products (HNPs) in different marine sponge species. For more than 50 years, environmental residues of anthropogenic polyhalogenated compounds have been studied. This targeted analysis focused largely on polybrominated flame retardants, chlorinated pesticides, polychlorinated biphenyls (PCBs) and dioxins. However, several recent studies have demonstrated that there are a larger number of polyhalogenated chemicals in environmental samples that are not routinely detected hence are poorly understood. One subset of rarely studied compounds are the HNPs. Over 4,500 HNPs have been identified with a small number repeatedly detected in marine mammals and fish. Their chemical structures are similar to anthropogenic polyhalogenated contaminants. Recent work has shown that these HNPs are bioaccumulative, persistent and possibly toxic. The predominant sources of these HNPs are sponges, algae and cyanobacteria. However, the characterization of HNPs in environmental samples is hampered by the large diversity of compounds, lack of authentic standards, control materials and inclusion into gas chromatography mass spectrometry (GC/MS) mass spectral libraries. To find, identify and characterize new HNPs we screened different sponge species from the Caribbean as possible producers of accumulating HNPs. A non-targeted screening approach using GC coupled to electron impact MS (GC/EI-MS) in the selected ion monitoring (SIM) mode was applied. This method focuses on the detection of the molecular ion as most valuable information for the identification of unknown compounds. The method further provides univocal mass spectra even at trace levels. Over 70 polyhalogenated compounds with a molecular weight > 300 amu were detected with the majority of compounds detected being brominated. The distribution and concentrations of HNPs varied dramatically in 10 sponge species examined. Compounds with properties similar to the persistent organic pollutants (POPs) such as methoxylated polybrominated diphenyl ethers and possible polybrominated dioxins were identified and further GC/MS experiments are in progress to verify these compounds. Anthropogenic compounds such as PCBs and other legacy POPs were only detected at trace levels in the samples. Results from this work will help to elucidate different types and concentrations of HNPs, which possibly bioaccumulate in higher marine organisms.

TP127 Targeting Persistent and Bioaccumulative Chemicals of Concern in the Environment through Non-Targeted Analyses

K. Jobst, E. Reiner, V. Taguchi, Ontario Ministry of the Environment; L. Shen, Brock University; A. Muscalu, Ontario Ministry of the Environment; M. Pena, A. Myers, University of Toronto; P.A. Helm, Ontario Ministry of the Environment / Environmental Monitoring and Reporting Branch; D. Poirier, T. Watson-Leung, D. Morse, Ontario Ministry of the Environment

Advances in chromatographic and mass spectrometric techniques and instrumentation have helped environmental chemists begin to face one of the remaining major challenges in environmental analytical chemistry; the characterization of complex mixtures of chemicals present in environmental matrices. However, questions of which compounds are environmentally relevant accompany the capability of characterizing a large number of compounds in the environment. Non-targeted analyses using high resolution gas chromatography and high resolution mass spectrometry offer complimentary approaches to *in silico* screening exercises to prioritize chemicals of concern. This presentation highlights examples of non-targeted and screening analyses of key environmental matrices which have identified compound classes and components within such classes that are occurring but not captured in targeted analyses. Mass defect plots, generated using Fourier Transform Ion Cyclotron Resonance mass spectrometry (FTICRMS), were constructed by graphing nominal mass vs. mass defect to identify approximately 100 polyhalogenated compound classes, about 50 of which contain both chlorine and bromine, occurring in deposits of ash from an industrial fire. These included halogenated polycyclic aromatic hydrocarbons (PAHs), furans, dioxins, and thiophenes. Other examples utilizing this approach include the identification of mixed chlorinated and fluorinated compounds from fluorochloropolymer combustion experiments, and confirmation of halogenated flame retardant analogues in lake trout and stream sediments. Complimentary to mass deflect plots, which cannot distinguish between isomers, are analyses using high capacity chromatographic methods like GCxGC. For example, GCxGC-HRMS results for fish exposed to the combustion ash confirmed the presence of multiple isomers of halogenated anthracenes and pyrenes. GCxGC-TOF and GCxGC-ECD analyses, complimented by FTICR-MS, are also non-target approaches to screen for potential chemicals of concern. Traditional targeted analyses can then be applied for compounds occurring frequently, in key matrices, and/or with greater responses in the preliminary analyses. Standards can be purchased or synthesized to confirm identity and to conduct quantitative analyses to support chemical assessments.

TP128 Comparison between a novel hybrid mass spectrometer and a hybrid high resolution mass spectrometer for non targeted analysis of emerging contaminants

J.R. Beck, Thermo Fisher Scientific / Environmental and Food Safety Marketing; T. Zhang, Thermo Fisher Scientific; L. Ferguson, Pratt School of Engineering / Department of Civil and Environmental Engineering, Duke University; G.J. Getzinger, Duke University / Nicholas School of the Environment

The recent emergence of HPLC-high-resolution accurate-mass mass spectrometry (HR/AM MS) into the field of environmental analytical chemistry has allowed for the comprehensive characterization of organic pollutants present at trace levels in complex environmental media. Current analytical platforms employed for these analyses suffer from several limitations that hinder complete realization of non-targeted analysis (NTA); the most notable of which are insufficient mass accuracy and resolution and limited tandem MS coverage. To address these shortcomings, we have developed a novel analytical workflow, utilizing a state-of-the-art hybrid HR/AM MS, for the NTA of emerging contaminants. The goal of this presentation is to demonstrate advances in NTA achieved through methods deployed on a new hybrid HR/AM MS. The newly developed instrument incorporates a quadrupole MS, a HR/AM Orbitrap MS, and a linear ion trap. The fusion of these three MS into one instrument allows for implementation of novel MS experiments, offering data that was previously unobtainable on a single instrument. In the current work, we compare results obtained from the NTA of wastewater extracts on an LTQ-Orbitrap Velos to those acquired with the new triple-hybrid HR/AM MS. Preliminary results indicate that the novel scanning experiments available to the new instrument greatly improved our ability to characterize the micropollutant composition of wastewater effluent. Specifically, we demonstrate improved MS analysis in the following ways: Full-scan acquisition with R=450k FWHM at m/z 200 at 15Hz, providing more and higher quality full-scan data for confident molecular formula assignment. Internal calibration, by a dedicated ion source, facilitating excellent and consistent mass accuracy (< 1ppm) for both full-scan and tandem MS data. Comprehensive HR/AM tandem mass spectra coverage by all ion fragmentation (AIF) in the quadropole MS followed by detection in the Orbitrap MS, greatly improving spectral library searching capabilities, especially for low abundance ions that are not selected during conventional data-dependent analyses. Data-dependent Ion Trap MS^n acquisition, acquired in parallel to HR/AM full-scan and AIF data, for structural elucidation of chromatographic features where MS^2 data alone is insufficient for confident identification. Rapid polarity switching, for comprehensive characterization of structurally diverse micropollutants in one chromatographic run.

TP129 Environmental fate of S-metolachlor in its pure form and as a part of commercial product – Mercantor Gold®: biodegradation and sorption onto sediment

E. Baginska, Leuphana Universitat Luneburg; L. Gutowski, A. Haiss, O. Olsson, K. Kuemmerer, Leuphana University Lüneburg

S-metolachlor, an herbicide, is an active compound in many commercially available products. One such product is Mercantor Gold® which is a combination of S-metolachlor with a mixture of organic solvents. The aim of the following study was to investigate whether this mixture has an influence on environmental fate of S-metolachlor namely in its biodegradation and elimination by sorption onto sediment. Biodegradation and sorption studies were performed using the screening biodegradation Water sediment test (WST). WST is mostly based on OECD Guideline No. 308. However the test was further modified by creating an artificial matrix to which all components were standardised based on their corresponding existing OECD Guidelines. Concentration of compound and mixture was 40 mg/L of the theoretical oxygen demand (ThOD). The S-metolachlor concentration was 19 mg/L (pure) and 17 mg/L (Mercantor Gold®). Additionally analytical assessment of S-metolachlor removal from water phase by high performance liquid chromatography coupled with UV-detector was performed. Biodegradation level of S-metolachlor was 8%±19% (n=6) and Mercantor Gold® was 27%±12% (n=3). Neither Mercantor Gold® nor S-metolachlor was toxic to the test bacteria (biodegradation in toxicity controls in both cases was above 30%). At day 28 of the WST, the elimination of S-metolachlor from the water phase was 60% in test series as a pure substance with a corresponding 62% in the abiotic control and 53% in test series as a component of Mercantor Gold® with a corresponding 45% in the abiotic control. S-metolachlor pure and in Mercantor Gold® showed similar sorption rates. In conclusion this study showed that additives in the Mercantor Gold® have not influenced the biodegradation of the compound S-metolachlor. Higher biodegradation level of Mercantor Gold® is attributed to biodegradation of some constituents of the organic solvent mixture. S-metolachlor removal from water phase in the WST also proved that the organic solvent mixture had no influence on sorption. Therefore, WST provides a promising tool for the assessment of the fate of pesticides in water sediment systems.

TP130 Predicting Long Term Impacts of Oil/dispersant Exposures on Human Health and Higher Trophic Organisms

A. Temkin, Medical University of South Carolina / Marine Biomedicine and Environmental Studies; D. Spyropoulos, Medical University of South Carolina / Pathology and Laboratory Medicine; J. Kucklick, National Institute of Standards and Technology

Obesity is a growing health epidemic among adults, adolescents and children. While a shift in genetics cannot account for this epidemic, epigenetics, including diet, exercise and lifestyle choices have been implicated. In addition, early life and fetal exposure to environmental "fat inducers" or "obesogens" has emerged as an additional contributing epigenetic factor. Identification of these obesogenic compounds is of critical importance to effectively reduce exposure. We have focused on identifying components of crude oil that may have big impacts on obesity in order to develop more targeted and effective oil spill clean-up strategies. To assess the obesogenicity of crude oil, human, alligator and pygmy sperm whale stem cells will be exposed to multiple concentrations of unfractionated oil/dispersant mixture and mixture fractions, and then monitored for fat cell differentiation. In this model, stem cell differentiation serves as a surrogate for fetal growth, which we predict will incur the most profound and lasting health impacts of oil/dispersant exposures. Crude oil samples will be fractionated using alumina/silica columns to separate the aliphatic and aromatic hydrocarbon components. Selected hydrocarbon classes, polycyclic aromatic hydrocarbons, aliphatic hydrocarbons, hopanes and steranes, in the fractions will be determined by gas chromatography mass spectrometry. Stem cell lines will be dosed with subsamples of the oil fractions. Initial results show a dose-response induction of fat cell differentiation in response to exposure to unfractionated oil/dispersant in alligator, pygmy sperm whale and human stem cells.

TP131 Fractionation of passive sampling device extracts explores contribution of PAHs to zebrafish toxicity

A.J. Bergmann, Environmental & Molecular Toxicology; R.L. Tanguay, K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology

Polyethylene passive sampling devices (PSDs) deployed in the Portland Harbor Superfund megasite have previously been used to correlate PAH concentrations with effects in embryonic zebrafish (*Danio rerio*). However, spatial and temporal variation in bioactivity was not completely explained by PAH differences. The current work describes HPLC fractionation of PSD extracts and subsequent bioassay testing to further investigate the role of PAHs in the toxicity of similar samples. *D. rerio* embryos were exposed to the whole extract, fractions, and reconstituted samples with more than 20 morphological and physiological endpoints recorded. Separated by gel permeation chromatography, replicated fractionation of a sample from Portland Harbor found that specific fractions demonstrated a reproducible response. With qualitative and quantitative GC/MS methods, we determined PAHs were the component sampled in the highest abundance. Other constituents of interest included chlorpyrifos, p,p'-DDE, and the provisional identification of pendimethalin and musk compounds. Fractions containing PAHs did not exhibit a response in *D. rerio*. We have shown that blank PSD extracts fractionated with HPLC and the associated sample manipulations do not elicit a response in this *D. rerio* assay. Other lines of evidence for bioactivity are under investigation and will be discussed.

Geospatial Approaches to Issues in Environmental Toxicology and Chemistry

TP132 Chloride concentrations in runoff before and after road-brine application at Mammoth Cave National Park, Kentucky, 2011-2013

D. Solomon, Tennessee State University / Civil Engr; L. Johnson, Mammoth Cave National Park / Science & Resources Management; B. Carson, Mammoth Cave National Park / S&RM; R. Toomey, Mammoth Cave National Park / Science & Resources Management; L. Sharpe, Tennessee State University; T.D. Byl, US Geological Survey / Department of Interior

Mammoth Cave National Park in south-central Kentucky is the world's largest known cave system and is host to many unique and threatened species including the Kentucky Cave Shrimp. The National Park Service operates Mammoth Cave National Park to encourage tourists while still protecting the unique and fragile ecosystem in the cave. MACA has begun the first phase of application of road deicers on primary roads through the Park. As part of that project, the Park in partnership with Tennessee State University and USGS has established a monitoring system to characterize chloride concentrations before and during the use of brine or other road treatments. The scope of this investigation included six surface monitoring stations and 2 sites in the cave. These sites were selected because of their location with respect to primary roads targeted for treatment, their drainage qualities, and close proximity to critical cave shrimp habitat. This project began in 2011 prior to any road treatments in the Park, and has continued through the winter of 2013 when 3 brining events took place. Specific conductance meters and auto-samplers were used to monitor the runoff waters. The 2011 monitoring established winter storm conditions prior to brine application. There were no winter snow storms in 2012 and no brining events. The 2013 winter had 3 brining events in January. Only small increases of short duration (<12 hours) were observed during the brine runoff events. The results of the monitoring established that even when brine was applied, the runoff at all the monitoring stations was well under the 600 mg/L chloride threshold for chronic exposure. Chloride never exceeded 200 mg/L during the runoff events. These results indicate that brine application on the Park roads did not significantly raise chloride levels in the resulting runoff or the cave streams.

TP133 The Use of Land Cover Data in the Refinement of the Chronic "Water + Fish" Arsenic Standard Recalculation, North Fork Gunnison River, Colorado

S. Skigen, GEI Consultants; D. Guth, GEI Consultants, Inc.; S.D. Baker, GEI Consultants, Inc.; S.P. Canton, GEI Consultants, Inc. / Ecological Division

The United States EPA has not updated the ambient water quality criteria for arsenic since 1980. The EPA-recommended water + fish criterion of 0.02 µg/L total recoverable arsenic was derived using equation parameter values which are not specific to conditions in Colorado. In Oregon's recently updated water + fish criteria, they utilized the EPA's calculation method (2000) for deriving human health criteria for arsenic, while incorporating updated and regionally appropriate values for each of the metrics, based on current EPA standards, newly published information, and regionally significant values for water and fish consumers in Oregon. A fundamental element of the state of Oregon's recalculation was the use of a revised human health risk factor, from 10^{-6} to 10^{-4}. In 2005, the state of Colorado modified the EPA's 2000 methodology to include a 30% inorganic factor and a bioconcentration factor of 1, resulting in a chronic standard of 0.02 µg/L for total recoverable arsenic, no revision was made to the EPA's human health risk factor of 10^{-6}. We initiated a study in 2011 involving the collection of both fish tissue for arsenic speciation and water column samples to evaluate total recoverable arsenic concentrations in a portion of the North Fork of the Gunnison River, Colorado. Due to the presence of elevated arsenic concentrations in the underlying geology of the area, partitioning of background arsenic concentrations occurring naturally within the surrounding watersheds remains an essential element of substantiating our recalculation, which revises various parameters of Colorado's 2005 criteria calculation, including the human health risk factor. A detailed land use analysis has been initiated to help identify present and future land cover, which will assist in answering two key questions: Firstly, what are the existing land use designations within the watersheds of concern? Secondly, what is the potential for future land conversion within the focus area? The intention of the recalculation is to address the importance of site-specific conditions, while also providing a chronic standard which is adequately protective of human health. An overview of our recalculation approach as well as the methods and results of the land use analysis will be presented.

TP134 Modeling Sucralose, an Ideal Anthropogenic Tracer in an Effluent Dominated Watershed with the Soil and Water Assessment Tool during an Extreme Drought

C. Breed, Baylor University / Department of Environmental Science, Center for Spatial Research; B. Du, Baylor University / The Institute of Ecological, Earth, Environmental Sciences, Baylor University / Department of Environmental Science, Center for Reservoir and Aquatic Systems Research; J.G. Arnold, Grassland Soil and Water Research Laboratory, US Department of Agriculture; K. Chambliss, Baylor University; B.W. Byars, Center for Spatial Research, Baylor University; B.W. Brooks, Baylor University / Department of Environmental Science

Contaminants of emerging concern (CECs) in rapidly urbanizing watersheds represent an area of increasing attention. The North Bosque watershed, an area of interest and research for the US Department of Agriculture, is ideal for testing the assumptions of water quality modeling systems. The Soil and Water Assessment Tool (SWAT), first calibrated in the North Bosque watershed, has been used for compound fate and transport assessments, but relies on many assumptions. Here we examined an anthropogenic wastewater tracer compound, sucralose, in the North Bosque watershed with a SWAT simulation to examine the utility of using a common model system to understand CEC transport during an extreme drought when instream flows downstream from Stephanville, Texas, was dependent on effluent discharge. The pesticide function in SWAT was used to insert physicochemical properties and concentrations of sucralose and other CECs. Our modeling outputs were compared to field observations (quantified by isotope dilution LC-MSMS), which highlight the opportunities and challenges associated with using SWAT for CEC modeling at the watershed scale. Whereas the simulation sucralose outputs are similar at the point discharges (waste water treatment plants), downstream of the city of Stephenville, Texas, in-stream sucralose concentration decreases drastically. A number of non-simulated factors in SWAT can affect contaminant fate in the environment such as in-stream loss or alluvial presence. Our findings suggest that CECs are entering the local Trinity aquifer in a primary recharge area through the alluvium downstream of the city of Stephenville. Because major factors like alluvium permeability are not accounted for in SWAT and other watershed models, this omission may introduce significant uncertainty during watershed scale risk assessments of CEC and other contaminants, particularly as effluent dominated and dependent watersheds experience drought conditions. Future research is necessary to account for these variables during water quality assessments and management activities.

TP135 Statewide assessment of estrogenic contaminants and intersex in North Caroline Streams and Rivers

C. Lee Pow, North Carolina State University / Department of Environmental and Molecular Toxicology; W. Cope, North Carolina State University / Department of Toxicology; D. Aday, T. Kwak, J. Rice, North Carolina State University / Department of Biology; M. Law, North Carolina State University / Department of Population Health and Pathobiology; S. Kullman, North Carolina State University / Department of Environmental and Molecular Toxicology

Estrogenic endocrine disrupting compounds (EDCs) span a multitude of chemical classes, and have been detected in surface waters throughout the United States. Exposure to estrogenic EDCs in wastewater treatment plant effluent and agricultural runoff has been linked to the development of oocytes within the testis (ovatestis) of male fish and is demonstrated to increase the risk of reproductive impairment and population decline. In this study, we aim to determine the incidence and severity of intersex in fish throughout the state of North Carolina, and assess the relationship between intersex and proximity to point and nonpoint sources of estrogenic EDCs. To select sites for this study, a geographic information system (GIS) map was used to identify potential sources of estrogenic EDCs to the aquatic environment. The map was generated using data collected from the National Pollutant Discharge Elimination System (NPDES), the US Environmental Protection Agency (USEPA), and North Carolina Department of Environmental and Natural Resources (NCDENR). Potential sources of EDCs were identified and quantified based on their potential impact to the water system. A total of 16 impacted sites were selected for sampling, taking into consideration the size (permitted discharge) of sources, number of sources, and point versus nonpoint sources. 4 sites considered "clean", with little to no EDC input from the headwaters, were selected as reference sites. Sampling was then conducted between March – May 2012. Male sport fish of three different species (black bass, sunfish and catfish), selected based on their commercial and recreational relevance to the state of NC, were collected at each site. Histopathology was conducted on testis in order to determine the incidence and severity of intersex. To assess the levels of EDCs at each site, passive sampling devices were deployed for one month and sediment samples were collected for quantification of steroidal hormone, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, legacy organochlorine pesticides, and current use pesticides. Additionally, grab samples of water were collected for assessment of estrogenic activity using the T47D-KBLuc *in vitro* bioassay. The correlation of the occurrence and severity of intersex with the GIS-based EDC source classifications, estrogenic activity of the water, and contaminants within the sediment and water will be discussed.

TP136 Spatial approaches to refine agricultural chemical use areas for endangered species assessments: Study with California Tiger Salamander in California

J. Amos, Waterborne Environmental, Inc. / GIS Specialist; R. Vamshi, C.M. Holmes, Waterborne Environmental, Inc.; J. Bang, Syngenta Crop Protection / Environmental Safety

The geospatial analysis of a threatened and endangered species risk assessment will be presented by highlighting a tiered approach to characterizing the potential exposure of the California Tiger Salamander (CTS) to an herbicide use. The studied herbicide use was recorded in 35 California counties from 2001 to 2010 (Pesticide Use Reporting Database, California Department of Pesticide Regulation), while CTS critical habitats defined by the US Fish and Wildlife Service were located in 21 counties. Using herbicide applications to orchards as an example for refining species/pesticide co-occurrence beyond the county-level screening, multiple approaches to characterizing spatial relationships between potential herbicide use sites and species habitats were performed. Best available geospatial data for species habitat, crop, vegetation, hydrology, wetlands, topography, and more were incorporated step-wise to represent realistic spatial relationships while documenting the impact (and uncertainty) each refinement made. Pesticide transport modeling was used to determine the distance that potential exposure may affect the species or its habitat. Refinements to this modeling further reduced the geographic extent of potential exposure. The tiered

approach offers a step-wise refinement to quantify the potential overlap of pesticide usage with widely distributed species locations from the state-wide scale to the field scale. This was performed in a programmatic, documented and transparent way, allowing for full retrieval of all details.

TP137 A geospatial toolbox for higher-tier endangered species exposure assessments during pesticide registration review

R. Vamshi, Waterborne Environmental, Inc.; J. Amos, Waterborne Environmental, Inc. / GIS Specialist; C.M. Holmes, V.L. Seed, Waterborne Environmental, Inc.

Going beyond the screening-level proximity assessment, several challenges are faced in higher-tier analyses for a threatened and endangered species risk assessment conducted as part of USEPA's registration review of pesticides. Presented here is a toolbox for spatial analysis that offers a suite of approaches depending on the species being studied and the mode of chemical transport in order to characterize and refine the possible interaction between endangered species habitats and pesticide use areas. Potential pesticide use areas can be spatially located using best available agricultural land cover data from USDA and state-level sources. However, aspects such as classification accuracy and the temporal nature of cropping need to be taken into account when defining the final data layer. Opportunities exist to spatially refine endangered species habitats after the screening level assessment. Examples of higher tier refinements to species location data include distinguishing the specific aquatic or terrestrial habitat suitable for breeding based on life history information, distinguishing the habitat types preferred by adults that are within a specific migratory distance from spawning sites, and using elevation data to limit species range. In addition to refining species habitats and pesticide use sites, the spatial relationships between them can be characterized using novel approaches. For instance, vegetation between crops and species location can be characterized in order to identify features that may impede pesticide runoff or drift; the magnitude of co-occurrence can be quantified by calculating the proportion of species habitat potentially exposed; pesticide application timing can be assessed in relation to sensitive species life stages; and other landscape factors affecting pesticide transport such as intervening slope (when erosion is the concern) or wind speed/direction (when drift is the concern) can be examined. The approaches utilized from this toolbox will depend on the specific aspects of exposure being examined and provide a useful mechanism to refine the scope of potential pesticide exposure to protected species, and focus energies on those specific areas in which mitigation or stewardship are of greatest value.

TP138 A refined crop-footprint approach to characterize potential use sites of pesticide applications for endangered species assessments

K. Budreski, Stone Environmental, Inc.; M.F. Winchell, Stone Environmental, Inc.; J. Bang, Syngenta Crop Protection / Environmental Safety

A refined crop footprint approach was applied to characterize potential use sites of pesticide applications in California using the best available land cover data and pesticide application records for endangered species assessments. Over the past several years, the cropland data layer (CDL), developed by the National Agricultural Statistics Service (NASS), has been enhanced with improved accuracies, allowing for crop-level analysis on a broad scale. NASS also produces detailed agricultural statistics at the county and crop level on a five-year cycle, through the Census of Agriculture report. Also, the pesticide use reporting (PUR) database maintained by the California Department of Pesticide Regulation (CDPR), includes a comprehensive record of publically available pesticide use data at the Public Land Survey System (PLSS) section level for agricultural applications. These three datasets were used to estimate potential and historic pesticide use sites by labeled crop within California. Five-years of data were used to get comprehensive footprints of potential use and probabilistic historic use site locations. The crop footprints were then used to calculate the spatial extent of potential and historic pesticide use sites to evaluate exposure potential to terrestrial and aquatic endangered species and their habitats in a co-occurrence analysis. The crop footprints were then used to assess the potential proximity of pesticide applications outside of habitat areas, but within a distance subject to modeled aerial and ground spray drift. Finally, we assessed the potential for exposure from potential and historic upstream uses sites to the species habitats. Finally, the downstream dilution of pesticide applications to potential and historic use sites was analyzed. Incorporating the CDL and PUR datasets in the development of refined crop footprints with verification using the Census of Agriculture report led to an improved understanding of the crop-level spatial extent of potential and historical use sites of pesticide applications for endangered species assessments.

Characterization and Processes of Atmospheric Pollutants

TP139 Vehicular contribution to atmospheric concentrations of Black Carbon and Fine Particulate Matter in Sao Paulo

I.G. Hetem, IAG – USP / Atmospheric Sciences; M.D. Andrade, IAG – University of Sao Paulo / Atmospheric Sciences

Brazil is one of the few countries in the world that has a fleet vehicle that uses ethanol as fuel on a large scale, and this poses a challenge to estimate vehicular emissions. According to the 2010 report by the Environmental Agency of Sao Paulo State vehicles powered by hydrated ethanol represented 30% of the fleet in the Metropolitan Region of São Paulo (MRSP) and burning gasoline (mixture of 22% ethanol and 78% gasoline) represent 58% and, therefore, the alcohol corresponds to 47% of the fuel consumed. Vehicles such as "flex fuel" (dual fuel), launched in the market in 2003, corresponds to 31.6%, and motorcycles account for 17%. Already the diesel vehicles account for 6% of the fleet, and are primarily responsible for the emission of NOx and fine particles. In this context, this study seeks to estimate the impact of vehicle emissions to the formation of fine particles (MP2.5) in the atmosphere of the MRSP, specially with a fleet surpassing 7 million vehicles. On open urban areas fine particles compose up to 60% of the inhalable fraction of particulate matter. Measurements show that concentration of these fine particles can exceed four times the EPA's standards (reaching up to 56 micrograms per cubic meter with an average of 20.8 micrograms per cubic meter). On road traffic tunnels the average concentration of fine particles doubles (for light duty traffic) and increases by the eightfold for heavy duty traffic. Calculations of the chemical mass balance and concentration of PM2.5 and Black Carbon of the Sao Paulo atmosphere will be presented, splitted between vehicular emission, pavement ressuspension and environmental contribution, and then compared.

Current Trends of Perfluoroalkyl Substances in the Environment

TP140 Determination and Characterization of PFOS in Environmental Samples Using Travelling Wave Ion Mobility Mass Spectrometry

M. McCullagh, K. Graham, D. Roberts, L. Dillon, M. Hodgkinson, Waters Corporation; I. Ericson Jogsten, B. van Bavel, MTM Research Centre, Orebro University; K.J. Rosnack, Waters Corporation

MRM transition based LC-MS/MS analyses have been used previously to investigate PFOS. Benskin *et al.* reported a common matrix interferent (taurodeoxycholate [TDCA]) that can complicate PFOS quantitation and tends to co-elute with PFOS, giving a positive bias. The use of high definition mass spectrometry (HDMS) is explored as an important tool for unequivocal identification of PFOS isomers in environmental samples. This technique offers some unique advantages to profiling complex matrices. The assay is based on the analysis of environmental sample extracts, mink liver and fish. These samples were injected on to a ultra performance liquid chromatography BEH C_{18} (1.7µm, 2.1x100 mm) analytical column. In addition, a mixture of PFOS isomer solvent standards were injected on column. The chromatographic conditions were comprised of a 35 minute H_2O (2mM Ammonium Acetate) (A) : 80:20 MeOH:ACN (B) gradient provided from a chromatographic system (equipped with PFC kit) operating at 0.45 ml/min and sample injection volumes of 5µl. Negative ion electrospray with ion mobility MS^E data acquisition was performed using an Synapt G2-S HDMS mass spectrometer. The results obtained to determine the presence of PFOS in mink, clearly show the benefits of using HDMS. It is possible to separate co-eluting analytes and increase the peak capacity using ion mobility. The PFOS isomers were resolved from the interfering components as they have vastly different mobility drift times. This approach negates the need for complex chromatography, extensive sample clean up or highly specific MS experimental design. All of the mass spectral information is retained, precursor and fragmentation information is acquired simultaneously and drift times enabling further characteristic profiling. With this information, it has been possible to create a characteristic assignment profile of PFOS isomers that co-elute with the cholic acid interferences. Using a prototype software platform, the target retention times were profiled to automatically generate the precursor and fragmentation spectra as well as the drift times for the

identified PFOS isomers. The results obtained warrant further exploration into the use of ion mobility as an approach to confirming the presence of PFOS isomers in the environment where confidence can be had that no contribution from isobaric interference's is made.

TP141 Occurrence of Perfluorinated chemicals and their precursors in Wastewater Matrices

K. Dasu, USEPA / National Risk Management Research Laboratory / Department of Agronomy, Crop, Soil and Environmental Sciences, NRC Post Doc at USEPA / Office of Research and Development; M.A. Mills, USEPA, ORD / Remediation and Pollution Control Division, NRMRL; K. Tadele, USEPA / Student Contractor to National Risk Management Research Laboratory, Student Service Contractor; B. Crone, Student services, USEPA

Perfluorinated chemicals (PFCs) are detected globally in many environmental matrices such as surface water, ground water and wastewater matrices (influents, effluents and biosolids). PFCs are persistent, bioaccumulative and shown to have developmental toxicity in laboratory animals. Wastewater treatment plants (WWTPs) play an important role in the transport of PFCs into the environment. There is a growing concern about the potential risks associated with the release of treated effluents and land-application of biosolids to the aquatic ecosystems and ultimately humans. Once land-applied, the emerging contaminants (ECs) present in biosolids can enter the surface and ground waters or accumulate in the plants grown on such soils and this increases the risk of potential exposure to humans and aquatic ecosystems. The current work encompasses an interdisciplinary effort to monitor and quantify ECs in effluents and biosolids from different WWTPs. Several classes of contaminants monitored include PFCs and their precursors, steroid hormones, alkylphenol ethoxylates, alkylphenols, pharmaceuticals and personal care products along with metals and microbial pathogens. The current presentation focuses on the analysis and concentration trends of PFCs and their precursors in treated effluents and biosolids. Grab samples of effluents and biosolids are collected from 9 WWTPs across the United States in four sampling events. The effluent and biosolid samples are extracted separately for PFCs and their precursors and are analyzed on UPLC/MS/MS and GC/MS/MS, respectively. Evaluation of fluctuations of the levels of these chemicals in each plant was done in quarterly sampling events during a period of one year. Based on the concentrations and compositions of PFCs, seasonal variations, differences in the treatment processes, additives and other factors will be evaluated. The data will aid in understanding the fate and transport of PFCs in wastewater discharges and the results will provide information for the risk management of these chemicals in the environment. The data on the occurrence and concentrations of different PFCs and their precursors in wastewater matrices will be discussed in detail.

TP142 Temporal trend of Poly- Perfluoroalkyl Substance in the Canadian Arctic Atmosphere

M.E. Shoeib, Environment Canada / Atmospheric Science and Technology Directorate; H. Hung, Environment Canada / Science and Technology; H. Li, C. Geen, P. Fellin, Airzone One Ltd

The atmospheric and oceanic long range transport (LRT) from source regions to remote areas led to the global distribution of poly and perfluoroalkyl substance (PFAS). The Stockholm Convention on Persistent Organic Pollutants, listed perfluorooctane sulphonate (PFOS) and its precursors as POPs in 2009, to eliminate or reduce their production and usage. Canada added PFOS and precursors to the Canadian Environmental Protection Act in 2006, which prohibits most uses of PFOS within Canada. To better understand and evaluate the effectiveness of the regulations, temporal trends are required. The atmospheric concentrations of several precursor compounds have been measured in remote areas over the last decade in different studies, often on board of ship cruises crossing different locations, which make deriving temporal trends from these studies complicated. The Northern Contaminant Program (NCP) was initiated to monitor pollutants in the Arctic and to assess the risk they posed to the ecosystems. The NCP master station of Alert, Nunavut, Canada (82° 30' N, 62° 20' W) provides an ideal location to track changes in the PFAS air concentrations in response to emission/ban of these chemicals and to develop temporal trends. Eighty – four air samples were collected at the Alert station between August 2006 and October 2010 for screening of 7 precursors (6:2, 8:2 and 10:2 fluorotelomer alcohols (FTOHs) methyl and ethyl perfluorooctane sulfonamide (MeFOSA and EtFOSA) and methyl and ethyl perfluorooctane sulfonamidoethanols (MeFOSEs and EtFOSEs). The 8:2 FTOH was the most dominant compound detected in 100 % of the samples with air concentrations ranging from 0.10 to 9.73 pg/m^3. Among the FOSA and FOSEs, MeFOSE was the most abundant, with air concentrations ranging from BDL to 2.63 pg/m^3. In terms of seasonality, generally we see spring maxima which may be associated with the increase in particulate input during the Arctic Haze. Summer maxima are also apparent for FTOH and MeFOSE which may be related to volatilization due to higher temperatures. The MeFOSE and EtFOSE temporal trends show slight declining tendencies probably in response to their ban in North America. In contrast, the FTOHs, which are not regulated, showed increasing tendencies in air at the Alert station.

TP143 Occurrence, transport and fate of Perfluorinated compounds in the tropical and subtropical Global Oceans

B. Gonzalez-Gaya, Institute of Environmental Assessment and Water Research; B. Jimenez, Institute of Organic Chemistry – CSIC / Instrumental Analysis & Environment; J. Dachs, Institute of Environmental Assessment and Water Research -CSIC / Environmental Chemistry

Perfluoroalkyl compounds (PFC) are environmental contaminants that have received worldwide attention due to their toxicity and widespread occurrence. Compared to the legacy persistent organic pollutants, most PFC have high water solubility and lower lipophilicity, being as well extremely persistent. As a consequence, the hypothesis that the open-ocean water column must be their main final global sink has been recently developed. Three PFC families, perfluorinated carboxylic acids, perfluorosulfonates and perfluorosulfonamides, were measured in surface seawater and at 100 m depth in the global tropical and subtropical oceans in order to identify their occurrence and elucidate their transport and fate. The samples were taken during the Malaspina 2010 circumnavigation cruise, providing the first synoptic sampling effort for the North Atlantic and Pacific oceans, and the south Atlantic, Pacific and Indian Oceans, between 35ºN and 40ºS, thus allowing a direct comparison of levels and patterns in the different oceanic basins. The Atlantic Ocean appeared as the most polluted basin in our global study; followed by the Pacific and then the Indian Ocean. Mean PFC concentrations (as the sum of all the compounds measured) found in the Northern hemisphere were lower than values found in the Southern hemisphere, suggesting differences in emission patterns in both Hemispheres. Nevertheless, each family showed singular distributions and levels over the global ocean, being remarkable the high abundance of perfluorooctanoic sulphonate (PFOS) and perfluorooctanoic acid (PFOA) in the overall PFC levels. The concentrations of individual PFC are in agreement with previously reported PFC concentrations in some marine areas. The comparison of concentrations and patterns in coastal regions, the center of the five oceanic subtropical gyres, and oceanic currents and biogeochemical provinces allow to elucidate processes driving their transport and cycling in the marine environment. Furthermore, the PFC concentrations measured at 100 m depth allow investigating on the processes responsible for the vertical transport of PFC in the ocean. An estimation of the global oceanic sink due to vertical eddy diffusion will be given for individual PFC compounds.

TP144 Historical Trends of Inorganic and Organic Fluorine in the North American Great Lakes

G. Codling, University of Saskatchewan / Toxicology Centre; J.P. Giesy, University of Saskatchewan / Toxicology Centre and Department of Veterinary Biomedical Sciences; P.D. Jones, University of Saskatchewan / School of Environment and Sustainability; A. Vogt, T. Wang, University of Saskatchewan / Toxicology Centre; A. Li, University of Illinois at Chicago / School of Public Health; N. Sturchio, University of Illinois at Chicago / Department of Earth and Environmental Sciences; P. Wang, University of Saskatchewan / Toxicology Centre; Y. Lu, Chinese Academy of Sciences / State Key Lab of Urban and Regional Ecology; K.J. Rockne, University of Illinois at Chicago / Department of Civil and Materials Engineering; K. Ji, University of Saskatchewan / Biomedical Veterinary Sciences and Toxicology Centre, Seoul National University; J. Khim, University of Saskatchewan / Dept. Veterinary Biomedical Sciences; J.E. Naile, USEPA / Ecosystems Research Division

Though first manufactured in the late 1940s PFCs really began mass production in the 1970s and estimations of the global release of PSOF from 1970-2002 is thought to be ~45 250 t with the majority from manufacturing. Total Fluorine (TF), Extractable Organic Fluorine (EOF) and perfluoroinated compounds (PFCs) were measured in surface and core sediments taken from 3 of the Great Lakes (Michigan, Superior, and Huron)

from 2010 to 2012, (Lake Ontario and Erie are to be sampled over the coming two years). The aim of the study was to investigate both temporal and spatial variability of PFCs within the sediment of the Lakes, and to relate this to the TF and EOF. Cores of sediments (N~25) and PONAR grab samples (*n*~80) were collected as part of the Great Lakes Sediment Survey Project (GLSSP) from the R.V Lake Guardian. Sediments provide a chemical 'sink' for PFCs, removing them from the overlying water. When combined with measurements of ^{210}Pb, ^{241}Am, ^{226}Ra, and ^{137}Cs, dated cores can be used to reconstruct a historical record of deposition of PFCs. Measurement of TF and EOF, by combustion Ion Chromatography indicated that there is a large proportion of non extractable fluorinated material in sediment compared to the EOF and that $\sum_{29}$PFCs, measured in this project account for < 0.2% of the EOF. This is differing from biological studies where PFCs account for a large proportion of fluorinated material, For instance, in a study of Cetaceans ~23% of EOF were accounted for by the commonly studied PFCs. Mean concentration of PONAR grab surface PFCs from Lake Michigan was 2.4 ng g^{-1}, this is similar to reported concentrations in other sediment studies where there is no known local sources. The range for PONAR samples was from 0.9 to 4.9 ng g^{-1} and indicated a low variability across the Lake. Core concentration mean in 1900-1910 was 2.1 ± 0.9 ng g^{-1} dw with the majority of compounds below the method detection limits (MDL) to 10 ± 3.9 ng g^{-1} dw around 2000, the trend seen closely resembles modelled PFC production from 1970 to 2000. Variability in concentrations noted in cores may be products of local sources and changes in emission and waste management. To date this study represents the most comprehensive study of the historical record of PFCs in sediments of freshwater lakes and indicates that there is a large volume of unknown fluorinated material in the Great Lakes.

TP145 Quantitative Determination and Time Trends of Perfluoroalkane Sulfonates and Sulfonamide-based Precursors in Herring from the Swedish West Coast

S. Ullah, Stockholm University; S. Huber, Norwegian Institute for Air Research / Department of Chemistry; A. Bignert, Swedish Museum of Natural History; U. Berger, Stockholm University

Perfluoroalkane sulfonic acids (PFSAs) and their sulfonamide-based precursors, namely perfluoroalkane sulfonamidoacetic acids (FASAAs), sulfonamides (FASAs), and sulfonamidoethanols (FASEs), are an important group of environmental contaminants. Among the PFSAs, perfluorooctane sulfonic acid (PFOS) and its sulfonamide-based derivatives (PreFOS) have gained extensive attention from scientists and regulators due to their high production volume, persistency and global occurrence. PreFOS can be transformed to PFOS in the environment as well as via biotransformation. The ubiquitous presence of PFOS is thus the result of a combination of direct emissions of PFOS and emissions and subsequent transformation of PreFOS. Likewise, the total internal human exposure to PFOS is the result of both exposure to PFOS and PreFOS. The aim of this study was to investigate the potential importance of PreFOS exposure via fish consumption over time. To this end, an analytical method was developed for simultaneous analysis of PFSAs and their precursors in fish muscle. The fish sample was spiked with labeled internal standards and extracted with acetonitrile. The extract was cleaned up and fractionated into two fractions (containing neutral compounds and acids, respectively) by solid phase extraction. Both fractions were analyzed by UPLC/MS/MS in electrospray negative ionization mode.The fraction containing neutral compounds was additionally analyzed by GC/MS in positive chemical ionization mode. Relative recoveries calculated at spiking levels of 0.1 and 1 ng/g were 50-108 and 60-115%, respectively, for all analytes except for FASEs (UPLC/MS/MS). Recoveries for FASEs were around 150% (GC/MS), reflecting the presence of matrix effects. The method limits of detection ranged from 0.05 to 5 pg/g for MeFBSA, FOSA, PFSAs and FOSAAs in UPLC/MS/MS, and from 35 to 100 pg/g for MeFOSA, EtFOSA and FOSEs in GC/MS. The method was applied to investigate the time trends of PFSAs and their precursors in herring muscle samples originating from a sampling site (Fladen) at the west coast of Sweden covering the years 1991-2011. PFSAs, FOSA and FOSAAs were detected, all with decreasing or unchanged trends between 1991 and 2011. Shorter disappearance half-lives were observed for PreFOS compared to PFSAs, suggesting that the relative contribution of PreFOS to total human exposure to PFOS via fish consumption decreased over time. PreFOS may have constituted a significant indirect exposure to PFOS in the 1990s.

TP146 Spatial and Temporal Trends of Perfluoroalkyl Compounds in Fish Fillets Collected from Pool 2 of the Upper Mississippi River

J.L. Newsted, Cardno Entrix, Michigan State University / Wildlife Toxicology Laboratory, Department of Animal Science; R. Holem, Cardno Entrix

In a 2011 study, perfluoroalkyl compounds (PFCs) were analyzed in fish fillet samples taken from Pool 2 of the Upper Mississippi River, a 33 mile stretch of river inclusive of the Minneapolis/St. Paul, Minnesota metropolitan geographical area. Approximately 100 each of bluegill sunfish (BGS), freshwater drum (FWD), smallmouth bass (SMB), and white bass (WHB) were collected from 10 separate sampling reaches of approximately 3 miles in length. Fish fillet tissues were analyzed for perfluorinated carboxylic acids (PFCAs) (C4–C12), perfluorinated sulfonic acids (PFSAs) (C4, C6, and C8), and perfluorooctane sulfonamide (PFOSA). Perfluorooctane sulfonate (PFOS) was observed with the greatest frequency and at the greatest concentration in fish tissues ranging from 3.0 to 760 ng/g ww. Mean (geometric) PFOS concentrations in BGS, FWD, SMB and WHG were 20, 28, 29 and 58 ng/g ww, respectively. Concentrations of perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHS), PFOSA, and the nine C4-C12 PFCAs had species-specific geometric mean concentrations that were less than 5 ng/g ww. Spatially, concentrations in all fish species were consistent with their being two principle sources of PFCs to Pool 2. Temporal comparison of the fish data from this study showed that concentrations had decreased from previously measured values, specifically data from 2009. The reduction in mean (geometric) PFOS concentrations from 2009 to 2011 for BGS, FWD, SMB, and WHB were 60%, 60%, 43%, and 30% respectively. A subsequent study conducted in 2012 confirmed the finding of the current study that fish PFC concentrations are decreasing throughout Pool 2. The measured declines in fish population PFOS levels for the Mississippi River Pool 2 region are consistent with the >10-year cessation of manufacturing of products based on perfluorooctanyl chemistry and with ongoing efforts in Minnesota to effectively control sources of PFCs to the Mississippi River.

TP147 Fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids, perfluoroalkane sulfonic acids and their precursors: crossroads or dead end?

Z. Wang, Swiss Federal institute of Technology / Institut fur okologische Chemie, ETH Zürich / Institute for Chemical and Bioengineering; M.I. Gomis, Stockholm University / Department of applied environmental science; I.T. Cousins, Stockholm University / Applied Environmental Science (ITM); M. Scheringer, ETH Zürich / Institute for Chemical and Bioengineering; K. Hungerbuehler, ETH Zürich

Long-chain perfluoroalkyl carboxylic acids (PFCAs) and perfluoroalkane sulfonic acids (PFSAs), especially perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS), are a group of global contaminants of high concern due to their persistence, bioaccumulative potential and global presence in the environment, biota, humans and food items. As a consequence of this high concern, after 2002, the global industry started to replace long-chain PFCAs, PFSAs and their precursors with short-chain homologues or other types of (non)fluorinated chemicals. It is important to note that among all alternatives, fluorinated substances that have a similar structure as long-chain PFCAs, PFSAs and their precursors are extensively used, particularly in situations where extremely low surface tension and/or durable oil- and water-repellence is needed. In this study, we address the question: are the fluorinated alternatives safe for humans and the environment? We first review information on fluorinated alternatives that is available in the public domain and identify over 20 new fluorinated substances that are applied in various industry branches (e.g., fluoropolymer manufacture, surface treatment of textile and food contact materials, metal plating, etc.) and consumer products. We then summarize the current knowledge on environmental releases, persistence, exposure of biota and humans of these substances, and on their potential adverse effects on biota and humans. We used a quantum chemistry-based model, COSMOtherm, to estimate the physicochemical properties of these fluorinated alternatives. The physicochemical properties were then used as input parameters for a global-scale multimedia fate model to estimate the overall persistence and long-range transport potential of the fluorinated alternatives. In the light of the information collected and properties estimated in this study, it is still unclear whether fluorinated alternatives are fully safe for humans and the environment. We provide guidance on what further steps are needed to tackle the remaining issues regarding the safety of fluorinated alternatives.

TP148 Accumulation and depuration of perfluorinated compounds in Chironomus riparius larvae

D. Bertin, B. Ferrari, Irstea / Groupement de Lyon; P. Labadie, H. Budzinski, University of Bordeaux 1 / UMR 5805 EPOC Equipe LPTC; J. Garric, Irstea Lyon / Division Qualite des Eaux, CEMAGREF Lyon / Groupement de Lyon, Irstea; M.P. Babut, Irstea / Groupement de Lyon

Long chain perfluoroalkyl acids or sulfonates and a few precursors have been found in different media of the Rhone river downstream of Lyon (France). Although most of these compounds are considered as bioaccumulative, the exposure pathways of aquatic biota and the accumulation kinetics remain poorly understood. The objectives of this study are (i) to assess the accumulation and depuration kinetics of C9 to C14 carboxylic acids (nonanoic PFNA to tetradecanoic, PFTeDA), C8 to C10 sulfonates (perfluoro-octane, PFOS to perfluoro-decane PFDS) and 3 precursors, in a benthic invertebrate species, the midge *Chironomus riparius*; (ii) to evaluate the influence of temperature on these processes. Chironomids are an important food source for other species; their life cycle involves a larval stage in contact with sediment and use to feed on detrital carbon food source. Natural sediments from a deposition site along the Rhône river (France) downstream of an industrial site releasing amounts of PFASs were collected with a Van-Veen grab, sieved at 2 mm, pooled in polypropylene jars and stored at 4°C. Chironomids were exposed either at their optimum temperature (21°C) or at lower ones (12°C and 17°C). Two series of experiments were conducted, dealing with uptake and depuration respectively. PFASs were analysed in water, pore water, sediment and organisms by LC-MS/MS. The accumulation of all the tested perfluoroalkyl substances (PFASs) was measurable already at the first intermediate time (two days), and was enhanced at higher temperatures. Transfers from sediment to surface or pore water as well as chironomid larvae can be demonstrated. Biota to sediment accumulation factors appear lower than those published for *Lumbriculus variegatus*. These results and those obtained for $\delta^{13}C$ and $\delta^{15}N$ allow drawing a conceptual model of PFASs distribution in abiotic compartments, and determine the pathways and dynamics of accumulation by chironomids. Tentative uptake and depuration rates were calculated on the basis of the uptake experiment and a partition model ([1,3]). Experimental depuration rates will be presented, and the perspectives to develop a complete kinetic model will be discussed.

TP149 Toxicity of perfluoroalkyl phosphonic acids (PFPAs) in the green algae Chlamydomonas reinhardtii

D. Sanchez, Sciences biologiques; M. Houde, Environment Canada / Centre Saint-Laurent; M. Douville, Environment Canada; J. Verreault, Université du Québec à Montréal / Department of Biological Sciences

Recently, perfluoroalkyl phosphonic acids (PFPAs), a new class of perfluoroalkyl substances, were reported for the first time in surface waters and wastewater treatment plant effluents in Canada as well as in surface waters from the Netherlands and Germany. PFPAs that are constituted of chain lengths of 6, 8 or 10 carbon atoms, have been used primarily in the industrial sector as surfactants. Toxicological effects of PFPAs in aquatic organisms have as yet not been investigated. Thus, an integrated toxicological approach, combining physiological, biochemical, and genomic endpoints, was elaborated to evaluate the impacts and modes of action of PFPAs in phytoplankton. The green microalgae *Chlamydomonas reinhardtii*, a primary producer in aquatic/marine ecosystems, was used to study the toxicity of the high-production volume perfluorooctylphosphonic acid (C8-PFPA) as well as perfluorodecylphosphonic acid (C10-PFPA). Algal cultures were exposed to different concentrations (31-250 μgL^{-1}) of C8-PFPA and C10-PFPA for 72 hours. Potential adverse effects on *Chlamydomonas reinhardtii* were assessed using esterase activity (cell viability), chlorophyll content, reactive oxygen species (ROS) production and associated transcription of antioxidant enzyme genes (i.e., GPX, GST, CAT and Sod1) and genes related to the photosynthetic process (i.e., Lhcb, Lhca and CHLI1). Results of esterase activity suggested that C8-PFPA and C10-PFPA did not affect cell viability. However, ROS production was stimulated in cultures exposed to the highest concentration tested for C10-PFPA, whereas no significant effect was observed for C8-PFPA. Moreover, the chlorophyll a and chlorophyll b contents of *C. reinhardtii* exposed to C8-PFPA and C10-PFPA were significantly lower than those control ($p < 0.05$). Results for the transcription of seven genes related to oxidative and photosynthetic processes and possible relations with cellular activities will be presented at the conference. Preliminary results suggest that exposure to C10-PFPA in *Chlamydomonas reinhardtii* may induce excessive generation of ROS, causing oxidative damage to cells.

Linking Formulation and Use Data to the Occurrence and Impacts of Home and Personal Care Products

TP150 Wax Ester Emollient Profiles in Personal Care Products

J.A. Layshock, Battelle Memorial Institute / Analytical Chemistry Services; C. Usher, Battelle; F. Pala, Battelle Memorial Institute

How a lotion feels on your skin is largely the result of the type and composition of emollients in the product formulation. Natural emollients like Shea butter and cocoa butter consist of distinct compositions of wax esters. Synthetic wax esters can also be manufactured via chemical reactions of fatty acids and alcohols. The analysis is of emollient composition is of interest to formulators as they strive to meet market demands of more natural or green products. The composition may also be of interest to consumer and environmental advocacy groups concerned about the use of certain ingredients. As part of a larger market intelligence project for consumer care products, methods of analyses were developed to characterize wax esters. A brief description of the developed methods and analytical challenges for emollient quantification using GC/MS will be presented. Measured emollients range from C24 to C48 saturated and unsaturated wax esters with acid and alcohol chain lengths ranging from C12 to C24. Emollient profiles of commercial lotions are compared to each other and to ingredient labels. In general, the detected wax ester composition agrees with the labeled ingredients but often includes additional emollients. Since individual compositions of naturally derived emollients are generally not listed on labels, the compositional analysis provides insight on the complexity of naturally-derived chemicals used in formulations.

TP152 What's out there in consumer products and the environment? A survey of home and personal care product ingredients and related safety information

P.C. DeLeo, American Cleaning Institute; M.C. Ciarlo, EA Engineering, Science, and Technology, Inc.; S. Williams, Baylor University; W. Greggs, Soleil Consulting, LLC; W.L. Goodfellow

The American Cleaning Institute® (ACI) is the industry trade association that represents the formulators of more than 90% of the consumer cleaning products in the United States. Recently, ACI set out to define and publish the universe of ingredients used in its members' products with the goal of identifying the publicly available hazard data for every ingredient and developing an associated exposure assessment and screening-level risk assessment. This presentation reviews the methodology used to develop the Ingredient Inventory for cleaning products, which was completed as the first stage in this multi-part study (http://www.aciscience.org/IngredientInventory.aspx). Over 900 products in the categories of laundry care, dish care, and hard surface cleaners were surveyed and all listed ingredients recorded. Data were captured using a rigorous set of quality assurance criteria, and naming was consolidated to eliminate redundancy and facilitate the search for hazard data. Over 800 unique ingredients were identified, not including fragrance and dye compounds. This presentation will review the challenges encountered and lessons learned during ingredient information collection, and provide insights into the makeup of the Inventory. Key tasks included consolidation of the ingredient list, resolving differences in ingredient naming, and developing a strategy for initial grouping into chemical classes. A number of factors had to be considered when assigning a grouping; these included consideration of groupings established by previous studies, product labels which listed a general chemical category as opposed to a specific ingredient name, broad chemical categories, and functional categories. Initial reviews indicate that chemical hazard data is available for numerous categories of compounds. The presentation will include demonstration of the end product for the first phase of the effort, which is a web-based database allows access to ingredient listings and basic identification information. This will form the basis for expansion to include hazard, fate and effects data for the nearly one thousand chemicals represented.

TP153 The evolution of phosphate-free detergents toward a more environmentally friendly alternative

F. Pala, J.D. Seyfert, C. Usher, W. Li, S. Darkey, Battelle Memorial Institute

Sodium tripolyphosphate (STPP), an efficient and cost-effective water softener, has been used as the main builder and cleaning agent in many detergents and household cleaners. Phosphate is not significantly removed by wastewater treatment and consequently is discharged to the aquatic environment, where it is the main cause of eutrophication in rivers and lakes. With

the US Water and Sewage Phosphate Detergent Act (1989, amended in 1992), STPP was banned from US laundry products. In Europe, STPP was completely removed from use in Germany, Italy, Switzerland, Australia, Norway, and the Netherlands but not in other countries. The last challenge to STPP occurred in the US in 2010, when automatic dishwashing detergent formulators began formulating phosphate-free products through the use of effective and economic alternatives. In Europe, STPP will be banned from dishwashing detergents starting in 2016. This talk focuses on the evolution of phosphate-free formulations of laundry and dishwashing detergents. Battelle Detergent Program data are used to investigate the chemical composition and environmental fate of phosphate-free detergents commercialized in the US and Europe.

TP154 Market Forensics as an Environmental Exposure Tool: Using Sales Data to Determine the Contribution of Personal Care Product Ingredients to WWTPs

S.M. Mudge, Exponent International Limited; P.C. DeLeo, American Cleaning Institute

A sampling and analytical campaign in 2011/2 investigated the regional differences in the removal of fatty alcohols from personal care products (PCPs) and detergents entering wastewater treatment plants (WWTPs); the results clearly showed significant differences in the influent composition across the eco-regions of the USA. At that time, it was suggested that this might be due to differences in the usage of consumer products in the catchment. To investigate this effect, the sales data for over 4000 products were purchased for four USA regions along with the national data for the 24 weeks immediately prior to the sampling periods. A Market Forensics approach was applied to these data to determine which products make the greatest contributions to the influents and the fatty alcohol analytical data used to reconstruct the profiles for each treatment plant. The results indicate a relatively small difference between regions overall although individual products may vary by between -15% and +10% compared to the national data. The greatest contributor to the influents are the liquid laundry detergents followed by the hand dish detergents making up >85% of the total fatty alcohol load. Using data for the fatty alcohol profile for faecal matter and the profiles in each region, it was possible to determine the contribution that the consumer products make to WWTP influents. These did vary significantly by region with two regions only receiving ~25% of their fatty alcohols from the consumer products while in two other regions the contribution is 50% of the influent load. The per capita usage rates have also been calculated and compared to the national average. These data allow a national inventory of fatty alcohols from consumer products to be calculated. This approach again demonstrates that Market Forensics, the use of market sales data to estimate the environmental exposure for consumer product chemicals, can be a cost effective and efficient alternative to field experimental campaigns.

TP155 Development of a mass balance model to predict the fate and transport of home care chemicals in domestic wastewaters

F. Pala, Battelle Memorial Institute; M.J. Benotti, Analytical and Environmental Chemistry, Battelle; J.A. Layshock, Battelle Memorial Institute / Analytical Chemistry Services

Detergents, such as laundry, dishwashing, and surface cleaner products, are used daily in the care of homes globally. Once the cleaning cycle is complete, these products are discharged into domestic wastewater and are a significant source for chemicals (such as surfactants, solvents, polymers, bleach activators, whitening agents, metal chelants, inorganic salts, and other compounds). Therefore, the need exists for a tool that can predict changes in domestic wastewater composition within a specific sewershed as a function of changes in the composition and consumption of home care products. This talk presents the initial phases of development of a mass balance model to predict the fate and transport of home care chemicals discharged into domestic wastewaters. The first module of the proposed model combines input data from Battelle's Detergent Program, which provides a historical record of the chemical composition of the most popular home care products commercialized yearly in the US, and worldwide, with consumption data provided by consumer intelligence surveys to predict input changes of home care chemicals in wastewaters.

TP156 Making Sense of Scents: Assessing Health and Ecological Hazards of Fragrances in Consumer Products Submitted under the USEPA DfE Recognition Program

C. Rudisill, SRC, Inc.; L. Morlacci, SRC Inc.; M. Adams, USEPA / Office of Chemical Safety and Pollution Prevention, Design for the Environment; C. Baier-Anderson, USEPA / Design for the Environment; B. Drake, E. Sommer, USEPA / Office of Chemical Safety and Pollution Prevention, Design for the Environment; J.L. Tunkel, SRC, Inc. / Environmental Health Analysis

The US Environmental Protection Agency's (EPA) Design for the Environment (DfE) Safer Product Labeling Program allows partners to use the DfE label on products whose ingredients meet or exceed hazard-based criteria for their functional class. The program also maintains the Safer Chemical Ingredient List (SCIL) (www.epa.gov/dfe/saferingredients.htm), which lists chemicals allowed in DfE-labeled products by functional class. In determining which fragrance chemicals are acceptable under the new criteria and for inclusion on SCIL, available human health, ecological health, and environmental fate data were examined for chemicals in this functional class. The first goal of this presentation is to provide a snapshot of the status of fragrance chemicals evaluated under DfE criteria with a focus on High Production Volume (HPV) chemicals that were submitted for DfE recognition. Results from this analysis indicate that a large portion of HPV and non-HPV fragrance chemicals submitted to DfE for recognition did not meet new criteria even though the updated criteria were more flexible. Critical data gaps proved to be a complicating factor in adequately assessing these chemicals. Where appropriate, fragrance chemicals were examined under the scope of a cluster analysis in order to ensure consistent and conservative calls in the face of these data gaps. Of the chemicals that were found to be acceptable and posted on SCIL, many have been assigned quantity limits within the formulation due to dermal sensitization and chronic toxicity concerns. The vast majority are ineligible for use in products that are released directly to the environment, mainly due to aquatic toxicity concerns. This presentation will help make "scents" of the available data and provide insight on how it can be used to create safer consumer products. The second goal of this presentation is to discuss results from a focused search of HPV fragrance chemicals in the available monitoring data, under the premise that fragrances used in the highest quantities are more likely to be detected in monitoring studies. Highlights from the literature review will be compared with the associated DfE status, and whether the results from this comparison can be used to identify emerging contaminants will be discussed.

TP157 Characterizing the sensitivity of Daphnia magna to "Green" home and personal care products: linking product formulation to hazard

A.D. Gray, Department of Biology; J.E. Weinstein, The Citadel / Department of Biology

Although it is generally assumed that green home and personal care products contain individual ingredients that are less toxic and/or more degradable than conventional formulations, little laboratory research has been conducted to validate these assumptions. Juvenile freshwater cladocerans, *Daphnia magna*, were exposed to non-degraded and photodegraded treatments involving six green household products: Green Works All-Purpose Cleaner, Green Works Dish Detergent, Earth Option Insect Killer, Tom's Mouthwash, Martha Stewart Bathroom Cleaner, and Seventh Generation Laundry Detergent. Sensitivity of *D. magna* to these products was assessed using 48-hour static acute toxicity tests. The results of these tests were then statistically analyzed in order to determine median lethal concentration, LC50s, and compared to one another using an LC50 ratio test. Of the six household products tested only three (Green Works Dish Detergent, Tom's Mouthwash, and Martha Stewart Bathroom Cleaner) were significantly less toxic after the degradation treatment. In the case of one household product (Raid Earth Options Insect Killer), the degraded product was significantly more toxic than the non-degraded product. And, in two cases (Green Works All Purpose Cleaner and Seventh Generation Laundry Detergent), the toxicity of the degraded product was not significantly different than that of the non-degraded product. Toxicity results from these green products were also compared to that of conventional product formulations, and these results then correlated to product ingredients in an effort to understand the drivers of the toxic response. This research demonstrates that green home and personal care product formulations are not necessarily less toxic and/or more degradable than their conventional counterparts.

TP158 Cleaning products ingredient safety initiative: Process for acquiring publicly-available human health hazard data and assessment of data availability

S. Williams, Baylor University; M.C. Ciarlo, EA Engineering, Science, and Technology, Inc.; B. Greggs, Soleil Consulting; C. Horne, EA Engineering, Science, and Technology, Inc.; K.A. Connors, Baylor University / Institute of Biomedical Studies, ; B.W. Brooks, Baylor University / Department of Environmental Science; W.L. Goodfellow; P.C. DeLeo, American Cleaning Institute

The American Cleaning Institute (ACI) is the industry trade association that represents the formulators of more than 90% of the consumer cleaning products in the United States. Recently, ACI set out to define and publish the universe of ingredients used in its members' products with the goal of identifying the publicly available hazard data for every ingredient and developing an associated exposure assessment and screening-level risk assessment. This presentation details the process of acquisition for hazard data that is relevant to human health. The goal of this task was to acquire a complete screening information data set (SIDS) for human health for over 900 chemical ingredients identified in consumer cleaning products. A tiered process was created consisting of primary and secondary data gathering followed by alternative approaches to address data gaps. Tier 1 data sources included EPA's HPVIS, OECD's HPV chemical archive, and ECHA REACH dossiers. Tier 2 sources include the FIFRA Inerts Database, TOXNET and the Hazardous Substances Databank, EPA's Integrated Risk Information System, and the peer-reviewed literature. Endpoints of interest included acute and repeated dose studies (oral, dermal, and inhalation), skin and eye irritation, skin sensitization, toxicity to development and reproduction, mutagenicity, and carcinogenicity. In numerous cases, primary data constituting a complete SIDS was not available, and much decision-making relied on grouping, read-across, and category approaches. Previous efforts on grouping and categorization were gathered and incorporated alongside ACI's initial grouping approach, in order to address gaps in primary data. Data availability scoring will be discussed, noting the frequency of application for primary data or read-across approaches.

TP159 What ingredients are present in everyday home cleaning products? Preliminary findings and trends from the Cleaning Product Ingredient Safety Initiative

P.C. DeLeo, American Cleaning Institute; M.C. Ciarlo, C. Horne, EA Engineering, Science, and Technology, Inc.; W. Greggs, Soleil Consulting, LLC; S. Williams, Baylor University

The American Cleaning Institute® (ACI) is the industry trade association that represents the formulators of more than 90% of the consumer cleaning products in the United States. Recently, ACI set out to define and publish the universe of ingredients used in its members' products with the goal of identifying the publicly available hazard data for every ingredient and developing an associated exposure assessment and screening-level risk assessment. This presentation reviews the preliminary findings from the development of an Ingredient Inventory for consumer cleaning products, which was the first part of this multi-year study (http://www.aciscience.org/IngredientInventory.aspx). The approximately 900 unique cleaning product ingredients were assigned to one of 60 different preliminary chemical groupings. Unique ingredients were also assigned to one of three categories (laundry care, dish care, hard surface cleaners) which were then broken down into more specific product use categories (e.g., all-purpose cleaner-spray, diluted). A number of trends were evident in the ingredients associated with different product and ingredient uses. This presentation will look at the basic trends regarding types of ingredients found and number of ingredients found within each chemical grouping, as well as how the ingredients they are distributed across product use, the number/type of ingredients per product per category, and number/type of ingredients per category.

TP160 A summary of publicly-available primary and categorical human health hazard data for cleaning products ingredients

S. Williams, Baylor University; M.C. Ciarlo, EA Engineering, Science, and Technology, Inc.; W. Greggs, Soleil Consulting, LLC; C. Horne, EA Engineering, Science, and Technology, Inc.; K.A. Connors, Baylor University / Institute of Biomedical Studies, ; B.W. Brooks, Baylor University / Department of Environmental Science; W.L. Goodfellow; P.C. DeLeo, American Cleaning Institute

The American Cleaning Institute (ACI) is the industry trade association that represents the formulators of more than 90% of the consumer cleaning products in the United States. ACI's recent effort gathered publicly available hazard data for each ingredient identified during the inventory phase (comprising over 900 substances). During acquisition of hazard data relevant to human health, endpoints included acute and repeated dose studies (oral, dermal, and inhalation), skin and eye irritation, skin sensitization, toxicity to development and reproduction, mutagenicity, and carcinogenicity. Dose descriptors included NOAELs, LOAELs, LD50s, LC50s, and judgments on irritation and sensitization. Studies that were judged as reliable without restrictions or reliable with restrictions (Klimisch 1 or 2) were acquired, while studies that were considered invalid or not assignable (Klimisch 3 or 4) were discarded. A summary of the available data for each endpoint will be provided; when available, hazard data will be provided in the context of chemical groupings. Decision-making on hazard noted the absence of primary data for a number of ingredients and in many cases grouping/categorization, QSAR, or other read-across approaches were employed. A web-based clearinghouse has also been developed to provide references for publicly-available hazard data for each ingredient.

Groundwater–Surface-Water Interface (GSI) Investigations used in Site Characterization and Assessment

TP161 Improved Detection of Groundwater Seepage Rates for Groundwater-Surface-Water Interface and Sediment Risk and Remediation Investigations

B. Chadwick, SPAWAR Systems Center San Diego; R. Paulsen, C. Smith, J. Groves, Coastal Monitoring Associates; P. Chirivas, Flexim Americas; J. Radford, Zebra-Tech Ltd.

Understanding the rates and variability of groundwater discharge is fundamental to assessment of Groundwater-Surface-Water Interface (GSI) sites, as well as to the proper design of sediment remedies such as capping. For example, seepage rates determine the residence time and thus the potential for natural attenuation of VOCs as they migrate through the GSI, and seepage rates of even < 1 cm/day can have significant implications for cap design and success. However, quantitative detection of groundwater seepage rates at these environmentally relevant levels remains a challenging problem. A range of different methods have been developed to address this challenge, ranging from indirect measurements such as natural tracers and piezometers, to direct measurements primarily with various types of seepage meters. Here we report on recent evolutionary improvements to the original ultrasonic seepage meter methods originally developed and reported by Paulsen et al, 2001. These improvements, including the incorporation of larger amplification funnels, and an improved ultrasonic transit-time flow meter, have led to significant improvements in the accuracy and precision of seepage rate measurements in the range below 1 cm/day. Controlled testing in this low range indicates calibrated accuracy of 1-2% RPD (~0.1 mm/day) and precision of 4-7% RSD (~0.4 mm/day). Results from the developmental testing of these new systems will be presented, as well as field results across a range of lake, river and coastal environments over the last year.

TP162 Investigations of Upwelling Groundwater in the Columbia River Hanford Reach, WA

B.L. Tiller, Environmental Assessment Services; R. Paulsen, Coastal Monitoring Associates

In south-central Washington State, the Columbia River flows through the US Department of Energy (DOE) Hanford Site. Groundwater beneath the Hanford Site discharges to the Columbia River via springs and subaqueous (below the riverbed) groundwater upwellings. Past waste management and waste disposal practices at the Hanford Site have resulted in the presence of several contaminated groundwater plumes. Beginning in 2009, a new technology has been successfully used to systematically identify where contaminated groundwater is upwelling in the river bottom and sample pore-water and sediment in those areas to support remedial investigations, risk assessments, and natural resources damage assessments of Hanford Site Releases to the Columbia River. The advanced liquid-tip Trident Probe[1] is a field research tool that has been adapted for use in the currents and depths of the free-flowing Columbia River in south-central Washington State. Additionally the probe's ability to distinguish subtle differences between two freshwater systems (groundwater and surface waters) enabled the probe to identify where groundwater is seeping into the Columbia River and to better understand groundwater upwelling patterns from shore to shore. Information and sample results from the field investigations are being used

to characterize current conditions within the Columbia River and are being combined with existing data to develop and monitor clean-up activities and perform ecological risk and natural resource damage assessments. Field sampling guidelines and quality controls used in this project helped minimize the influence of river fluctuations and bank recharge so that results could be compared with one another. Significant levels of indicator contaminants were reported in pore-water samples collected from a number of off-shore stations. Indicator contaminant sample results were not normally distributed across space, suggesting preferential pathways of groundwater movement to off-shore locations and non-uniform sources of the contamination. Additional field studies are currently being developed to further characterize the spatial and temporal variations of contaminated groundwater upwelling into the Columbia River.

TP163 A Multi-Phase and Multi-Technique Groundwater-to-Surface Water Interface (GSI) Study of Chloride Contamination

D.R. Lavoie; J.J. Tortomasi, J. Johnson, CH2M Hill

Historical investigations at an industrial facility in the Midwest identified elevated groundwater chloride concentrations. In 2006, on/offsite CPT/EC logging and groundwater sampling suggested chloride might be venting into an adjacent lake at concentrations exceeding a state water quality criterion. Although EC probes showed indirect chloride concentrations in the lake at the GSI were lower than in deep grab samples, no direct chloride measurements in shallow sediment pore water had previously been collected. Therefore, 2 phases of sediment pore water sampling were conducted to survey chloride venting zones using the Trident Probe, a direct-push, integrated conductivity/temperature (CT) sensor and GSI sampler. Phase 1 (2008) was conducted to verify the existing hydrogeological CSM, identify specific venting hotspots and gauge concentration magnitude. Phase 2 (2010) was conducted to refine the delineation of Phase 1 hotspots, establish a potential relationship between onsite groundwater and offsite sediment pore water, and support potential corrective action measures. For both studies, the Trident Probe was configured with 3 pore water sample probes (4", 16" and 29") and CT sensors at the shallowest and deepest sample points. Each probe was attached to peristaltic pumps to facilitate slow-flow sampling. Both Phase 1 and 2 results were consistent with the existing CSM. Two venting zones, where chloride exceeded the criterion at the GSI, were identified and delineated in the lake nearshore. Data from these studies clearly established the site-relatedness of the venting chloride and facilitated a path forward for onsite corrective measures (extraction wells). In 2012, additional pore water sampling (PushPoints), a hydraulic pressure survey (PushPoints with manometer) and a seepage meter study further refined the understanding of venting exfiltration water from process water retention ponds. This study revealed: (a) pore water quality was similar to pond exfiltration water and different from river and lake surface water; (b) consistent upwelling in nearly all lake and pond locations; and (b) an estimated groundwater-to-surface water venting rate (mass balance between the estimated exfiltration rate and venting rate accounted for < 20% of the exfiltration water). Based on these and local aquifer hydraulic data, it was thus concluded that exfiltration water from the pond is not migrating to areas other than the adjacent lake and river.

TP164 Use of Pore Water Chemistry and Seepage Velocity to Inform In-River Active Cap Design

S. Vaughn; M.S. Greenberg, USEPA / Environmental Response Team

The Lower Passaic River Study Area is a 17-mile stretch of the river from its mouth in Newark Bay up to Dundee Dam near Garfield, New Jersey, and is part of the Diamond Alkali Superfund Site. Approximately 20,000 cubic yards of highly contaminated sediment is being removed from the top two feet of a mud flat in the river approximately 11 miles upstream of its mouth at Newark Bay. The contamination includes dioxins, PCBs and mercury and elevated concentrations will remain under the dredged area. As such, a 2-foot thick active cap—containing activated carbon as a sorbent material—will be placed over the dredged area. Site-specific concentrations of contaminants in the sediment pore water were measured and Darcy velocities were determined through the use of seepage velocity devices. This information was helpful in selecting an appropriate thickness for the design of the active layer of the cap. A numerical simulation model developed to inform cap designs was then used to predict the potential transport of select contaminants through the active cap using the site-specific values as input parameters. This presentation will cover the chemical and geophysical characterization of the sediment in the mud flat and how the data and information on ground-water surface-water interactions were needed and used in the design of the removal action.

TP165 Development of a Passive Sensor for Measuring Water and Contaminant Flux in the Hyporheic Zone

L. Layton, K. Hatfield, M. Annable, M. Newman, J. Cho, H. Klammler, University of Florida; R. Gonzalez, University of Yucatan

Aquatic sediment sites contaminated with semi-volatile organic compounds are often difficult to characterize and manage due to the tendency for the contaminants to be retained within the sediments at these sites for long periods of time because of the hydrophobic nature of some of the key compounds, such as polycyclic aromatic hydrocarbons and polychlorinated biphenyls. Currently, technologies are able to identify groundwater discharge zones and infer estimates for contaminant mass flux based on total contaminant concentration in bulk sediment, though it is generally accepted that freely dissolved concentration in pore water is a better measure of potential exposure. The purpose of this research is to demonstrate a new tool to provide more accurate characterization of sediment pore water and bioavailable contaminant fluxes through direct in-situ measurement. The sediment bed passive flux meter (SBPFM) is designed for passively and directly providing direct in-situ measurements of volumetric water and contaminant mass flux vertically through the upper surface sediment layer and into the overlying water column. The SBPFM consists of an internal permeable sorbent which is impregnated with several water soluble tracers and is contained in a drive-point with an upper and lower screened opening. This will allow vertical flow through the device if there is a pre-existing vertical gradient between the sediment bed and the water column. Once deployed, the tracers are displaced from the sorbent at rates proportional to the average vertical specific discharge; thus, the mass loss of the tracers during deployment can be used to calculate water specific discharge. Similarly, the cumulative mass of sorbed contaminants provides a direct measurement of the vertical contaminant flux during deployment. The SBPFM prototype will initially be tested and validated through bench-scale box aquifer experiments, of which the initial results show agreement between the SBPFM estimated and actual measured fluxes through the box-aquifer. Following will be full-scale field deployments of the SBPFM to sites with manageable conditions and previous contaminant characterization to demonstrate the ability to measure contaminant flux through the aquatic sediment bed, as well as develop optimal inlet and outlet sizes, evaluate sorbent and tracer performances, create efficient deployment and recovery strategies, and derive of transport theory.

TP166 Chlorinated VOC degradation at the groundwater-surface-water interface in stream hyporheic zone

G.L. Mills, University of Georgia / Savannah River Ecology Laboratory; J. Williams, South carolina State University

Natural physical, chemical, and biological processes often act to reduce the concentration, mobility, and toxicity of contaminants in the environment. Monitored natural attenuation (MNA) assesses the contribution of these processes in the cleanup of contaminated soils and groundwater. MNA is often considered as part of an overall remediation plan that includes the use of engineered treatment technologies to achieve acceptable levels of risk to human health and the environment. Saturated floodplain soils and sediments adjoining streams generally provide conditions favoring microbial degradation of volatile organic carbon (VOC), including perchloroethene (PCE) and trichloroethene (TCE), and have been the focus of many MNA studies within the Southeastern Coastal Plain. However, few studies have explicitly examined natural attenuation processes within the subsurface stream sediments that are directly linked to stream surface water. This region is called the hyporheic zone and has been shown to play a critical role in controlling the flux of groundwater solutes to surface waters. With regard to MNA, it is the final interface before contaminants outcrop into regulated receiving waters. We investigated the distribution and degradation of PCE and TCE in the hyporheic sediments of Pen Branch stream located on the DOE's Savannah River Site near Aiken, SC. Our study focused on the region of stream reach where it intersects with a contaminated groundwater plume originating in an upland waste site. Seven cores were collected and selected sections between 10 and 150 cm were analyzed. Over 200 volatile analyses were performed. Significant concentrations of PCE and TCE were detected in the sediments in the mid -region of the plume impacted area. PCE concentrations ranged from < 0.1 to 1662 ppb while TCE value were < 0.3 to

506 ppb. Dichloroethene (DCE) and vinyl chloride (VC), products of microbial reductive dechlorination, were detected in many samples. One of the most detailed core profiles was collected in the location of maximum plume impact. PCE concentrations decreased from 960 ppb at 130 cm to 0.4 ppb at 7 cm while DCE initially increased from 14 to 50 ppb in the 130 to 40 cm interval, then decreased to < 0.1 ppb at 7 cm below the stream interface. These results indicate assessing degradation processes in the hyporheic zone at the GSI is critical for predicting further migration of contaminants and evaluating the longer term feasibility of natural attenuation.

TP167 An artificial sweetener, acesulfame: Candidate of chemical tracers for evaluation of sewage exfiltration in groundwater

Y. Watanabe, T. Orisikida, H.M. Nakata, Kumamoto University / Graduate School of Science and Technology; T. Hosono, Kumamoto University / Priority Organization for Innovation and Excellence; M. Kagabu, M. Ono, T. Tokunaga, J. Shimada, Kumamoto University / Graduate School of Science and Technology

In recently years, large amounts of low-calorie sweeteners have been used in a wide variety of beverages and foodstuffs in the world. Our previous studies reported the wide spread distribution of artificial sweeteners, acesulfame and sucralose, in wastewater, river water and groundwater samples because of their persistent and water-soluble properties. However, high concentrations of acesulfame were randomly detected in groundwater samples collected from downtown area of Kumamoto city, Japan. This may imply the leakage of wastewater into groundwater, due to breakdown of aging sewer pipes. Because groundwater is an important resource used for drinking water in this city, the conservation and safety management of groundwater are of great concerns. Based on these backgrounds, we collected and analyzed persistent water-soluble compounds, artificial sweeteners, in aqueous samples including wastewater, river water and groundwater samples in Kumamoto to re-confirm the results obtained from previous study. We also estimated the exfiltration rate of sewage in groundwater. Furthermore, X-ray contrast media, diatrizoic acid, was analyzed in wastewater and groundwater samples collected from hospital to evaluate a possibility of this compound as chemical tracer. Acesulfame was detected in almost wastewater and groundwater samples at mean concentrations of 907 ng/L and 3.29 ng/L, respectively. The acesulfame concentrations of 7 groundwater samples collected from downtown area were more than 20-folds greater than the background levels, although the distance of these sampling points were close. This result is similar to that of previous study, possibly due to the occurrence of sewage exfiltration in groundwater. We estimated the sewer exfiltration rate based on acesulfame concentrations in wastewater and groundwater, and referred groundwater recharge volume in Kumamoto. As a result, it was estimated that approximately 2.1 percent of the sewage in annual amount of wastewater is leaked into groundwater. The exfiltration rate in Kumamoto was close to the values of other cities, such as Tokyo, Japan (0.4 to 0.8%) and Nottingham, UK (2.0 %), which were calculated by pharmaceuticals in groundwater. Diatrizoic acid was detected in both wastewater and groundwater samples collected from hospital. While further research is needed, diatrizoic acid may become a chemical tracer to evaluate the hospital wastewater leakage into groundwater.

Fate and Effects of Metals

TP168 A comparative analysis of aquatic HC05 values for aluminum using Biotic Ligand Model- vs. hardness-normalized toxicity data

J. Gondek, GEI Consultants; R.W. Gensemer, GEI Consultants / Ecological Division; B.A. Stubblefield, Oregon State University / Environmental and Molecular Toxicology; A.S. Cardwell, Oregon State University / Faculty Research Assistant; R.C. Santore, HydroQual, Inc., HDR|HydroQual, Inc.; A.C. Ryan, HDR | HydroQual; B.J. Adams, Rio Tinto / Product Stewardship; E. Nordheim,

The toxicity of aluminum (Al) under circumneutral conditions depends on a variety of water quality parameters including: temperature, pH, dissolved organic carbon, and hardness. While most aquatic life protection criteria in the US are single fixed values, two states now use hardness equations to derive criteria. Since these criteria were derived, chronic species sensitivity distributions (SSD) are now available for derivation of 5% effect concentrations (HC_{05}). Utilizing toxicity data from tests conducted at pH 6 to 8 for fish, invertebrates, and algae, a Biotic Ligand Model (BLM) was used to normalize toxicity results to various conditions representing standard waters often used for metals risk assessments in Europe. HC_{05}s were derived using distribution fitting software including @Risk, ETX, and EPA's method for deriving a Final Chronic Value (FCV). These results were then compared to the hardness-based water quality standards for New Mexico and Colorado. We found that the HC_{05} can vary considerably depending on the program and distribution model used to fit the data. Overall, @Risk fit the data better than ETX or the EPA models at the HC_{05} level because of the flexibility in selection of the most appropriate statistical model. HC_{05} values from @Risk and ETX were lower than those using the EPA FCV method largely because EC_{10}s were used to derive HC_{05}s, whereas EPA methods recommend EC_{20} –based chronic values. In addition, calculated FCV values were under protective of algae and would have to be lowered to protect algae under the majority of water conditions evaluated. For water conditions at pH 7-8, hardness-based criteria based on a largely acute toxicity dataset were much higher than HC_{05}s estimated by any of the SSD methods which were based on a much larger chronic toxicity database. However, hardness-based criteria may be overly restrictive when pH is less than 7 due to State criteria requiring a fixed chronic criterion concentration of 87 µg Al/L. Consideration should, therefore, be given to BLM-derived criteria as they better account for all water quality parameters that drive Al speciation and toxicity, and because a much more robust chronic database is now available.

TP169 Application of Lead and Arsenic Bioavailability in Human Health Risk Assessment for a Sediment Site

C. Liu, CDM Smith; N. Luke, CDM Smith Inc.

Lead smelting slag was used to construct seawalls and jetties in a waterfront park approximately 40 years ago and battery casing wastes were disposed at the site. As a result, soil, groundwater, surface water and sediment have been contaminated with lead and arsenic. To evaluate the physical and chemical characteristics of lead and arsenic detected at the site and to determine the degree to which lead and arsenic are available for uptake into the human body, a site-specific *in-vitro* bioavailability and speciation study was performed. The *in-vitro* bioavailability test involved a laboratory procedure that is designed to mimic some of the conditions of the human digestive tract. Quantitative electron microprobe analysis (EMPA) was used to determine chemical speciation, particle size distribution, association of the metal-bearing forms, frequency of occurrence, and relative mass. Results of the *in-vitro* study indicated that bioavailability of lead and arsenic varied widely among different samples, both within and across each area. The relative bioavailability for lead ranged from 12% to 84% and averaged about 56%. Similar to lead, *in-vitro* bioaccessibility for arsenic was also highly variable, ranging from 0.3% to 63%, with an average of 14%. The EMPA study revealed that lead and arsenic were mainly associated with iron oxyhydroxide, which is expected to be moderately bioavailable. Site-specific bioavailability factors were developed that resulted in more realistic human health risk characterization results and remediation goals.

TP170 Arsenic speciation in environmental samples from the Youngsan River Estuary, Korea: A comparison between freshwater and saltwater

S. Hong, Seoul National University / Division of Environmental Science and Ecological Engineering / School of Earth and Environmental Sciences; J. Park, Seoul National University / School of Earth and Environmental Sciences (Oceanography); S. Song, Seoul National University / Division of Environmental Science and Ecological Engineering; B. Kwon, Seoul National University; J. Jung, Korea University; S. Choi, Ulsan National Institute of Science and Technology (UNIST) / School of Urban and Environmental Engineering; J. Khim, Korea University / Division of Environmental Science and Ecological Engineering, Seoul National University / School of Earth and Environmental Sciences

Differences in distribution, partitioning, and bioconcentration characteristics for arsenic species between freshwater and saltwater systems are still not well understood. In order to determine the characteristics of distribution and behavior of arsenicals, various environmental samples including surface water, suspended particle (0.45 – 30 µm), zooplankton (> 100 µm), sediment, and porewater were collected from inner (5 sites, freshwater) and outer (5 sites, saltwater) regions of estuary dike in the Youngsan River of Korea during November of 2012. Six organic and inorganic arsenic species were separated and measured by use of HPLC-ICP/MS equipped with an anion exchange column. Concentrations of arsenics in water samples of inner regions (mean = 1.5 µg L^{-1}) were significantly lower than those of outer regions (mean = 5.21 µg L^{-1}), while concentrations of arsenics in suspended particles of inner regions (mean = 13.9 µg g^{-1}) were much greater than those of outer regions (mean = 5.7 µg g^{-1}). It is indicated that another arsenic pollution sources could exist in outer regions of dike, as well as adsorption of

arsenic on suspended particles seemed to be affected by salinity (say salting-in effect). The As^V was found to be the major arsenic species in all water and particle samples both inner and outer regions. Meanwhile, cladocerans were the most dominant zooplankton species in freshwater, and cyclopoinds were predominantly found in saltwater. The arsenic concentrations in zooplankton were shown to be particle-concentration dependent, suggesting that dietary exposure plays a substantial role in the bioconcentration of arsenics. Inorganic arsenics such as As^V and As^{III} were the most dominant species found in zooplankton. Concentration and partitioning behavior of arsenics between porewater and sediment showed similar to those in water-particle distribution. Overall, the results of the present study would provide better understanding on fate and bioavailability of arsenics in land-ocean connections.

TP171 Arsenic speciation in water, suspended particles and organisms collected from the Taehwa River, Ulsan, Korea

S. Hong, Seoul National University / Division of Environmental Science and Ecological Engineering / School of Earth and Environmental Sciences; H. Kwon; H. Son, Ulsan National Institute of Science and Technology (UNIST) / Environmental Analysis Center; S. Choi, Ulsan National Institute of Science and Technology (UNIST) / School of Urban and Environmental Engineering; J. Khim, Korea University / Division of Environmental Science and Ecological Engineering, Seoul National University / School of Earth and Environmental Sciences

In order to determine the concentration, distribution, and bioconcentration characteristics of arsenic, surface water, suspended particle, and various organisms (fish, bivalve, crab, shrimp, gastropod, and algae) were collected from the Taehwa River, Ulsan, Korea. Suspended particles were segregated into three size fractions through filters with pore sizes of 0.45, 30, and 180 µm. Six organic and inorganic arsenic species were separated and measured by use of HPLC-ICP/MS equipped with an anion exchange column. In general, the concentration and spatial distribution of arsenicals in water indicated that their sources are closely associated with industrial and municipal activities. Concentrations of dissolved arsenic in water ranged from 0.05 to 4.7 µg L^{-1}, with an average of 1.4 µg L^{-1}. The As^V (~93%) was the most dominant arsenic species in water, followed by arsenobetaine (AB, 2.8%), and As^{III} (0.9%). Interestingly, concentrations of AB in water were significantly correlated with their corresponding salinities (r^2= 0.67, p < 0.01), suggesting that AB was released from aquatic organisms as a cellular osmolyte. The 0.45 – 30 µm size fractions held the most particulate arsenic at all sites, consisting of about 86% of As^V. Field-based partition coefficient of arsenic between water and suspended particles generally decreased with increasing salinity (viz., salting-in effect). The AB was found to be the most dominant arsenic species in fish, bivalve, crab, shrimp, and gastropod, while unknown arsenicals were predominant in algae. Overall, the results of present study would provide useful information on distribution, fate, and bioconcentration of arsenicals in aquatic organisms for future studies.

TP172 Assessing bioaccessibility of polycyclic aromatic hydrocarbons (PAHs) contaminated soil using silicone rods

K.J. James, University of Saskatchewan / Toxicology Group and Soil Science; V. Gouliarmou, Aarhus University, Science and Technology Faculty / Environmental Chemistry and Microbiology; P. Mayer, Technical University of Denmark / Department of Environmental Sciences; J. Wragg, M. Cave, British Geological Survey; S.D. Siciliano, University of Saskatchewan / Toxicology Group and Department of Soil Science

Polycyclic aromatic hydrocarbon (PAH) bioavailability varies significantly between soils. PAHs are an organic lipophilic class of contaminants where the bioaccessibility of ingested material is influenced by thermodynamic properties. Here we assess PAH oral bioaccessibility, using the FORE(h)ST model modified with the addition of silicone rods, and PAH oral bioavailability, using the juvenile swine model, and compare both models to the thermodynamic properties (fugacity capacity) of soil. Preliminary results show a strong relationship between in vivo bioavailability of individual PAHs and soil fugacity capacity. The regression between individual PAH bioavailability and soil fugacity capacity produce r^2 values between ~ 0.6 and 0.9, indicating that PAH bioavailability is strongly influenced by thermodynamic properties. PAH bioaccessibility data is yet to be analyzed; however preliminary results indicate that silicone rods can be used in two ways 1) to create an effective fugacity gradient to avoid limiting PAH bioaccessibility to the solubility of simulated gastro-intestinal fluids and 2) to efficiently separate and extract the bioaccessibility fraction from simulated gastro-intestinal fluids.

TP174 Contaminant Transport and Pathways Model for Assessment of Current and Future Risks in the Beaverlodge Lake Area, Canada

S.L. Fernandes, C. Hamer, H. Phillips, B. Halbert, SENES Consultants; M. Webster, Cameco Corporation

The Beaverlodge Lake region is an area in northern Canada which has been affected by past uranium mining and milling activities. Remediation activities have occurred; however, there are residual radionuclide and metal issues in the watersheds of the area. Modelling has been performed on a number of interconnected watersheds using a proprietary computer code called LAKEVIEW in order to predict future environmental conditions with the dual aim of communicating existing risks to area residents and informing further remediation of the site. LAKEVIEW consists of a contaminant dispersion model, which predicts future water column and sediment concentrations, and a pathways assessment model, which allows for an integrated approach to assess the risk to human and ecological receptors that may frequent the site. Uranium City is a small nearby community with a year-round population of approximately 70-100 people. A survey of the local community was conducted which involved consultation with approximately 91% of year-round and seasonal residents to determine region-specific information such as ingestion rates for country foods and hunting/fishing locations. In addition, regional sampling was conducted for berries as well as number of ecological receptors including small and large mammals, grouse and fish. Exposures or doses to ecological and human receptors were calculated within the model on a year-by-year basis and compared to toxicity reference values (TRVs) to determine specific risks throughout time. Results of the assessment indicate that, in the downstream environment, radionuclide concentrations are generally not a concern while there are potential risks to human health related to consumption of fish containing selenium and drinking water containing uranium in some water bodies. Potential risks to ecological receptors in these areas are due primarily to predicted bioaccumulation of selenium in fish and waterfowl with a fish-based diet. Further simulations indicate that the majority of these risks are unlikely to be quickly mitigated regardless of remedial strategy employed.

TP175 Derivation of ecologically relevant effects threshold concentrations for Pb in marine waters: a European experience

P. Van Sprang, ARCHE; J. Chowdury, ILZRO

In the REACH framework, Pb specific information on environmental toxicity and on environmental exposure/fate for key environmental compartments (water, sediment, soil) was compiled in order to assess the potential environmental risks related to the production and use of Pb in the European Union. Because only few reliable marine chronic toxicity data could be retrieved from literature a testing program was conducted aiming to generate the data necessary to 1) develop a species sensitivity distribution for Pb and 2) derive a safe threshold HC5-50 for the marine aquatic compartment. Consequently, chronic testing using 14 different marine species have been conducted, representing the most relevant taxonomic groups for the marine environment, i.e., crustaceans, molluscs, worm, echinoderms, unicellular algae, higher plant and fish. Where possible, the chronic toxicity experiments were conducted according to internationally accepted standard testing protocols (e.g., EPA, ASTM, ISO). Dissolved Pb levels were measured and reported effect levels (NOEC, EC10) were based on these measurements. No-effect levels for dissolved lead varied between 9.2 and 1234 µg Pb/L, i.e., a difference of a factor of 134 between the most and least sensitive species. The mollusc *Mytilus trossolus* was the most sensitive species. The least sensitive species were the algae *Dunaliella tertiolecta* and *Phaeodactylum tricornutum*. The HC5-50 that was associated with the Log-Normal Distribution that was plotted through the chronic data was 6.9 µg dissolved Pb/L.

TP176 Distribution of Heavy Metals Across the Northern Reaches of Grand Lake O' The Cherokees

S.A. Morrison, J.B. Belden, Oklahoma State University / Department of Zoology

The abandoned Tri-State Mining District (TSMD) is an historic superfund site that covers 6500 square kilometers and three states (Kansas, Missouri and Oklahoma) and is the source for trace metal loading of sediments within Grand Lake O' The Cherokees (hereafter referred to as Grand Lake). Elevated concentrations of lead, cadmium and zinc have been documented in sediments collected along several lake transects that included shallow and deep water; with no evidence of sediment toxicity to aquatic invertebrates. The current research goals addressed by this project are to develop a more

complete metal distribution map with emphasis on shallow water areas (≤ 20ft depth) located in the Northern reaches of Grand Lake where chances of sediment deposition from the TSMD is greatest. This distribution information is important because the shallow areas are subject to disturbance events (e.g., boat traffic, wave action and dock construction) and have a higher chance of becoming dry during low water periods. Changes in water chemistry and redox state of trace metals during these disturbance events could cause greater availability resulting in toxicity, bioaccumulation, and greater lake impacts.

TP177 Effect of fluoride on the uptake of a trivalent trace metal by the unicellular alga Chlamydomonas reinhardtii

A. Cremazy; C. Fortin, University of Quebec / INRS-ETE; P.G. Campbell, University of Quebec

Aluminum (Al) can be toxic to a variety of aquatic organisms, especially in acidic natural waters where its solubility is higher. Fluoro-complexes are important Al species in these waters, as fluoride strongly binds to Al. It is therefore important to characterize the effect of fluoride on Al bioavailability to aquatic organisms. According to the Biotic Ligand Model (BLM), metal biouptake should be proportional to the free ion activity. Thus, Al complexation by fluoride is expected to decrease Al uptake and toxicity to a predictable extent. However, the applicability of the BLM to trivalent metals such as Al is still largely uncertain, and there is yet no clear evidence whether or not Al fluoro-complexes are taken up by aquatic organisms. A major reason for this lack of knowledge is the absence of a suitable radio-isotope allowing for simple measurements at environmentally relevant Al concentrations. To overcome this limitation, the trivalent metal scandium (Sc) has been used in our laboratory as a substitute for aluminum. It shares chemical similarities with Al and it has a suitable radio-isotope (Sc-46, half-life of 83.8 d). Scandium was used in the present study to investigate the applicability of the BLM to Al (and more generally to trivalent metals) in the presence of fluoride. Scandium internalization fluxes (J_{int}) were measured in the green unicellular alga *Chlamydomonas reinhardtii* in short term (< 1 h) uptake experiments, at pH 5.00 and at different fluoride concentrations ranging from 0 to 10 µM. Scandium adsorption (Sc_{ads}) onto the algal surface was also determined in these experiments. The observed values of J_{int} and Sc_{ads} were then modeled with the BLM equation and the Langmuir isotherm respectively, as a function of the free Sc^{3+} concentration. The BLM failed to model Sc accumulation in *C. reinhardtii* in the range of fluoride concentrations tested. The observed values of J_{int} and Sc_{ads} increased for a same [Sc^{3+}] with increasing [F^-]. These results suggest that some fluoro-complexes participate in the Sc uptake flux and surface adsorption by *C. reinhardtii*. In a previous study, we also suggested that Sc hydroxo-complexes were internalized in *C. reinhardtii*, in addition to the free ion Sc^{3+}. There is thus growing evidence that the free ion concentration may not be adequate to predict the accumulation and toxicity of trivalent metals in aquatic organisms.

TP178 Effects of Environmentally Relevant Perfluorochemicals Concentrations on Fish Gene Expression

I.A. Rodriguez-Jorquera, University of Florida / Soil and Water Science; G.S. Toor, University of Florida, Gulf Coast Research & Education Center / Soil & Water Science Department; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology

Treated wastewater discharged into the Nation's streams and rivers contributes several contaminants of emerging concern in the form of complex mixtures of chemicals, making it difficult to elucidate the impact of individual chemicals on the exposed biota. Nonetheless, the fact that toxicity is preceded by alteration in gene expression in an organism allows the use of gene expression profiling (from microarray studies) to detect early toxic effects and identify mechanisms of action. This facilitates establishment of links between toxicants and effects on biota. In surface waters that receive wastewater, endocrine disrupting compounds (EDCs) are known to cause effects at very low concentrations. One common class of EDCs found in urban waters is perfluorochemicals (PFCs). Previously, we observed that urban waters with wastewater influence containing PFCs in the 300 ng/L range exerted effects in fish by altering the expression of 500 to 1100 genes and 12 to 19 altered biological processes that included cholesterol metabolism and DNA repair. To confirm our previous findings, we investigated the impact of 12 different types of PFCs in a controlled lab study by exposing fathead minnows for 48 h to low and high concentrations of PFCs, as described below. The most commonly found PFC in waterways is PFOS (perflourooctanesulfonic acid) and for this reason it was used alone in three concentrations in addition to a PFCs mixture. The five treatment groups were: (1) low PFOS (564 ng/L), (2) medium PFOS (1770 ng/L), (3) high PFOS (10965 ng/L), (4) PFCs mixture (11 types of PFCs: 393 ng/L), and (5) Control (0 ng/L). No fish mortality was observed in any treatment exposures, but gene expression was altred. Micorarray analysis yields information on altered molecular pathways that predict changes at higher levels of biological organization such as survival and reproduction. Micorarray analysis yields information on altered molecular pathways that predict changes at higher levels of biological organization such as survival and reproduction.

TP179 Fate, transport, and exposure: Radionuclides and metals in ecological field studies at an active uranium mine in northern Arizona, USA

J. Hinck, US Geological Survey / Columbia Environmental Research Center; G.L. Linder, USGS/BRD/Columbia Environmental Research Center, Heron-Works Field Office

Concerns about radionuclide contamination of southwestern water resources have re-emerged since the recent (2009) resumption of uranium mining near the Grand Canyon. Mining proponents have suggested that contamination risks are negligible based on the small footprint the mines and that milling takes place off-site. Data and information are insufficient; radionuclide and metal fate and transport in the local ecosystem and biological exposure pathways are largely unknown. Resource managers are limited in their ability to evaluate environmental impacts given these data constraints. Canyon Mine, located 10 km south of Grand Canyon National Park, has recently resumed production and represents a case study for focused studies intended to address data and information gaps. The USGS is leading an interdisciplinary study to determine whether levels of chemical and radiation are affected by mining activities sufficient to pose increased risk to biota relative to baseline. From a biological perspective, this requires collection of biota that are representative of the local food web before mining starts, during active mining, and after mine closure. Research efforts in 2013 focused on conducting baseline biological surveys and collecting tissues for contaminant analysis at the Canyon Mine. Contaminants of potential concern to biological receptors (As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Tl, U, and Zn for chemical toxicity and U and associated radionuclides for radiation toxicity) and known or suspected contaminant sources (vent shaft, ore storage and waste rock, haul road) were identified. Primary exposure pathways to be documented through sampling include direct contact uptake, ingestion, inhalation, and dietary transfer. Field studies included surveying and/or collecting plants, invertebrates, amphibians, reptiles, birds, and small mammals using a stratified sampling design. Survey data were used to understand the local food web and refine the list of target species for chemical contaminant and radiation analysis. Soil was collected from the mine site and along the haul road using a multi-increment sampling method. Sediment and water grab samples were collected from the mine detention pond and along stream channels. An anemometer was also installed on-site to record wind direction and velocity, which will be used to evaluate off-site contaminant transport. Results of 2013 field studies will be presented.

TP180 Free ion (Cu2+, Pb2+, Zn2+, Ni2+) measurements for predicting metal bioavailability to saltwater invertebrates at various DOC and salinity conditions

S.D. Smith, Wilfrid Laurier University / Department of Chemistry; T. Tait, Wilfrid Laurier University; C. Cooper, Wilfrid Laurier University / Chemistry; W. Chen, Trent University / Department of Chemistry; G. Webster, Wilfrid Laurier University / Department of Chemistry; C. Gueguen, Trent University; J. McGeer, Wilfrid Laurier University / Department of Biology

Developing quantitative bioavailability models to assist in the establishment of environmental regulation, as well as for risk assessment purposes, require specific understanding of the links between metal speciation and toxicity. The Biotic Ligand Model (BLM) has proven to be a useful tool for this purpose, especially for freshwater environments. For saltwater matrices BLM approaches are less well developed/tested. Here we test the BLM framework for saltwater application by measuring free ion at EC50 values for single exposures of four metals (Cu, Zn, Pb and Ni). Acute toxicity tests were preformed for marine invertebrate including mussel embryo tests (*Mytilus galloprovincialis*) or euryhaline rotifer (*Brachionus plicatilis*) or mysid (*Americamysis bahia*). Test organisms were selected to give sensitive effect concentrations below solubility limits for the metals of interest. At a fixed

salinity, solutions of variable dissolved organic matter concentration should demonstrate constant free metal concentration if BLM is a valid approach for salt water. In addition, for applicability without including a quality factor the free metal should be constant for variable source of organic matter as well. Here, free metal is estimated using ion-selective electrode (Pb^{2+}, Cu^{2+}), fluorescence quenching (Cu^{2+}, Ni^{2+}), Absence of Gradients and Nernstian Equilibrium Stripping (AGNES) (Zn^{2+}). Advantages and disadvantages of each technique for free metal estimation are highlighted along with representative results for solutions appropriate to salt water toxicity testing. Specific results include total dissolved lead for the mytilus tests spanned a range of 63-157 µg Pb/L for samples with dissolved organic carbon in the range 2 to 10 mg C/L. For these same samples free lead was approximately constant measured at $10^{-7.6\pm0.3}$M. For copper and rotifer toxicity testing, free copper at the LC50 for samples collected at 9 different estuarine sites, with variable fluorescence quality indices, remained constant (average of $10^{-10.14\pm0.3}$ M), within a factor of two, while the LC50 values ranged from 333 to 980 nmol Cu/L (factor > 3). These results highlight the potential applicability of BLM bioavailability modelling in saltwater environments. This research is funded by NSERC through a CRD grant in partnership with ICA, CDA, ILZRO, NiPERA, IZA, Teck, Vale and Xstrata Zinc.

TP182 Interactions of waterborne metals in binary mixtures on short-term metal and ion uptake, and toxicity in rainbow trout (Oncorhynchus mykiss)

S. Niyogi, Department of Biology, & Toxicology Program; S. Nadella, A. Ng, McMaster University / Biology; C.M. Wood, McMaster University / Department of Biology

The current Biotic Ligand Models (BLMs) do not account for the effects of metals in mixture, although organisms inhabiting the metal-contaminated natural waters are almost always exposed to multiple metals at elevated concentrations. Metals are known to share common uptake pathways in fish gill. For example, cadmium and zinc are both taken up by calcium transport pathway, whereas copper and silver are absorbed by sodium transport pathway. Thus, metals sharing a common uptake pathway should exhibit competitive interactions when they are in mixture, and metals with different uptake pathways should not interact in the gill. The BLM framework is based on the short-term gill-metal binding characteristics of the fish gill. The objective of the present study was to examine the interactive effects of metals (cadmium, copper, nickel, silver and zinc) in binary mixtures on short-term (3-hr) branchial uptake of metals and ions (calcium and sodium) in juvenile rainbow trout (*Oncorhynchus mykiss*) during acute waterborne exposures. We found that both copper and zinc reduced cadmium uptake and vice versa, however nickel did not have any effect on cadmium uptake. Interestingly, silver was found to stimulate copper uptake, whereas copper had no effect on silver uptake. The inhibitory effect of cadmium and zinc in mixture on calcium uptake was greater than that of cadmium or zinc alone. Similar additive inhibition of calcium uptake was also recorded with cadmium and copper in mixture. The inhibitory effect of copper and silver in mixture on sodium uptake was greater than that of copper or silver alone. Cadmium did not affect sodium uptake, and no additive inhibition of sodium uptake was observed with cadmium and copper in mixture. Acute toxicity assessments conducted with cadmium and zinc in mixture demonstrated that zinc did not affect the toxicity of cadmium, but cadmium increased zinc toxicity in an additive manner. Overall, our findings indicate that gill-binding characteristics of metals in binary mixture do not always follow the predicted pattern, nevertheless toxicity can be reasonably explained by simple fractional response additions. These observations may have useful implications for adopting the BLM approach to assess metal-mixture toxicity in fish. (Acknowledgements: Environment Canada MITHE Program, NSERC)

TP183 Lead accumulation by Chlamydomonas reinhardtii in the presence of citrate: importance of discriminating truly internalized from adsorbed metal

P. Sanchez Marin, Institut National de la Recherche Scientifique / Centre Eau Terre Environnement, University of Aveiro & CESAM / INRS-ETE; Z. Chen, C. Fortin, P.G. Campbell, INRS

Low molecular weight organic metabolites, such as citric acid, can enhance metal bioavailability despite the fact that they form hydrophilic metal complexes that cannot cross the plasma membrane by passive diffusion. Documented exceptions to the BLM involving this kind of ligands include cases where the metal-ligand complex is taken up by piggy-back transport (fooling the anion transporter) or cases where the metal uptake flux is higher than the diffusive flux of the free metal, and therefore labile complexes dissociate and contribute to the metal internalization flux. In this study, we show that the lead (Pb) apparently internalized by *C. reinhardtii* in the presence of citrate is higher than expected on the basis of the BLM, and higher than the expected considering the possible contribution of piggy-back uptake of Pb-citrate complexes (based on measurements of assimilated ^{14}C-citrate). The same effect was observed for a wall-less strain of *C. reinhardtii*, which takes up Pb at a lower rate. Time-course experiments revealed that the extra-Pb accumulated in the presence of citrate was not truly internalized but strongly associated to the external surfaces. Lead subcellular distribution in the algae in the absence and presence of citrate was further assessed by gentle cell disruption and differential centrifugation. These results imply that the apparent enhanced bioavailability of lead in the presence of citrate may not have implications for the alga itself (if adsorbed Pb is not toxic) but should be considered for assessing the risk of Pb transfer to predators in the food chain.

TP184 Lead poisoning in children from townships around a lead-zinc mine in Kabwe, Zambia

S.M. Nakayama, Hokkaido University / PhD. Student; J. Yabe, University of Zambia; Y. Ikenaka, Hokkaido University; K. Muzandu, University of Zambia; J. Ntapisha, Kabwe District Health Office; A. Mweene, University of Zambia; A. Kabalo, Kabwe District Health Office; T. Umemura, M. Ishizuka, Hokkaido University

Childhood lead (Pb) poisoning is a serious public health concern worldwide. Young children under the age of 7 years are particularly vulnerable to Pb poisoning because of behavioral factors, such as frequent hand-to-mouth activities and biological factors including greater gastrointestinal absorption compared to adults and developing neurological systems. Lead exposure among children is associated with developmental abnormalities including impaired cognitive function, reduced intelligence, impaired hearing and reduced stature. In Kabwe, the capital of Zambia's Central Province, extensive contamination of Pb in soils, wild rats as well as offal of cattle and chicken in townships in the vicinity of a lead-zinc mine has been reported and poses a serious health risk to children in these townships. Therefore, this study investigated blood lead levels (BLLs) in children in townships around the Pb-Zn mine in Kabwe and to identify children with BLLs that require medical intervention so as to mitigate the toxic effects of Pb. The study was approved by the University of Zambia Research Ethics Committee and the Ministry of Health, Zambia. After informed and written consent was obtained from the parents or guardians, blood samples up to 3 mL (17 samples at Chowa, 100 samples at Kasanda and 129 samples at Makululu) were collected by qualified laboratory technicians from the children at clinics in the study areas. For each child, data on the age, sex and residential area were recorded. The blood samples were promptly transferred and stored at -20 °C at the laboratory of the Kabwe District Health Offices. The samples were transported to Japan and analyzed for Pb concentrations by ICP-MS. Almost all of the sampled children in the current study had indications of Pb poisoning, with BLLs exceeding 10 µg/dL. When children were grouped according to age, younger children between the ages of 0 – 3 years accumulated higher BLLs than their older counterparts (4 – 7 years). Significant negative correlation between age and BLLs supported this finding. This study demonstrated that childhood Pb poisoning in Kabwe is among the highest in the world. Although clinical cases and deaths due to Pb poisoning among children in Kabwe are rare, these findings indicate that more studies are needed to establish the health effects of Pb poisoning in children exposed to Pb pollution in townships around the Pb-Zn mine in Kabwe.

TP185 Mitigating effects of dissolved organic matter on Cu toxicity to Americamysis bahia in estuarine waters

R. Nasir, Wilfrid Laurier University / Biology; J. McGeer, Wilfrid Laurier University / Department of Biology

In estuarine waters the concentration of ions, particularly Na^+ and Cl^-, vary tremendously and this can have an important modifying effect on Cu toxicity. Dissolved organic matter (DOM), another important toxicity modifying factor for Cu in fresh water, can also vary in estuarine conditions. This study evaluates the acute and chronic effects of Cu on *Americamysis bahia* and the role of DOM quantity and quality on responses. Neonates of ages 2-4 d or 7 day were used for either 96 h acute toxicity tests or 7d chronic tests. The tests were static (acute) or in the case of 7 d tests, static renewal with

daily water changes and survival, growth and fecundity (sexual maturation) as the end points for the latter. The protective effects of DOM source and concentration (as dissolved organic carbon (DOC)) were characterized at different salinities. DOM from freshwater sources that discharge into estuaries were collected just upstream of the saline mixing zone by reverse osmosis concentration at five sites in Atlantic Canada. A concentration dependent protective effect of DOC was observed with additions of 2, 4 and 10 mg C/L. At 15 ppt up to a 3-fold reduction in acute toxicity was observed. DOC mitigation of Cu toxicity was tested at salinities of 15, 25 and 35 ppt and the degree of protection generally declined as salinity increased. The degree of acute toxicity mitigation varied with source. In the 7 d chronic tests DOM offered significant protection against the effects of Cu and this was evident in terms of both survival and growth. DOM sources were characterized using optical measures (specific absorption coefficient, fluorescence index and excitation emission matrix spectroscopy followed by parallel factor analysis) to determine linkages with toxicity mitigation capacity. This study is contributing to the development of an estuarine BLM for water quality guidelines/criteria for Cu. This research is funded by NSERC through a CRD grant in partnership with ICA, CDA, ILZRO, NiPERA, IZA, Teck, Vale and Xstrata Zinc.

TP186 PAH Interactions with Soils and Effects on Bioavailability to Humans

H. Xia, UMBC / Dept. of Chemical, Biochemical, and Environmental Engineering; J.L. Gomez-Eyles, UMBC; M. Ruby, Integral Consulting; Y. Lowney, Exponent; U. Ghosh, University of Maryland Baltimore County / Department of Chemical, Biochemical, and Environmental Engineering

Polycyclic aromatic hydrocarbons (PAHs) are released into soils within differently sorbing domains (e.g., soot, char, coal tar or NAPLs) that are likely to alter their bioavailability to humans. Weathering and further interaction with native soil particles (e.g., black carbons) may reduce this bioavailability further. In this study we constructed a number of artificial soils with a range of native organic carbon and black carbon types and concentrations. PAHs were introduced into these soils within a variety of different source materials (soot, coal tar-based skeet particles, and fuel oil). The soils were then weathered in the laboratory before being characterized. The partitioning characteristics of the PAHs within their different source materials were investigated by exploring the equilibrium partitioning of the PAHs in both a water and a simulated human gastrointestinal fluid matrix. Aqueous equilibrium partitioning experiments using polyoxymethylene solid phase extractions (POM-SPE) were also performed for the weathered soils. The bioaccessibility of the PAHs within the weathered soils was determined using an *in vitro* physiologically-based extraction test that provides an estimate of PAHs in soil that may be available for absorption following ingestion. PAH partitioning into the aqueous phase was greatly enhanced in the presence of simulated gastrointestinal fluid relative to water for most source materials. The simulated gastrointestinal fluid contains various ingredients that are responsible for this enhanced desorption. Bile salts, for example, can form mixed micelles and serve as a surfactant mobilizing PAHs from their source materials. This effect was greater for matrices like fuel oil and soot, as well as for the more carcinogenic 4-, 5-, and 6-rings PAHs. The aqueous equilibrium experiments reflected large variability in freely dissolved PAHs from the constructed soils: over 2 orders of magnitude differences in soil K_D were observed among soils spiked with different source materials. Specifically, the presence of additional black carbon in the soil enhanced K_D by an order of magnitude. As part of a separate study, the effect of different source materials and soil matrices is also being evaluated for effects on bioavailability in an *in vivo* rat model developed by the University of Florida. The preliminary *in vitro* data generated in this effort will serve as a starting point for validating an *in vitro* method against data from animals.

TP187 Predicting aluminium toxicity as a mixture of effects from dissolved and precipitated metal

R.C. Santore, HydroQual, Inc., HDR|HydroQual, Inc.; A.C. Ryan, HDR | HydroQual; F. Kroglund, Norwegian Institute for Water Research (NIVA); H. Teien, Norwegian Univ of Life Sciences; P. Rodriquez, CIMM; B.A. Stubblefield, Oregon State University / Environmental and Molecular Toxicology; A.S. Cardwell, Oregon State University; W. Adams, Rio Tinto; E. Nordheim,

Aluminum (Al) bioavailability and toxicity to aquatic organisms is strongly affected by water chemistry. In particular, pH, dissolved organic carbon (DOC), and hardness can change the observed toxicity of Al to aquatic organisms resulting in wide variation in observed effects. These types of bioavailability interactions have typically been well-described by the biotic ligand model (BLM) framework in previous applications to cadmium, copper, cobalt, lead, nickel, silver, and zinc. The BLM framework for metals considers how chemical changes can impact the chemical speciation of dissolved metal and the interaction of metal ions with biological surfaces, thereby resulting in changes in metal bioavailability. A review of Al toxicity data, however, shows that concentrations of Al sufficient to cause toxicity are frequently in excess of solubility limitations. Al solubility is strongly pH dependent, with a solubility minimum near pH 6 or 7, depending on temperature and other water chemistry characteristics (e.g., DOC concentrations). Conceptually, the BLM framework should be a valid description of metal bioavailability when toxicity is a result of exposure to dissolved Al, but the mechanistic framework needs to be extended to allow toxicity from a combination of dissolved and precipitated Al. We have developed a modified BLM for Al that considers Al solubility and combines effects due to dissolved and precipitated Al when water chemistry conditions indicate that both forms of Al would be required to reach a specified toxicity effect level. This model determines, for given chemistry conditions, if the solubility of Al is sufficient for Al to accumulate at the biotic ligand (BL) to a critical level associated with a specified toxic effect. In cases where solubility is limiting, a combination of dissolved and precipitated Al is considered. In these cases, a response-additivity calculation is performed to determine the predicted effect concentration that results from both dissolved and precipitated metal. This approach requires a modified BLM parameter list that includes the specification of concentration-response relationships based on Al bound to the BL and on the basis of precipitated Al.

TP188 Relationships between oral absorption efficiency in mammals and nature of elements

Y.T. Le, Radboud University Nijmegen / Environmental Science, Institute for Water and Wetland Research; J.A. Hendriks, Radboud University Nijmegen / Department of Environmental Sciences

Oral uptake is the dominant source for the accumulation of a number of elements, especially metals, in humans. Therefore, it is important to predict chemical accumulation via this uptake route. In current frameworks established in Europe, the US, and many other countries, exposure assessment is carried out by comparing the total daily intake with the tolerable daily intake. However, intake might not represent the actual accumulation level in organisms because of the variability in the absorption efficiency between elements. The bioaccumulation of elements occurs as a balance of uptake and elimination via different pathways. Mechanistic models simulating these fluxes provide more accurate estimations of chemical accumulations. Absorption efficiency is required in these models. We developed a relationship between the oral absorption efficiency collected from well-standardized studies and chemical properties of elements. The model is based on the hypothesis that bioavailability of elements for absorption in the gastrointestinal tract is determined by reactions of the elements with biotic ligands. Moreover, the absorption efficiency is expected to be related to the electron dissociation as ions are readily adsorbed to biotic ligands in the tract. Moreover, the rate of dissociating electrons depends on ionisation energy and is limited by the internal energy. Consequently, absorption efficiency was related to ionisation energy. Approximately 94% of the variability in absorption efficiency between elements collected in ICRP publications could be explained by ionization energy. Additionally, the predicted absorption efficiencies were within a factor of two of the values judged by ICRP. The model also showed good predictive potential in the assessments based on independent data on humans and ruminants. Besides chemical properties of elements, absorption efficiency depends on exposure conditions, particularly chemical forms of intake and diet composition. However, these factors were not covered in the model yet. Despite this advantage, the model provides initial insight into a mechanistic understanding of the relationship between absorption efficiency and chemical properties of elements. Moreover, the model might create progress in human exposure assessment. For example, the predicted absorption efficiency can be used to estimate oral uptake, which then can be combined with other uptake routes to provide reliable estimations of chemical accumulations in mammals.

TP189 Relative Bioavailability Study of PAHs in Coal Tar Pitch of Clay Target Fragments

G.C. Hoeger, ARCADIS / Environmental Science, Assessment and Planning; B.H. Magee, ARCADIS

Thousands of trap and skeet ranges are located across the United States with elevated concentrations of polycyclic aromatic hydrocarbons (PAHs) in surface soil. The source of PAHs at these ranges is the deposition of fragments from clay pigeon targets that are made from coal tar pitch and limestone. Existing literature indicates that the matrix of the coal tar pitch can reduce the bioavailability of PAHs following incidental ingestion; however, no information is available to determine the degree to which the coal tar pitch/limestone matrix of the clay target fragments can affect the bioavailability of PAHs. An oral bioavailability study has been initiated to determine the relative bioavailability of benzo(a)pyrene (BaP) and other potentially carcinogenic PAHs between two treatment groups: (1) diet with soil containing target fragments sieved to < 250 micron to mimic human exposure to site soils, and (2) diet with solvent extracts of sieved soil containing target fragments to mimic the Culp *et al*. (1998) two-year carcinogenesis assay in mice, which will likely serve as the basis for the EPA's new oral slope factor for BaP. Site soil samples have been collected for a pilot study of test article preparation from site soil samples, as well as evaluating in-life and analytical procedures. Soil sample drying and sieving procedures have been tested to determine the appropriate methods for preparing a < 250 micron test article that is representative of site soil conditions for dosing to laboratory animals and preparing extracts for relative bioavailability testing. BaP concentrations ranged from 48 – 91 ppm in the < 250 micron fraction of the soil providing sufficient doses to measure hydroxylated PAH metabolites in urine of test animals with relative bioavailability factors as low as one to ten percent of the administered dose. The relative bioavailability study will use laboratory grade BaP and pulverized target fragments as positive controls. Laboratory method development to quantitate hydroxylated PAH metabolites in rodent urine is underway as is a pilot feeding study with a limited number of animals to provide confirmation of the methodology. The goal of the pilot study is to provide data to modify the final investigation plan prior to implementing the main study. Results to date will be presented.

TP190 Salinity and dissolved organic matter mitigation of Cu toxicity in estuarine environments

J. McGeer, Wilfrid Laurier University / Department of Biology; R. Nasir, J. Cunningham, Wilfrid Laurier University; J. Duncan, Wilfrid Laurier University / Biology; S.D. Smith, Wilfrid Laurier University / Department of Chemistry

In freshwaters the BLM approach has proven to be extremely successful in advancing the understanding of how site specific factors influence the bioavailability and toxicity of metals. However for estuarine waters it is not clear whether the same modelling framework, which integrates geochemistry and physiology to estimate bioaccumulation at the site of toxicity, can be successfully applied. Key uncertainties include the geochemical behaviour of metals in estuarine systems as well as how adjustments in organism physiology across salinity gradients influence the uptake and accumulation of metal. This presentation will focus on the latter of these two uncertainties through an examination of Cu bioaccumulation and toxicity in euryhaline invertebrates (primarily Americamysis bahia). Acute lethality (96 h) and 7 d growth and reproductive development tests were done with mysids across a range of salinities from 5 to 40 ppt and with the addition of dissolved organic matter (DOM). Acute tests demonstrated that toxicity was reduced as salinities increased towards the organism's generally accepted salinity optimum (20-25 ppt). Above 30 ppt the toxicity of Cu was increased but this was associated with an osmoregulatory disruption induced by the high salinity. Acclimation of cultures to either 15 or 25 ppt and subsequent testing at a range of salinities illustrated that mysids are able to adapt rapidly to changes in salinity and responses to Cu were not significantly different. In 7 d growth and development tests the impact of Cu was greater at lower salinity but this was only evident for survival (e.g., the LC50 at 15 ppt was approximately 50% of the LC50 at 25 ppt). Interestingly the effect of Cu on growth was not influenced by salinity, those that survived at 15ppt grew as well (relative to controls) as those at 25 ppt. DOM provided protection against Cu toxicity that was concentration (up to 10 mg DOC/L) and source dependent. Cu bioaccumulation was characterized (whole body dry weight basis) to validate the theoretical underpinning of the BLM (LA50 values) and its applicability in estuarine conditions. This research is funded by NSERC through a CRD grant in partnership with ICA, CDA, ILZRO, NiPERA, IZA, Teck, Vale and Xstrata Zinc.

TP191 Seawater and sediment nickel toxicity to amphipods and copepods under controlled DOC and sulfide conditions

T. Chandler, University of South Carolina / Department of Environmental Health Sciences; K. Washburn, University of South Carolina / Environmental Health Sciences; L. He, University of South Carolina; C.E. Schlekat, NiPERA; E.R. Garman, NiPERA / Ecotoxicologist; J. Ferry, University of South Carolina / Dept. of Chemistry and Biochemistry

Sediment biogeochemical controls on nickel toxicity to two marine crustaceans were evaluated using partial to full lifecycle bioassays in silt:clay organic-rich sediments and, for comparison, in seawater alone. This study used novel drip-through sediment microcosms to measure nickel uptake and toxicity to *Leptocheirus plumulosus* and *Amphiascus tenuiremis* in the context of life history/stage and sediment use. In sediment-free partial lifecycle exposures, juvenile *A. tenuiremis* are 2-3 times more sensitive to nickel than juvenile *L. plumulosus* even though similar in body size. As body size increases with *L. plumulosus* growth (max. size = 1.5 cm), the disparity in species nickel sensitivity increases in seawater alone, but for more typical sediment-associated exposures, nickel differential toxicity was attenuated and more dependent on multiple interactions of biogeochemistry and organismal ecology. Both species experience similar but importantly different routes of sediment and porewater metal exposure influenced by feeding and lifestyle ecologies. In this study both species were exposed over the lifecycle to 4% OC sediments incubated and rolled for 95 days under anoxic conditions to equilibrate divalent nickel with e.g., sulfides, carbon, Fe:Mn, and other moieties. Resulting porewater nickel concentrations were near seawater background in most treatments at the start of bioassays and monitored weekly for 28 days for change relative to [AVS], [DOC] and bioturbation. Bioassays were run flow-through with ~100 turnovers per day. Porewater nickel concentrations in sediments up to 740 ug-Ni/g-dry sediment remained low (< 150 ug-Ni/L) across a five concentration exposure series that, based on short-term sediment incubations, should have proved highly toxic to both species. In seawater-only exposures, DOC amendments at measured porewater field concentrations (55 ng-DOC/mL) sharply attenuated lifecycle nickel toxicity to copepods in microplate assays (survival and development). Sediment assessment of divalent metal toxicity should consider long-term anoxic incubation of sediment:metal spikes prior to bioassay of muddy organic-rich sediments in order to achieve higher realism to long-term bedded sediments typical of suboxic estuarine basins. Short incubations under oxic conditions likely stimulate porewater nickel release and may over estimate nickel bioavailability and toxicity to these and similar infauna.

Risk Management, Remediation, or Science Policy

TP192 A Proposed Framework for an Environmental and Toxicology Assessment of an Unleaded Piston Engine Aviation Fuel

G.M. Huddleston, Cardno Entrix; J.H. Rodgers, Clemson University / School of Agricultural, Forest, and Environmental Sciences; A.D. McQueen, Clemson University

A preliminary framework was developed for an environmental and toxicology assessment of an unleaded fuel for piston engine aircraft. Detailed Federal Aviation Administration guidelines for conducting environmental and toxicology assessments for piston engine aviation fuels do not currently exist, so this assessment provided a new proposed framework. The purpose of the assessment was to characterize the relative environmental (human health and ecological) risks associated with a new unleaded 100 octane aviation fuel. The fuel is a mixture of pure hydrocarbons, and is undergoing evaluation to replace or provide an alternative to the commonly used leaded aviation gasoline (avgas). Currently, 100LL avgas contains tetraethyl lead, ethylene dibromide, and multiple hydrocarbon components that vary depending on petroleum source and refining process. This assessment compared the relative risks from exposure to the two fuels in the workplace and environment. Due to the limited availability of data on the aviation fuels, certain assumptions regarding the environmental fate and potential toxicity of the fuels were made using current information on the fuel components and best professional judgment. This environmental and toxicology assessment was based on USEPA ecological and human health risk assessment guidelines. The assessment concluded that adoption of the unleaded fuel would pose less risk for human health and wildlife than continued use of leaded avgas, driven primarily by the toxicity of tetraethyl lead and ethylene dibromide. This environmental and toxicology assessment framework may be formalized

and applied toward evaluation of other new piston engine aviation fuels as they are developed for the aviation industry.

TP193 Biodegradability of human pharmaceuticals by activated sludge from sewage treatment plants in Tokyo

T. Suzuki, Department of Pharmaceutical and Environmental Sciences; Y. Kosugi, M. Hosaka, D. Nakae, Tokyo Metropolitan Institute of Public Health; T. Nishimura, Teikyo Heisei University

Effluents of sewage treatment plants (STPs) are main sources of human pharmaceuticals and personal care products (PPCPs) in urban rivers in Tokyo. In order to refine the predicted environmental concentration of PPCPs on the environmental risk assessment, the estimation of the degradability of PPCPs at STPs is necessary.Target PPCPs were selected to be sulpyride, amantadine, epinastine, carbamazepine, lorazepam, candesartan, and propranolol, because they have been detected in urban rivers in Tokyo. The biodegradability of PPCPs was examined according to OECD TG 314B. The concentrations of the activated sludge and PPCPs were set as 1 g/L and 5 micro-M, respectively. 1-naphtol is used as reference compound. The removal rate of 1-naphtol after a 5- day incubation was 87% under the laboratory conditions, and its estimated half-life (T1/2) was 2 days. This suggested that this biodegradation system is adequate to test biodegradability of chemicals. Under this laboratory conditions, removal rates of propranolol and candesartan after a 5- day incubation were 25 and 7%, respectively. The other PPCPs were not degraded after a 5-day incubation. The removal rates of these PPCPs at STPs in Tokyo were as follows; sulpyride 15%, amantadine 6%, epinastine 7%, carbamazepine 77%, lorazepam 0%, candesartan 34%, and propranolol 0%. Differences seen among carbamazepine, candesartan, and propranolol depended by conditions of the biota and its acclimatization in activated sludge, and the composition of degradation systems.Further biodegradability study in the laboratory conditions is necessary in order to confirm suitability of the conditions.

TP194 Characterization of the relationship between unregulated PAHs in aquatic organisms and passive sampling devices

L.B. Paulik, Environmental and Molecular Toxicology; G.R. Wilson, N.D. Forsberg, Oregon State University / Environmental and Molecular Toxicology; K.A. Anderson, Oregon State University / Environmental & Molecular Toxicology

Benzo[c]fluorene is not currently regulated, but it has the third highest relative potency factor (RFP) in the US Environmental Protection Agency's 2010 Integrated Risk Information System (IRIS) document. Preliminary analysis of crayfish collected in the Portland Harbor Superfund site shows the presence of benzo[c]fluorene and other unregulated polycyclic aromatic hydrocarbons (PAHs). Superfund remediation of a contaminated environmental site is often a long and expensive process. Investigators need efficient, reliable tools to expedite these efforts. Traditional methods of sampling aquatic organisms are resource and time-intensive, and collecting organisms can have additional negative impacts on the ecosystem. Lipid free tubing (LFT) passive sampling devices (PSDs) are designed to mimic the uptake of the bioavailable fraction of contaminants in organisms. When deployed at aquatic sites, LFT provide information about PAH concentrations in aquatic organisms, including benzo(c)fluorene. This study investigates the ability of LFT to reliably and precisely mimic contaminant concentrations in crayfish, *Pacifastacus leniusculus*, at Superfund sites in the Pacific Northwest, United States. Previous research suggests that PAH concentrations in LFT can be used to predict concentrations in crayfish. This study employs a novel analytical method using GC-MS to quantify over 60 PAHs, including 23 of the 26 PAHs identified in the 2010 IRIS document. A model relating concentrations in LFT to concentrations in aquatic invertebrates would enable investigators to replace collecting organisms with deploying LFT, drastically reducing the cost and time associated with characterizing concentrations in aquatic organisms.

TP195 Deriving Generic Soil Preliminary Remediation Goals and Remedial Action Levels for the Protection of Terrestrial Ecological Receptors

L.H. Judd, ERM; S.C. Peterson, Environmental Resources Management (ERM); K. Fletcher, ERM

With the promulgation of Ecological Soil Screening Levels (Eco-SSLs) by USEPA, procedures for addressing terrestrial ecological risk as a component of State or Federal site remediation projects have become increasingly standardized in recent years. Whereas the Eco-SSLs are a convenient tool for generic use to identify and manage ecological risk issues, according to USEPA, it is inappropriate to use the Eco-SSLs as cleanup standards. However, no acceptable alternatives are offered. In many cases, either for reasons of cost or time, defining appropriate ecological preliminary remediation goals (Eco-PRGs) to address ecological risk and ensure the long term protection of terrestrial ecological receptors must be accomplished in the absence of site-specific information on constituent toxicity or bioavailability. This paper outlines an approach for using the Eco-SSLs to derive generic Eco-PRGs for soil, and further provides an approach to demonstrate compliance with these goals. For avian and mammalian receptors, Eco-SSLs are based on the geomean of peer-reviewed no observed adverse effect levels (NOAELs) for growth and reproduction across all test species. Similar to other published reports, generic Eco-PRGs may be based on the geomean of the NOAEL and the lowest observed adverse effect level (LOAEL). Important considerations in deriving the Eco-PRGs include the bounding of potential effects between the NOAEL and the LOAEL, and the selection of appropriate effect endpoints and indicator species. Using this methodology, generic Eco-PRGs are tabulated for common constituents in soil. Compliance with generic Eco-PRGs focuses on the derivation of a remedial action level (RAL), which is defined as the maximum concentration that can be left in place within a study area that would result in average exposure to soils (estimated by the arithmetic average or an upper confidence limit on the mean) equal to or less than the generic Eco-PRG. An example of the application of this methodology is provided.

TP196 Developing Guidance for Alternative Chemical Assessments

J.Y. Tanir, HESI

The ILSI Health and Environmental Sciences Institute (HESI), a global branch of the International Life Sciences Institute (ILSI), currently has a sustainability-focused project on alternative chemical assessments. With a vision of creating science-based solutions for a sustainable, healthier world, HESI recognizes sustainability as vitally important as it identifies and resolves global health and environmental issues through engagement with scientists from academia, government and industry. Alternatives assessment is a process for identifying and comparing potential chemical and non-chemical alternatives that can be used as substitutes to replace chemicals or technologies of high concern. Generally, alternatives assessment compares human health and safety, environmental health, lifecycle thinking, as well as social, economic, and technical performance factors. In the fall of 2011, HESI initiated the Emerging Issues Subcommittee on Frameworks for Alternative Chemical Assessment and Selection of Safer, Sustainable Alternatives. The mission of the project is to evaluate and identify key elements/criteria and tools to help trigger and guide the selection of safer, sustainable alternatives while minimizing the likelihood of regrettable substitutions. A workshop was held in February 2013 which focused on developing guidance on three aspects of alternatives assessment: 1) Attributes and tools, 2) Decision-making and weighing and 3) Data gaps. A summary of the guidance and recommendations developed by the subcommittee will be presented.

TP197 Environmental Damages Associated with Wildland Fires

S. Semenova, Exponent; T.L. Deardorff, Exponent; C.A. Menzie, Exponent

"Intangible" environmental damages associated with wildland fires in California present a category with the widest range of potential liability, where no quantification technique currently exists for use in fire litigation. Recent cases show that "intangible" damages could range from $18/acre to $1,600/acre of burned forest for similar fires. A careful analysis of environmental conditions prior and post the fire is necessary to determine a reasonable amount of the damage claim on the scale of financial liability. We identified major claim categories for which monetary damages are typically recovered and categorized them into fire suppression, environmental (market-based), environmental (non-market), and interest/ penalties. Our "scaling approach" for estimating environmental damages associated with wildfires is based on the premise that there is a relationship between the magnitude of damages and the pre-fire non-market resource values provided by the forest. We developed a scale (in dollars per acre) for "intangible" environmental damages that reflects a wide spectrum of environmental characteristics from poor pre-fire conditions due to prior burns of the area and invasive species to very valuable conditions associated with presence of threatened/endangered species.

TP198 Environmental risk minimization method based on lifecycle risk assessment and alternative assessment for HBCD in products

S. Managaki, Musashino University; T. Kobayashi, Yokohama national university / Graduate School of Environment and Information Sciences; H. Hondo, Yokohama national university; T. Nakarai, Yokohama national university / Graduate School of Environment and Information Sciences; A. Miyake, S. Masunaga, Yokohama national university

In order to minimize environmental risk and to select better a chemical among alternatives, grasping various risks all through the life stage is very important. In this research, we are developing a material flow based comparative assessment method by studying on a brominated flame retardant, hexabromocyclododecane (HBCD) as a case. First, we constructed a material flow of HBCD in Japan through its life cycle. By adopting emission factors, environmental emissions of HBCD were estimated for every life stage. Also, in the case of no data available (e.g., emission from incineration process and fire), empirical researches were conducted to support the predicted emission. Then five potential alternative flame retardants (FRs) were identified for the major HBCD use categories, namely expanded polystyrene (EPS), extruded polystyrene (XPS), curtain and car fabric. Environmental emissions and exposure were estimated for those alternatives and compared with those of HBCD. The results showed that considerable amount of HBCD will be stocked as consumer products (26,000 ton in 2020) due to their long life time, in spite of the introduction of alternatives and indicated that products containing HBCD would be a potential emission source for a long time. The results also indicated that replacement of HBCD would reduce FR emission from polystyrene foam, while it would slightly increase FR emission from curtains. As a next step, we compared products with HBCD with other products that have similar functions without use of HBCD. Products selected were glass wool and urethane foam as thermal insulating material and flame retardant yarn as a material of textile. Comparison in flame retardant emssion, CO_2 emission, landfill space demand for disposal, and price indicated some alternative products have good prospect of reducing enviromental impact. This study showed life-cycle assessment of both risk and other environmental impacts, although it has large uncertainties, would provide useful information for selecting a better option among possible alternatives.

TP199 Is Adsorbed Contaminant "Remediable"?

J. Wang, Department of Environmental Sciences; J. Gan, University of California Riverside / Environmental Sciences

Chemical treatments, including especially oxidation or reduction-based reactions, have been frequently used to remove contaminants during soil remediation. It is known that adsorption of the target contaminant to soil generally decreases the treatment efficiency. In this study, we quantitatively explored the effect of adsorption on chemical remediation effectiveness, and derived a simple model to predict the effect of adsorption on contaminant removal. The previously well-characterized reductive dehalogenation reaction between halogenated pesticides and thiosulfate was used as a model reaction. A soil was amended with activated carbon at different levels, and adsorption coefficients K_d were then measured in the amended soils for 1,3-dichloropropene, methyl bromide, methyl iodide, and chloropicrin. The same soils were treated with these chemicals, and then spiked with thiosulfate to initiate the chemical remediation. Degradation of fumigants consistently decreased in the soil as K_d increased. Regression analysis of the first-order degradation rate constant μ and K_d showed that degradation was almost entirely attributable to the reaction in the solution phase, and that degradation of adsorbed fumigant by thiosulfate was essentially zero, suggesting that adsorbed chemical is not "remediable". The effect of K_d on the chemical removal may be predicted using a factor of $1 + (\varrho/\theta)K_d$. That is, if the reaction rate of a chemical in the solution phase is known as μ_L, the actual degradation of the chemical in a soil would be $[1 + (\varrho/\theta)K_d]\mu_L$. This relationship may be used for predicting the effectiveness of chemical remediation in soils or sediments.

TP200 Making Product and Chemical Safety Decisions from Varying Stakeholder Viewpoints

K.M. Hitchcock, Cardno ChemRisk; E. Shay, Cardno ChemRisk; J. Panko, Cardno ChemRisk

With sustainability and product stewardship issues entering public dialogue, many product and chemical assessment tools have become available. These tools can be used by a variety of stakeholders such as consumers, product formulators, and risk assessment professionals when deciding which is the most environmentally preferred chemical alternative or product formulation. The methods used (hazard-based vs risk assessment) and the type of results generated by the tools (numerical score vs. pass/fail) differ. As such, it becomes difficult to compare the results generated among the various tools. Additionally, the potential exists for the stakeholders to make differing decisions based on the tools they chose. Using a household cleaner with a publically available MSDS, we compared three tools representing three stakeholder groups – GreenScreen (product formulators), Ecetoc TRA (risk assessment professionals) and GoodGuide (consumers). Here we compare and contrast the information available, the methods used, and the results generated by these three tools. In addition, we discuss the data needs and solutions for filling the gaps among these three viewpoints.

TP201 Maximum Contaminant Levels (MCLs): Not Always the Appropriate Remedial Goal for Groundwater

B.J. Locey, J. Smith, ARCADIS / ESAP; T. Rubis, ARCADIS / Great Lakes; P. Kurzanski, CSX Transportation, Inc / Environmental Remediation

Many state environmental programs regulate groundwater assuming it is a useable drinking water resource and use the federal maximum contaminant levels (MCLs) as screening levels to determine whether measured concentrations are acceptable. MCLs may serve as good screening levels; however, they are not based on the same considerations as risk-based screening levels used in environmental programs. They may not always be the most appropriate final cleanup goal. MCLs are legally binding standards for potable water supplies and are derived consistent with federal Safe Drinking Water Act (SDWA) requirements with consideration of the cost, feasibity and benefit of implementing in public drinking water systems that serve communities across the US. MCLs are not all risk-based, not consistently set at the same level of protectiveness, and some are set based on the best available treatment technology. If the science underlying the original assessment changes because of new study data or approaches recommended for evaluating risks and hazards, the MCL may not change if there is no benefit to making the change for regulated systems. For example, the MCL for 1,1-DCE was developed in 1987. Initially, this assessment served as the basis for the USEPA Integrated Risk Information System (IRIS) assessment; however, the IRIS assessment was revised in 2002. The MCL is now significantly lower than the health-based concentration that could be derived using current toxicological information, including the USEPA's Regional Screening Level (RSL) for Tapwater. The Office of Drinking Water (ODW) has updated the Health Advisories for 1,1-DCE, but has not revised the MCL. When the MCL was evaluated for revision, it was not frequently detected in regulated waters, and when detected, it was at very low levels. The ODW determined there was no benefit to revising the MCL. However, the MCL continues to be used today in environmental programs where these considerations are not relevant. Many state environmental programs use MCLs as screening levels, however in some, these are promulgated and there is no option to base the final cleanup goal on the best available science. This poster will provide an overview of the process for developing and revising of MCLs compared to development of risk-based screening levels, provide a detailed comparison for 1,1-DCE, and an overview of regulation in several states.

TP202 Natural Attenuation Rates of Cutting Oil and Methanol in a West Texas Stream

A. Pawlisz, Conestoga-Rovers & Associates; D.W. Smith, CRA, Inc.

Catastrophic releases of hazardous materials into rivers and streams as a result of transportation accidents necessitates an appropriate and commensurate emergency spill response and cleanup. Of particular challenge is finding the right balance between aggressive, and often habitat-destructive, source material removal and what can be left in place via natural attenuation processes. Information on the type of chemical, its physicochemical properties, and receiving water body characteristics can be used to predict its fate and attenuation rates. However, the level of confidence in this prediction may be insufficiently low to base costly remediation decisions upon. This presentation discusses the results of extensive and prolonged analytical monitoring efforts associated with a spill of cutting oil and methanol into a west Texas stream. Levels of both constituents in sediment and water are tracked over time and the associated data are used to calculate natural attenuation rates. Site-specific half-lives are compared to those from literature and fate models to gauge the relative effectiveness of the existing predictive tools. A discussion is provided as to the appropriate remediation strategy for the case study site and others like it.

TP203 PCBs: Old and New Sources

H. Davies, Washington Department of Ecology / Waste 2 Resources; B. Morrissey, Washington State Department of Health; D. McBride, Washington State Dept. of Health; J. Grice, Washington Department of Ecology / Waste 2 Resources Program; A. Stone, Washington Department of Ecology / Chemist; D. Norton, Washington State Dept of Ecology / Environmental Assessment Program; K. Patora, Washington Dept. of Ecology / Economic Analysis Department; G. Palcisko, Washington State Dept. of Ecology; D.A. Delistraty, Washington State Department of Ecology; A. Borgias, C. Niemi, J.W. White, Washington State Dept. of Ecology

Washington State addresses persistent, bioaccumulative and toxic chemicals (PBTs) using chemical action plans (CAPs) and the most recent CAP focuses on polychlorinated biphenyls (PCBs). PCBs are distributed widely throughout Washington. For humans, the primary exposures to PCBs are from food (e.g., fish). For fish and wildlife, exposure is linked to both diet and abiotic media (e.g., sediments which serve as an environmental sink). The overall objective of the CAP is to reduce exposures to PCBs, thereby protecting human health and the environment. The final report will contain recommendations for a series of initial actions that address the worst sources of exposures, and options for future actions. How important is new generation of PCBs compared to legacy PCBs? Current exposure is derived from both old and new sources. PCBs were produced for commercial uses from about 1929 to 1977. The largest use of PCBs was for heat transfer fluids in electrical transformers and capacitors. PCBs were also used as plasticizers, flame retardants, wax and pesticide extenders, and lubricants. Many products used to contain PCBs at high levels (e.g., caulk used to seal cracks in homes and buildings). The 1976 Toxic Substances Control Act (TSCA) limits PCB uses and added restrictions on how equipment that contains PCBs must be maintained and disposed. Washington has additional state laws on disposal and cleanup. Since their phase-out, levels of PCBs in the environment have dropped dramatically. PCB exposures to continue however from PCBs circulating through the food web; from indoor PCB emissions from aging fluorescent light ballasts, caulk and joint sealants; and from generation of new PCBs. Inadvertent production of PCBs during manufacturing of chemicals (e.g., dyes and pigments) continues to be an ongoing source. TSCA largely restricts PCBs to less than 50 ppm, with inadvertent generation limited to an annual average of 25 ppm. These were seen as low levels at the time, but these levels are now known to be problematic.

TP204 Practical and Realistic PRGs

C. Julias, CDM / Chemical Engineer; N. Luke, CDM Smith Inc.

In feasibility studies (FS), to meet the remedial action objective for a specific site, chemical-specific preliminary remediation goals (PRGs) are developed to aid in defining the extent of contaminated media requiring remedial action. PRGs are based on applicable or relevant and appropriate requirements, risk-based levels (human health and ecological), and regional and site background concentrations, with considerations to analytical detection or reporting limits. Typically, PRGs are calculated by taking a risk equation and rearranging it to solve for the concentration of a particular chemical that corresponds to a specified level of risk for potential receptors at the site. These risk equations are used in human health and ecological assessments, which utilize data gathered during the remedial investigation (RI). Very often human health risk-based PRGs drive site soil cleanup levels; while sediment PRGs are typically ecological risk-based. Essentially, PRGs provide the basis for remedial goals and objectives selected by engineers and risk managers to develop potential remedial alternatives. Thus, strategic planning throughout the RI for FS purposes is critical to identify the cost and benefit for performing additional investigations to develop site-specific PRGs. Additional investigations may derive more realistic and practical PRGs to lower remediation cost. Thus, it is critical for risk assessors to present to risk managers the benefits in performing additional investigations which may take additional efforts up front, but in the long run, it will produce more cost effective remedial alternatives.

TP205 Risk Based Evaluation of the Chemical Constituents in Hydraulic Fracturing Fluid Using the Chemical Disclosure Registry FracFocus

M. Tosiano, J. Panko, P. Scott, Cardno ChemRisk

The rapid development of the Marcellus shale and other unconventional natural gas resources has been driven by more efficient extraction technologies such as hydraulic fracturing and horizontal drilling. The public is concerned by the toxicological risks associated with components of HF (hydraulic fracturing) fluid. The compositions of HF fluids in Pennsylvania, Ohio, and West Virginia were downloaded from FracFocus, a public disclosure website. Chemicals of interest (COI) were identified as those reported in at least 5% of wells, and present on at least one of the following hazardous chemical lists: IARC Class 1 or 2 carcinogen, California Proposition 65 list, EU REACH Candidate List of Substances of Very High Concern for Authorization (SVHC), Priority Persistent Pollutant List for Oregon, USEPA Regional Screening Level (EPA RSL) for tap water, USEPA National Primary Drinking Water Regulations (NPDWRS) Maximum Contaminant Level (MCL). Of the 185 unique chemicals that were identified, only 10 were identified as COI according to the prior criteria. Interestingly, the chemicals with the highest reporting frequency and greatest percent mass in the HF fluids, namely hydrochloric acid and hydrotreated light petroleum distillates (64742-47-8) did not qualify as COI because of their absence from hazardous chemical lists. The COI identified in this analysis are not necessarily chemicals of concern (COC); rather they should be evaluated further to understand their fate and transport properties in the environment as well as any potential environmental degradation byproducts. Additionally, of the chemicals in the FracFocus dataset 27 were listed on EPA's Design for the Environment (DfE) Safer Chemicals list including 6 of the top 20 by mass. Although much criticism has been directed at well operators and HF fluid suppliers because of non-disclosure of confidential chemicals, this dataset indicated that less than 1% of the mass of the HF fluid in the combined FracFocus dataset was associated with protected CBI information. While this evaluation was not meant to be a complete risk assessment of HF fluid, it demonstrates that traditional environmental risk assessment techniques are applicable to HF fluids and if chemical disclosure in FracFocus database were more complete, potential environmental risk could be properly evaluated and managed.

TP206 Toxicity of jet fuel in High Arctic, Greenland soils to soil invertebrates and microbial communities

N. Marleau; C. Boyle, R. Nhan, University of Saskatchewan; S.D. Siciliano, University of Saskatchewan / Toxicology Group and Department of Soil Science

Little is known about polar soil toxicity on Greenland. Here, we investigated the toxicity in two Greenland soils inadvertently contaminated with jet fuel. Jet fuel, a type of petroleum hydrocarbon (PHC), can have toxic effects on soil invertebrate communities depending on soil characteristics and degree of PHC contamination. Soil invertebrate communities are primary indicators of soil health and productively in these High Arctic soils. Greenland's soil invertebrate community structure and their response to PHC contamination is unknown, therefore we assessed jet fuel toxicity on soil toxicity test organisms found in Arctic soils. Toxicity tests were performed with soils from two different sites using four soil invertebrates with test durations for each as follows; oribatid mites (*Oppia nitens*) – 4 weeks, collembola (*Folsomia candida*) – 4 weeks, nematodes (*Caenorhabditis elegans*) – 24 hours, and enchytraeids (*Enchytraeus crypticus*) – 6 weeks. These four different invertebrates are representative of Greenlandic soil invertebrate communities and different trophic levels within the soil. Mite reproduction in these low organic carbon soils required the addition of vermiculite whereas other soil invertebrates were viable and reproduced in these harsh, High Arctic soils. The overall objective was to develop a recommendation for a site specific remediation objective for these soils and this will be presented.

TP207 Updates to EPA's AIM and ChemACE Computer Tools; New Algorithms for Identifying Analogs for Metals, Inorganics, and Organometallics

J.L. Tunkel, SRC, Inc. / Environmental Health Analysis; K.E. Mayo-Bean, USEPA / OPPT

The USEPA Office of Pollution Prevention and Toxics (OPPT) is responsible for implementing the Toxic Substances Control Act (TSCA). TSCA is the US law that regulates industrial chemicals in the US and OPPT evaluates both new chemicals entering commerce as well as existing chemicals that are already in commerce. In evaluating new and existing chemicals over the past 35+ years, OPPT has developed a strong knowledge base in structure-activity relationships (SARs), and is continually seeking ways to enhance these methodologies. OPPT has recently developed a set of tools that draw upon the well-established atom-fragment approaches used in these SARs to aid in identifying analogous chemicals to help fill data gaps and aid in the characterization of potential hazards. The Analog Identification Methodology (AIM) tool (www.epa.gov/oppt/sf/tools/aim.htm) allows a user identify structurally similar chemicals that have human health toxicology

data available in the public domain. The new Chemical Assessment Clustering Engine (ChemACE) – incorporates the AIM engine and highlights analogous chemicals for potential read across by organizing a user-defined list of chemicals into 'clusters of analogs.' This allows one to evaluate chemical space based on structure, properties, mechanism, and other factors. The ChemACE output is designed to determine if available data on physical-chemical properties, environmental fate, mammalian toxicology, and other endpoints are appropriate for some, all or none of the chemicals in a cluster. Recently, both the AIM and ChemACE methods have been updated with new algorithms that provide additional options for selecting analogs for inorganics, metals, and organometallic compounds. For metals and non-dissociating organometallics, new structural fragments and selection routines allow users to identify analogs based on either an exact element match (restrictive), or one that incorporates all other members of the same period (less restrictive). For dissociative organometallic species, these tools share the ability to identify analogs or analog clusters based on either the organic ion, the metallic ion (including other members of the period), or both. This poster will provide detailed information on these new methodologies, as well as case studies where they have been successfully utilized to derive hazard designations based on an analog approach.

TP208 Use of Chemical-Specific Relative Source Contribution Factors for Calculation of Ambient Water Quality Criteria

E.L. Osborn, Arcadis US, Inc.; D. Pfeiffer, Arcadis US, Inc.; P.D. Anderson, Arcadis US, Inc.; R. Stewart, Florida Pulp and Paper Association Environmental Affairs, Inc.

USEPA (2000) guidance recommends the use of a relative source contribution (RSC) factor to account for non-ambient exposures when deriving ambient water quality criteria (AWQC) for non-carcinogens. In general, RSCs have not been used to date when establishing AWQC. However, recent indications from USEPA suggest that RSCs will be used more widely as AWQC are revised in the near future. RSCs can be based on chemical-specific information or on an arbitrary default value of 0.2 (sometimes 0.5) when data or resources are not available to derive reliable quantitative estimates for all (surface water and non-surface water) relevant exposure pathways. However, if exposure estimates are available for all non-surface water related exposure pathways, the remaining exposure below the allowable daily intake or exposure (typically the reference dose, RfD) can be conservatively allocated to surface water sources. In certain cases, use of the default value can be demonstrated to be overly conservative, even though reliable exposure data from surface water sources is not available. This paper presents the calculation of chemical-specific RSCs for five compounds (beryllium, methyl bromide, 1,1-dichloroethene, chloroform, and diethyl phthalate) selected to represent a variety of chemical classes. All five chemical-specific RSCs are substantially greater than the default RSC of 0.2. Using the chemical-specific RSCs results in AWQC that are 2- to 4-fold greater than AWQC derived using the default RSC. Developing chemical-specific RSCs is not necessarily time or resource intensive, and can often be completed with information available in the literature. Use of chemical-specific RSCs has the potential to substantially affect AWQC, currently undergoing revision in several states.

TP209 Using Mercury Stable Isotopes to Assess Sources of Mercury Exposure in Humans

M. Li, University of Michigan / School of Natural Resources and Environment, Harvard School of Public Health / Environmental Health; L. Sherman, J. Blum, University of Michigan; E.M. Sunderland, Harvard University; J.P. Shine, Harvard School of Public Health / Department of Environmental Health

Methylmercury (MeHg) causes a variety of adverse health effects in humans and exposure for most individuals is mainly from seafood consumption. This study uses Hg stable isotopes for tracing MeHg exposure sources at an individual level and evaluates the utility of this method for estimating fish consumption pattern. Here we compare mercury stable isotope signatures in human hair and the seafood consumed by different individuals to assess sources of mercury exposure. Our analysis compares isotopic data in hair from two populations: 1) Faroese whalers whose MeHg exposure was mainly dietary intake of pilot whales; 2) Gulf of Mexico fishermen separated into several groups according to their seafood consumption preferences. Previous studies have shown that Hg stable isotope ratios are distinct between coastal and pelagic fish. We measured human hair samples to explore mass-dependent (MDF) and mass-independent fractionation (MIF) between diet and human biomarkers. The results indicate that MDF occurs within the human body (likely during MeHg demethylation) and results in an offset of ~1.75‰ in δ^{202}Hg between the consumed seafood and the human hair samples. Faroese hair samples demonstrated a narrow range of Δ^{199}Hg values which are very similar to pilot whales they consumed. This confirms that they receive MeHg from the single food source—pilot whales with a narrow range in Δ^{199}Hg values. Among the Gulf of Mexico fishers, results from an isotopic mixing model show that mercury isotopic compositions in hair are well matched with reported consumption patterns, with the exception of individuals who primarily consumed coastal fish. We propose that recall bias in fish consumption surveys provides a possible explanation for this discrepancy. Our data indicate that no MIF but significant MDF occurs during metabolic processes within the human body. We conclude that MIF can be used as a robust tracer of MeHg exposure via seafood consumption and suggest mercury stable isotopes are a powerful tool for discerning Hg exposure sources for individuals.

TP210 Biodegradation of Quaternary Ammonia Compounds by Biofilm and Free-Living Bacteria

Z. Ahmed, M.L. King Academic High School; T.D. Byl, US Geological Survey / Department of Interior

Quaternary Ammonia Compounds (QAC) are ubiquitous and commonly used in industrial, domestic, agricultural, and healthcare related fields, QACs are used in surfactants, fabric softeners, disinfectants and even pesticides. One consequence of its widespread use, however, is that 75% of these compounds are discharged into wastewater and little is known about their environmental fate or consequence to ecosystems. Despite their strong bacteriocide characteristics, QACs are vulnerable to the process of biodegradation. The objective of this project was to determine the role of surface area and biofilm development on biodegradation rates of QACs (didecyl dimethyl ammonium chloride and dimethyl benzyl ammonium chloride). To achieve this objective, bacteria resistant to QAC's were collected from a White-Nose Syndrome decontamination station at MammothCaveNational Parksaturated with QACs in 2012. An experiment was designed to test whether bacteria provided with approximately 2.6 times (527 cm^2) more surface area for biofilm development were more efficient at biodegrading QACs than free-living bacteria. There were 3 replicates per treatment. All microcosms received 250 mL of the same solution containing QAC and bacteria. The microcosms were allowed to equilibrate for 4 weeks to account for sorption and biofilm development. The QAC concentrations were measured through time. The microcosms with high surface area and biofilm development had a QAC half-life of 11 days. The microcosms without surface area for biofilm development had a QAC half-life of 37 days. Furthermore, the microcosms with high surface area tended to have lower standard deviations, suggesting they had a more predictable biodegradation process. The results indicated that surface area for biofilm development plays an important role in the rate of QAC biodegradation.

TP211 The Benefits of Using a Clustering Approach to Assess the Safety of Chemicals Used in Consumer Products

C. Rudisill, SRC, Inc.; L. Morlacci, SRC Inc.; M. Adams, USEPA / Office of Chemical Safety and Pollution Prevention, Design for the Environment; C. Baier-Anderson, USEPA / Design for the Environment; B. Drake, E. Sommer, USEPA / Office of Chemical Safety and Pollution Prevention, Design for the Environment; J.L. Tunkel, SRC, Inc. / Environmental Health Analysis; K.E. Mayo-Bean, USEPA / OPPT

Conducting hazard assessments for a large number of chemicals can be a difficult and time-consuming process, particularly when many of the chemicals under evaluation have critical data gaps. It is essential that company claims and regulatory decisions made in regards to product safety are scientifically-justified, but it is also imperative that the analyses used to support these claims are conducted within time and budget-constraints. When experimental data or QSAR models are inadequate, using structural analogs to evaluate data-poor chemicals can offer a reliable indication of hazard for these chemicals during prioritization exercises, alternatives assessments, and identification of testing needs. However, the narrow-approach of "one analog to evaluate one chemical" can lead to inconsistent and inaccurate results. Assessing hazards in the context of small groups ("clusters") of structurally-related chemicals increases overall confidence in the hazard calls assigned to these chemicals. This approach also lends to the identification of structural attributes that may influence toxicity (e.g., potential for skin sensitization or decreasing mutagenic potency across a series). The use of tools, such as

EPA's Chemical Assessment Clustering Engine (ChemACE), can save time and effort during the initial grouping exercise. Several USEPA programs, including the New Chemicals Program and Design for the Environment, use clustering approaches to assess chemical hazards. Cluster analyses are frequently implemented in chemical categories submitted to the USEPA HPV and OECD SIDS programs for the purpose of filling data gaps. This approach could be of further help for companies that are conducting hazard assessments under their own internal sustainability initiatives. This poster will provide examples of how clustering approaches are being implemented and how they contribute to identifying "best in-class" chemicals in regards to ecological, environmental fate, and human health contexts. The general characteristics of an effective clustering methodology will also be discussed.

Transitioning Nanosafety Science to Solutions

TP212 NanoEcoToxOntology

D. de Zwart, RIVM; H. Marvin, RIKILT; R. Lokers, Alterra; D. van de Meent, RIVM / Institute of Wetland and Water Research

In the Dutch research program NanoNextNL, two parallel information systems are being developed to support policy decision making regarding nanomaterials: one for human hazard ranking, and one for environmental risk ranking. The two systems share one knowledge base, in which relevant physical, chemical and toxicological information on nanomaterials in their various possible surroundings are stored. The two systems partly share knowledge rules, from which inferences are made of hazard and risk, based on (combinations of) measured characteristics. The data system uses a new (eco)toxicological ontology, adapted from the nanoparticle ontology for cancer research. In this poster, we report on the progress made for the environmental risk information system. We have built on RTI's Nanomaterial Registry, expanding it with ecotoxicologically relevant particle characteristics, making use of recently acquired insights in relationships between particle characteristics and nanoparticle-specific environmental process rates. As more information on fate and effects of specific nanomaterials in specific surroundings is collected in the system, the system trains itself to produce more and better inferences on the potential harmfulness of new nanosubstances in new applications, ultimately resulting in science-based environmental policy decisions for nanomaterials. The poster calls for international cooperation in acquisition and use of scientific information on hazard and risk of nanomaterials.

TP213 Modified test guidelines will broaden future applicability of standard toxicity data

A. Palmqvist, Roskilde University / Dept.of Environmental, Social & Spatial Change; A. Gergs, Roskilde University / Department of Environmental, Social and Spatial Change

Whereas populations, communities and ecosystems are protection goals for Environmental Risk Assessment (ERA) of chemicals, current ERA methods are based on individual level effects. The link between individuals and populations is represented by the application of a safety factor, considered to guarantee a protective risk assessment. In recent years it has become apparent that such simplified ERA methods may not fully provide the measures needed for value relevant RA or inform risk management decisions. Mechanistic effect models (MEMs) provide a more ecologically relevant tool for ERA of chemicals and may serve as an exploratory tool for developing risk mitigation strategies, as they allow extrapolation to untested scenarios and offer mechanistic explanation of observed tests. The aim of the study was to: 1) provide information on data requirements for parameterizing MEMs that can be used for nanoparticle (NP) ERA, 2) pinpoint current data gaps in NP effect data, and 3) suggest workable modifications to standard test guidelines that may provide the required data types and fill identified data gaps. Silver (Ag) NPs were selected as a case due to a comprehensive testing effort for this metal NP type. However, despite the large number of publications on toxicity of this NP, a targeted literature review for data on Ag-NP toxicity to parameterize selected MEMs revealed a general scarcity in applicable data for modeling NP toxicity. Most available Ag-NP effect data represent short-term exposures measuring lethality or sub-individual endpoints. Following standard test guidelines, a few available long-term studies registered sub-lethal effects in, e.g., growth and reproduction at the end of the exposure period. However, MEMs rely on effect data (i.e., growth, reproduction and survival) obtained during longer exposure periods with several census times. We advocate that minor modifications to experimental test guidelines may provide appropriate NP toxicity data that can be used directly as input to MEM parameterization. The focal (sub-)lethal endpoints and number of census times needed to parameterize MEMs depend on types and complexities (i.e., number of parameters) of the models. This has to be taken into consideration when suggesting modifications to existing test guidelines in order to broaden the field of application of standard toxicity data. In conclusion, we suggest minor modifications to existing test guidelines to provide data needed to model NP toxicity.

TP214 SimpleBox4 nano: modeling environmental behavior of nanomaterials

J. Meesters, Radboud University Nijmegen / Institute of Wetland and Water Research; D. van de Meent, RIVM / Institute of Wetland and Water Research; J. Quik, B. Koelmans, Wageningen University and Research; T. Traas, D. Sijm, RIVM

A new version of the multimedia mass balance model SimpleBox has been released. In SimpleBox 4, new insights in substance specific transport and transformation mechanisms, acquired since the previous release of SimpleBox 3.1 (2004), have been incorporated to allow exposure modeling of chemicals of more complex structures than the traditional hydrophobic organic comp pounds. Recently published approaches to modeling typical colloidal processes have been added to the model, which can now simulate environmental fate of nanomaterials. Functionality of the new "SimpleBox4nano" has been explored by modeling a suite of hypothetical nanomaterials, making use of slowly emerging measurement data from the literature. It appears that there are two dominant influences on exposure concentrations of nanomaterials in air, water and soil, which have not been accounted for in the traditional multimedia mass balance models, because they are irrelevant to conventional chemical substances: (i) rate of dissolution in water, and (ii) rate of aggregation and subsequent deposition. Particularly the latter may be important, since colloidal dispersions nanoparticles in air and water are usually rather unstable and rapidly form homo- and heteroaggregates, followed by often rapid deposition from atmosphere or water column. The results of this study demonstrate the importance of quantitative knowledge of the (in)stability of colloidal dispersions under environmental conditions. Ideally, rate constants for transport by aggregation/deposition (e.g., from water to sediment) should be written as explicit functions of physical-chemical characteristics of the nanomaterials and water chemistry, using well-established DLVO-theory of colloid stability. Practically, application of this theory to nanoparticles in environmental systems is still underdeveloped, so that predictions of environmental exposure concentrations needs to be based on reported empirical observations.

TP215 Extraction of Gold and Silver Nanoparticles from Tissues Using Tetramethylammonium Hydroxide Coupled to Single Particle ICP-MS Analysis (spICP-MS)

E.P. Gray, Colorado School of Mines / Environmental Science and Engineering; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; A.J. Kennedy, US Army Engineer Research and Development Center, CEERD-EPR; A.J. Bednar, US Army Engineer Research and Development Center / Environmental Laboratory; J.G. Coleman, US Army Engineer Reseach and Development Center, US Research and Development Center / Environmental Laboratory; C.P. Higgins, Colorado School of Mines / Civil & Environmental Engineering

The use of engineered nanoparticles (ENPs) in consumer products will inevitably lead to environmental exposures through the disposal of wastes, creating exposure potential for aquatic and terrestrial organisms. Current analytical methodologies are incapable of accurately quantifying ENP size, mass, and particle number distributions in complex environmental matrices. This work combines the highly sensitive analytical detection technique of single particle inductively coupled plasma mass spectrometry (spICP-MS) with a tissue extraction method to quantify and characterize ENPs in environmental and biological tissues. The tissue extraction was accomplished using the organic base, tetramethylammonium hydroxide (TMAH) to break down tissues, while not altering the primary particle size of ENPs in tissues. Method development was performed using ground beef and was verified in *Daphnia magna* (*D. magna*) and *Lumbriculus variegatus* (*L. variegatus*). ENPs investigated in this work include 100, 70 and 60 nm Au and Ag stabilized by polyvynylpyrrolidone (PVP). Spiking experiments showed high Au and Ag ENP recovery from all tissues tested, with values ranging from 84% to 121%. Additional spike recovery experiments suggest that particle mixtures (60 and 100 nm) can be extracted simultaneously while still allowing for

detection of both particle sizes. Aquatic exposures using 100 nm Au and Ag ENPs (*D. magna*) and 70 nm Ag ENPs (*L. variegatus*) were also conducted to verify the applicability of the method to exposed organisms. Size and particle number distributions were determined for *D. magna* exposed at 40 to 100 µg/L ENPs and *L. variegatus* exposed to 5 mg/L ENPs. The observed body burdens for *D. magna* were 7.7 x 10^3 µg/kg (Au) and 7.4 x 10^3 µg/kg (Ag), while *L. variegatus* body burdens ranged from 3.1 to 1.3 µg/kg for un-depurated and depurated worms. BAF values ranged from 86 to 3700 for *D. magna* exposed to Au and Ag ENPs. *L. variegatus BAF* values ranged from 0.0062 to 0.0026 across the depuration period. The exposure media was monitored throughout *D. magna* exposures using spICP-MS, allowing BAF values to be corrected for actual ENP dose. BAF values for *L. variegatus* were determined using the nominal dose.

TP216 Cytotoxicity of CuO Nanoparticles to Saccharomyces cerevisiae is Mediated by Cell Surface Attachment and Impact on Respiratory Metabolism

M. Maschock, A. Kappell, Marquette University / Milwaukee; K. Hristova, Marquette University

The potential mechanisms of toxicity of two types engineered CuO nanoparticles (NPs) to *Saccharomyces cerevisiae* were investigated and related to distinct physicochemical properties such as diameter, shape, dissolution, aggregation, and agglomeration. Spherical, 8 nm CuO NPs showed significant inhibition of yeast cells metabolic activity, whereas 50 nm CuO NPs with irregular morphology, and $CuSO_4$ showed much less effect. Differences in the dissolution, aggregate size and diameter range in the cell growth media of two NP types contribute to measurably different cytotoxicity. After chelation of Cuions, the8 nm CuO NPs caused significant metabolic inhibition, while the effect of both 50 nm CuO NPs and $CuSO_4$ was dependent on the presence of dissolved Cu^{2+} ions. Scanning electron microscopy (SEM) revealed NP attachment to *S. cerevisiae* cell surface after exposure toCuO NPs. SEM, inductively coupled plasma-mass spectrometry, and membrane damage assay identified greater NP-cellmembrane interaction of the 8 nm CuO and possible NP internalization, compared to 50 nm CuO NPs, explaining the greater toxicity of the smaller, round, smooth-surface NPs. Different susceptibility of the metabolic activity of single-gene deletion *S. cerevisiae* mutants in oxidative stress-response (*ctt1*Δ, *sod1*Δ, *grx1*Δ, *grx2*Δ,) and electron transport chain (ETC; *cor1*Δ, *cox4*Δ) genes were observed upon copper exposure. The primary difference between the ROS-mediated toxicity of the two NP types was different induction of oxidative stress; the intracellular ROS generated by exposure to 8 nm CuO NPs was increased by an active ETC compared to 50 nm CuO NPs. The present work provides useful data for further defining the relationship of CuO NPs physiochemical characteristics and the mechanisms of toxicity to *S. cerevisiae*.

Wastewater Treatment & Pulp and Paper Mill

TP217 Assessing the aquatic toxicity of a diverse series of silicone-based organic materials for use in hazard and risk assessment

C.A. Staples, Assessment Technologies, Inc.; E. Mihaich, Environmental and Regulatory Resources; G. Kozerski, Dow Corning Corporation

Silicone-based (Si-based) organic materials are widely used in commercial and consumer applications. These materials may be intermediates or reactive ingredients that undergo intentional chemical changes (e.g., polymerization) during use, or they may be used as non-reactive components of products to facilitate performance or appeal. Some Si-based materials are in uses that are disposed to wastewater treatment plants. Routes of entry into the environment include landfill disposal, effluent discharges, accumulation or adsorption to sewage sludge solids that are later applied to land, and volatilization into the atmosphere. Some Si-based materials undergo rapid hydrolysis, such that their intermediate or final degradation products may enter the environment, rather than the parent material. For the substances that may be released to surface waters, aquatic toxicity data are necessary for conducting risk assessment. Acute aquatic toxicity tests were performed with 16 Si-based materials. Tests followed OECD Guidelines 201, 202, and 203 and were conducted in conformance with Good Laboratory Practices. The results of the aquatic toxicity testing will be placed in context with the relevant physical-chemical properties and fate characteristics for each material. The possible relationships between structural features, properties and toxicity of these Si-based materials will be presented and discussed in terms of applicability to hazard and risk assessment.

TP218 Assessment of the relative sensitivity of two fish species exposed to Wastewater Treatment Plant Effluents in the Grand River, Ontario

P. Marjan, G. Tetreault, University of Waterloo; M.R. Servos, B.M. Smith, L. Bragg, University of Waterloo / Department of Biology

The Grand River in the southern Ontario, Canada, is a watershed with diverse anthropogenic inputs, including 30 Wastewater Treatment Plants (WWTPs). Traces of environmental contaminants observed in WWTP effluent have the potential to interfere with the endocrine system of fish at various levels and lead to alterations in fish morphology and physiology. Exposure to contaminants having endogenous hormone-like activity can lead to changes in gene expression, changes in sex steroid production, intersex and decreased gonad size. Previous studies have demonstrated a variety of effects in fish downstream of the wastewater outfalls including a dramatic increase in the incidence of intersex. However, preliminary studies have not demonstrated a major increase in vitellogenin (VTG), a common biomarker associated with MWWE exposure. The objective of this study was to assess the in vivo estrogenic potency of wastewater effluent in the central reach of the Grand River by contrasting the relative species sensitivity of Rainbow Trout (*Oncorhynchus mykiss)* and Fathead Minnow (*Pimephales promelas*) exposed to wastewater effluent in cages. Male fish were exposed to the wastewater effluent for 14 d at 2 sites upstream and 3 sites downstream of the wastewater discharge and plasma was collected for analysis of VTG by Enzyme Linked Imunosorbent Assay (ELISA). A subset of caged fish were injected with 17α-ethynylestradiol (EE2) a known potent estrogen at 10 mg/kg of wet-weight to evaluate the species responsiveness to a known estrogen. Wastewater effluent and river water samples were characterized for water quality and pharmeceuticals. Total estrogenicity was characterized using the Yeast Estrogenic Screening (YES) assay. Extracts were also used in an Effect-Directed Assessment (EDA) to isolate specific estrogenic chemicals. The outcome of these analyses will provide information that will help us better understand species-specific and site-specific responses in fish exposed to municipal wastewater effluents.

TP219 Benthic macroinvertebrate community assessments to evaluate pulp-and-paper discharge effects in the lower St. Johns River, Florida

O.T. Burgess, Environmental Resource Consultants / Fisheries and aquatic Sciences; T.S. Gross, USGS – Biological Resources Division / University of Florida; S. Lopez, Southern Sportsman Aquatics & Land Management; S.E. Holm, Cardno Entrix / Environmental Affairs; M. Wolfe, Environmental Resource Consultants

Georgia-Pacific Palatka initiated a series of mill upgrades beginning in 1998 that concluded in 2012 with the relocation of discharge from the historical Rice Creek site to the main-stem St. Johns River, Florida. To evaluate potential effects of this relocation, a six year monitoring study was developed and validated to assess multiple biological communities, including benthic macroinvertebrates. Due to their sedentary nature and role as a food source for higher organisms, macroinvertebrates are particularly valuable as indicators of the effects of environmental stress. They are also valuable indicators of water quality because some taxa respond differentially to pollution or disturbances. Results immediately following discharge relocation (30 days post) demonstrate that no changes in macroinvertebrate abundance have been detected. A change in Shannon Diversity Index was detected following discharge relocation, which was, however, a function of a seasonal increase in amphipods, skewing the evenness results, but was not a function of a change in species richness. This change in diversity was detected in all habitat types except in Rice Creek, near the original discharge site, the site of greatest exposure prior to discharge relocation. Other measures of community stress (e.g., species richness, percent composition of stress tolerant taxa, etc.) indicate that the macroinvertebrate community in the St. Johns River has not been adversely affected by discharge relocation. Results will also enable the assessment of effects at trophic levels above and below the macroinvertebrates. This monitoring study represents one of the most comprehensive ecosystem-based studies conducted in this region and on the St. Johns River. With more than 36 permitted wastewater dischargers, a host of other stressors in the Lower St. Johns River, and given the economic importance of this river, these types of monitoring studies should likely be required and conducted basin wide. Monitoring benthic macroinvertebrates appears to be a useful indicator of acute and chronic exposure to stress and the potential impacts to the aquatic community due to discharge relocation.

TP220 Biological community monitoring to evaluate ecosystem health and function in the lower St. Johns River, Florida

O.T. Burgess, Environmental Resource Consultants / Fisheries and Aquatic Sciences; T.S. Gross, USGS – Biological Resources Division / University of Florida; S. Lopez, S. Brown, Southern Sportsman Aquatics & Land Management; S.E. Holm, Cardno Entrix / Environmental Affairs

Beginning in 2008, a Florida Pulp and Paper mill was required to develop and execute a monitoring plan to evaluate effects of discharge on biological communities within the St. Johns River, Florida. The USEPA's rapid bioassessment protocol was initially utilized, but required extensive modifications and validations for its application in site and state specific conditions throughout the St. Johns River. A long term monitoring study was designed to evaluate effects of discharge relocation on multiple biological communities which included pre-planned comparisons that account for differences in years, seasons, distance from discharge, and habitat types. The monitoring study allowed for two years of preliminary technique evaluation and validation and baseline data collection, two years of assessment prior to discharge into the main-stem of the river, and two years of assessment after discharge relocation. Results demonstrate significant effects of season, year, habitat and climatic events such as drought and rainfall that must be considered in any evaluation of discharge effects. Results also indicate that this region of the St Johns River is a unique transitional zone between freshwater and marine ecosystems with high biological diversity and significant variance. The final study design will enable the detection and evaluation of the effects of multiple stressors including discharge relocation. This monitoring study represents one of the most comprehensive ecosystem-based studies conducted in this region and on the St. Johns River. With more than 36 permitted wastewater dischargers, a host of other stressors in the Lower St. Johns River, and given the economic importance of this river, these types of monitoring studies should likely be required and conducted basin wide. These monitoring studies will likely provide valuable insight into the health and function of the St. Johns River and an ability to assess and differentiate between effects of future basin stressors, such as water withdrawals and natural variance in the ecosystem.

TP221 Defining normal: using environmental monitoring data from reference sites to detect recovery of fish

T. Arciszewski, University of New Brunswick, Saint John / 5000+; M.E. McMaster, Environment Canada / National Water Research Institute; K.R. Munkittrick, Canadian Rivers Institute, Biology

An important topic in ecotoxicology is the occurrence of recovery after a disturbance. Since 2000 the Canadian pulp and paper industry has contracted by approximately 50%. With the permanent closure of many of these facilities comes the opportunity to more thoroughly study the recovery of fish previously exposed to pulp mill effluents. Downstream of a kraft mill in Smooth Rock Falls, Ontario, Canada (closed permanently in July 2006) impacts to fish reproduction were often found in white sucker (*Catostomus commersoni*) but in 2005 the pattern of responses resembled a nutrient enrichment effect in fish, including larger gonads. In 2011 the first collections of white sucker were done post-closure. We found that the fish exhibited a food limited response (lower condition, smaller livers and gonads, lower fecundity) compared to fish from the upstream reference site. This suggests that the effluent was an important resource for the ecosystem in the downstream area. Collections repeated in 2012 found no differences in liver size, gonad size, or condition, but the female fish had reduced fecundity and relative abundance was still low compared to upstream. The data from these two years of study suggest that the fish are adjusting to the new carrying capacity at the downstream site, but the historical reference data allows us to evaluate these changes compared to possible regional changes over time. Using data from 35 reference sites to define a normal range for the performance of white sucker also suggests regional declines and site-specific differences downstream of the closed mill. Augmenting the typical yearly analysis with historical reference data enhances the effectiveness of defining what is normal and when deviations from that normal range occur.

TP222 Development of biological community monitoring to evaluate pulp-and-paper discharge effects in the lower St. Johns River, Florida

T.S. Gross, USGS – Biological Resources Division / University of Florida; O.T. Burgess, Environmental Resource Consultants / Fisheries and Aquatic Sciences; S.E. Holm, Cardno Entrix / Environmental Affairs

Numerous upgrades at a Georgia-Pacific paper mill (Palatka, Florida 1998 – 2012) culminated in discharge relocation from its historic site in Rice Creek to the mainstem of the St. Johns River. Discharge permits required that Georgia-Pacific monitor for effects to the biological community that may result from this discharge relocation. The USEPA's rapid bioassessment protocol was utilized for protocol development, but site specific conditions required extensive modification and validation of techniques. A long term monitoring study was designed to evaluate effects of the relocation on multiple biological communities using pre-planned comparisons that account for differences in years, seasons, distance from discharge, and habitat types. Careful planning and sufficient technique validation were critical for creating a robust design. Long term monitoring of biological communities enables evaluation of effects of both acute and chronic stress. It was critical that the study design enabled a differentiation between differences detected, which may be part of the natural variation in the system, and adverse effects to the biological community that may be a result of the discharge relocation. These efforts demonstrate that application of rapid bioassessment protocols to Florida ecosystems required extensive modification and validation. Resultant protocols and designs are likely valid for the much of the St. Johns River basin and other Florida ecosystems. Initial results post discharge relocation did not demonstrate any effects of discharge relocation at any of the trophic levels from planktonic through higher aquatic vertebrates, while indicating significant effects of other stressors. Assessments will continue through two years post discharge relocation to evaluate both acute and chronic effects as well as effects of other environmental stressors within this basin. This comprehensive monitoring approach addresses biological effects at trophic levels that are of interest to regulatory agencies and the public, and likely should be applied to assess effects of the many stressors on these ecosystems.

TP223 Do polychlorinated dioxins and furans cause a legacy of contamination downstream of the Terrace Bay pulp mill in northern Ontario?

S. Cater, Department of Biology; R. Hall, University of Waterloo / Department of Biology; M.E. McMaster, Environment Canada / National Water Research Institute; K.R. Munkittrick, Canadian Rivers Institute, Biology; M.R. Servos, University of Waterloo / Department of Biology

In the early 1990's scientists measured polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzo furans (PCDFs) in fish tissue collected downstream of several pulp mills in northern Ontario. Male white sucker (*Catostomus commersoni*) captured in 1991 at Jackfish Bay, downstream of the effluent discharge from the Terrace Bay pulp mill on the north shore of Lake Superior, demonstrated a mean liver toxic TCDD equivalent (TEQ) of 84.4 ± 8.2 $pg{\cdot}g^{-1}$ with 2,3,7,8-TCDD and 2,3,7,8-TCDF representing the dominant congeners. Despite new effluent regulations and a shift towards total chlorine free (TCF) bleaching, previously released PCDD/Fs produced by pulp mills may remain a concern for the aquatic ecosystem due to their tendency to persist and bioaccumulate. PCDD/Fs measured in male white sucker liver samples collected in 2011 illustrate a decrease in mean TEQ to 7.2 ± 0.52 $pg{\cdot}g^{-1}$ approaching those in fish collected from the reference location at Mountain Bay (1.8 ± 0.16 $pg{\cdot}g^{-1}$) which has never received industrial or municipal effluent contamination. Concentrations of PCDD/Fs in surface sediment and dated sediment cores are being used to document the spatial and temporal patterns in relation to historic changes in mill processes and operations. Although below consumption guidelines, trace levels of PCDD/Fs persist in fish collected from Jackfish Bay and these concentrations are suspected to reflect sediment contamination during the operational period of the Terrace Bay pulp mill.

TP224 Efficacy of Composting at Mitigating Contaminants-of-Concern in Biosolids

L.T. Yonkos, G.K. Felton, University of Maryland / Environmental Science and Technology; D.J. Fisher, University of Maryland / Wye Research & Education Center / Environmental Science and Technology

Biosolids (BS) contain numerous contaminants-of-concern including natural (e.g., 17β-estradiol (E2) and estrone (E1)) and synthetic (e.g., 17α-ethynylestradiol (EE2)) estrogens and virtually the entire spectrum of human-use pharmaceuticals, many of which are little changed on excretion and wastewater treatment such that they remain bioactive upon environmental application as organic fertilizer to crop and pastureland. Such application is regulated under nutrient management plans that variously limit nitrogen and/or phosphorus without regard for other contaminants-of-concern. Significant precipitation events can mobilize and transport land-applied pharmaceutical and estrogen residuals directly to receiving waters and may explain, in part, recent mortalities and/or occurrences

of intersex in resident fish populations. Antibiotic residuals can modify microbial communities favoring pathogenic bacteria over commensal taxa and challenging immunocompetence of resident biota. Sub-pharmacological antibiotic residuals can promote resistance in resident bacteria such that receiving environments that permit significant human contact (e.g., recreation, commercial fishing) pose a heightened risk of difficult-to-treat infections. Preliminary investigations suggest that composting may reduce the environmental availability and/or bioactivity of these contaminants. Aqueous extracts of composted BS (via SPE and LC-MS/MS) were found to contain detectible antibiotics at concentrations an order of magnitude less than those from the material prior to composting (dry wt. basis). Estrogens (E1 and E2) in composted BS extracts (measured via SPE and GC-MS/MS) were 5 to 10-fold lower than in raw BS extracts. Similarly, estrogenicity (indicated by ELISA quantification of plasma vitellogenin following 9-d static exposure of mature male fathead minnows (FHM)), was significantly greater in the raw BS extract (80% of fish induced) compared to the composted BS extract (no induction). Metabolomic analysis of FHM polar liver extracts also revealed significant differences in levels of low molecular weight metabolites (measured via GC-MS/MS) following 9-d exposures to raw vs. composted BS extracts, suggesting meaningful changes in abundance, availability, or bioactivity of various BS constituents. Preliminary results and plans for future investigations will be presented.

TP225 Indirect Vitellogenin assay to assess potential estrogenic effects of urban and pulp mill effluents in a native freshwater fish of Southamerica

G. Chiang, Canadian Rivers Institute, Biology; C. Berrocal, EULA Chile Environmental Science Centre, University of Concepcion, Chile / Aquatic Systems Research Unit; M.E. McMaster, Environment Canada / National Water Research Institute; R. Barra, EULA Chile Environmental Science Centre, University of Concepcion, Chile / Aquatic Systems Research Unit; K.R. Munkittrick, Canadian Rivers Institute, Biology

As economic growth is related to human population and industrial development, it also has a series of possible impacts on wildlife. Sewage treatment plant and industrial effluents can load a series of compounds capable of alter reproduction (Endocrine Disrupting Chemicals, EDC) in aquatic species, especially fish. EDC´s are present in several zones of the world, but the assessment of the effects in wildlife, especially in aquatic ecosystems has been mainly focused in the northern hemisphere. Vitellogenin presence and different concentrations in female and male aquatic biota has been used a principal endpoint to assess the exposure and effects of EDC´s, especially in fish. Vitellogenin can be analyzed using ELISA assays, but the development of such assays and the antibodies required for it take a long time and are very expensive. The use of indirect methodologies, such as phosphoproteins in plasma and mucus of fish, to assess this problem raises a potential tool for native Chilean fish species, which are not a target for development of ELISA kits. We assess the possible estrogenic effect of Sewage Treatment Plant effluent (STPE) and Pulp and Paper Mill effluents (PPME) on a native small bodied fish from Chilean rivers (*P. irwinii*) in a static bioassay for 12 d of exposure, using river water, EE2 (10ng/L) and dechlorinated water as controls. The bioassay showed an increasing concentration of phosphoproteins and EROD activity in the fish exposed to the effluents and EE2 (PPME>STPE>EE2), after 7 and 12 d of exposure. In vitro estradiol concentrations showed a peak during the first 7 d, but at the end of the exposure time, no significant difference was observed with the controls. The use of an indirect assay to assess estrogenic induction of EDC´s for effluent appears to be a promising non lethal tool to be used along with other reproductive and biological endpoints for vulnerable fish species.

TP226 Removal of Pharmaceuticals and Personal Care Products in Mesocosm Constructed Wetlands

Y. Wang, B.C. Kelly, National University of Singapore

A large variety of pharmaceuticals and personal care products (PPCPs) are usually present in urban wastewaters, and since common WWTPs are not able to efficiently remove all PPCPs, many of them are released into surface water bodies. Technologies to remove PPCPs discharged into receiving waters like ozonation, reverse osmosis, and advanced oxidation processes can lower the level of pharmaceuticals but are extremely expensive. There is a growing interest in using constructed wetlands (CWs) as a low impact and economical alternative to treat contaminated waters. CWs remove contaminants through a series of complex physical, chemical and microbial interactions which involves a variety of processes including biodegradation, sorption, sedimentation, microbial and plant uptake. Though CWs has shown the ability to remove some PPCPs, the mechanisms involved are largely unknown. In this study, several mesocosm-scale CWs of different configurations are operated to assess their removal ability of PPCPs. The CWs differ in some design parameters, namely the presence or absence of plants, their species (*Thypha augustifolia vs Phragmites australis*) and the presence or absence of soil matrix. The efficiency comparison of different CWs is achieved by analyzing mass removal. The different behavior of each CW regarding the removal of selected PPCPs is monitored in order to suggest a more efficient type of wetland. The effect of different CW configurations on removal of every PPCP is also studied in order to propose elimination patterns. Generally, the presence of plants enhanced the removal of PPCPs and the rather efficient removal suggests that CW systems to remove selected pharmaceuticals with minimal land requirement may be practical in tropical regions.

TP227 Testing of wastewater effluent from City of Las Vegas, Nevada, for endocrine and reproductive effects to fathead minnow (Pimephales promelas)

B. Sharma, FMC Corporation, Agricultural Solutions; R. Patino, Geological Survey, Texas Cooperative Fish & Wildlife Research Unit; E. Orsak, US Fish and Wildlife Service / Arlington Ecological Services Field Office; S. Snyder, University of Arizona / Chemical and Environmental Engineering; D. Drury, Clark County Water Reclamation District; X. Deng, California Environmental Protection Agency / Department of Pesticide Regulation

Previous research has suggested the occurrence of endocrine disruption in fish of Lake Mead, Nevada, which receives effluent from Clark County Water Reclamation District (CCWRD). This study examined the endocrine and reproductive effects to fathead minnow of tertiary treated effluent (TTE) compared to effluent subjected to enhanced treatment by membrane filtration and ozonation (UOE) produced at CCWRD's Central Plant. Different effluent dilutions (in reconstituted tap water as dilution water, DW) were used to design experiments in two formats: (1) a short-term, 21-day assay using naïve (F0) adult fish as test subjects, and (2) a multigenerational assay that began with naïve (F0) hatchlings and continued through F1 juveniles (40-day posthatch). A positive control treatment consisted of DW spiked with ethynylestradiol (EE2, 10 ng/L). In the 21-day assays adult fish exposed to TTE did not appear to be affected in terms of their reproductive output, and also showed little if any changes in other indices of reproductive condition following exposure to TTE or UOE. Vitellogenin (Vtg) levels of males exposed to TTE were slightly elevated compared to those exposed to UOE. However, Vtg levels in all male fish were extremely low and the biological relevance of these observations is uncertain. Life-cycle exposures to TTE or UOE had no effect on pace of gonadal development or sex ratios, and reproductive output in adult fish also seemed unaffected. Interestingly, whereas plasma Vtg levels in adult males was unaffected by life-cycle exposure to TTE or UOE, Vtg levels in females declined considerably as the concentration of either effluent increased. Survival of F0 larvae at the end of the first month of exposure was impacted by TTE and UOE in a concentration-dependent manner, indicating that both types of effluent are toxic to naïve larvae. Overall, the results of this study revealed no evidence of reproductive impairment in fathead minnow exposed to wastewater effluents produced by CCWRD's Central Plant after short-term and multigenerational exposures. However, depending on the exposure format, toxicity of effluent to fish was observed during early development.

TP228 Using stable isotope signatures to trace pulp mill effluent exposure to white sucker (Catostomus commersonii) in Jackfish Bay, Lake Superior

J.A. Mendoza, University of Waterloo / Department of Biology; M.E. McMaster, Environment Canada / National Water Research Institute; K.R. Munkittrick, Canadian Rivers Institute, Biology; G. Tetreault, Environment Canada / Aquatic Ecosystem Protection Research Division; M.R. Servos, University of Waterloo / Department of Biology

The Canada-United States Great Lakes Water Quality Agreement has classified Jackfish Bay, Lake Superior, as an Area in Recovery (AiR) from its previous status as an Area of Concern (AOC). It was classified as an AOC in 1987 due to the degrading effects of the effluent it received from the bleached kraft pulp mill in Terrace Bay, Ontario. In the 1990s the pulp mill upgraded its processes to reduce the amount of dioxins, furans and nutrients in its effluent. In 1998, the Remedial Action Plan Report recommended that natural recovery was the best strategy to let the system recover. Jackfish

Bay was put under the new status as an AiR in 2011, partially due to the recovery of physiological effects of white sucker (*Catostomus commersonii*), a sentinel species that has been monitored at Jackfish Bay over the last 20 years. Jackfish Bay will be delisted as an AiR when monitoring confirms natural recovery of the system. However, this is complicated by the fact that collection of white sucker during spring spawning may include non-exposed fish from adjacent water bodies, which may give a false depiction of recovery. Measurements of pulp mill-derived chemicals in the body burden of fish have been unsuccessful as indicators of exposure during spawning since the fish depurate during long distance migration. Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) provide a unique signature to pulp mill effluent and can be traced in exposed fish. The objectives of this study are to compare $\delta^{13}C$ and $\delta^{15}N$ of exposed and non-exposed white sucker populations, to compare $\delta^{13}C$ and $\delta^{15}N$ of white sucker populations during mill operation and closure, and to determine if $\delta^{13}C$ and $\delta^{15}N$ signatures can distinguish exposed and non-exposed fish collected in the same location during spawning. Epaxial white muscle tissue and liver tissue of 40 male and 40 female white suckers were collected during spring spawning as well as during the fall at locations associated with effluent and reference sites. It is hypothesized that exposed populations will have depleted $\delta^{13}C$ values that reflect terrestrial carbon sources from the pulp mill. If $\delta^{13}C$ and $\delta^{15}N$ can distinguish exposed and non-exposed populations of white sucker during spawning, future biomonitoring can incorporate this technique to ensure that only exposed fish are used to represent the recovery of Jackfish Bay.

Anthropogenic Pollutants

TP229 A new empirical approach to quantifying the release of arsenic and selenium from coal fly ash into natural fresh waters

C. Wall, B. Sohst, G. Cutter, P. Sedwick, Old Dominion University

The interaction of coal fly ash with natural fresh waters can potentially have adverse ecological effects, by releasing a range of bioactive trace elements, including the potentially toxic elements arsenic and selenium. In this context, previous laboratory studies have typically employed batch-leaching methods, which are perhaps not readily extrapolated to the deposition of coal ash into rivers and lakes, where the solution to particle ratio is typically high. To address this problem, we have adapted a flow-through leaching protocol that has previously been used to estimate the fractional dissolution of trace elements from mineral aerosols in surface ocean waters. This rapid leaching technique may effectively mimic the interaction of coal ash with fresh waters, potentially allowing us to assess the release of trace elements from coal fly ash into rivers and lakes. We will present the results of a series of flow-through leaching experiments that were performed using coal fly ash from the TVA Kingston and Johnsonville power plants and freshwater samples collected from three rivers and one lake, and we will discuss the implications of these results for the release of arsenic and selenium from coal fly ash into various types of fresh waters in the southeastern United States.

TP230 Acute toxicity of benzotriazoles on the rainbow trout gill cell line, RTgill-W1

F. Zeng, University of Waterloo; J.P. Sherry, Environment Canada / Water Science & Technology Directorate; N.C. Bols, University of Waterloo / Department of Biology

Benzotriazoles are commonly used as UV stabilizer on plastic packages, corrosion inhibitors in aircraft deicing and anti-icing fluids (ADAF) etc., and have been frequently found in airport effluents and a variety of aquatic ecosystem. However, little is known about the their potential impacts on the aquatic environment. The current study aimed on an ecotoxicological characterization of benzotriazole (BT), 4-Methyl-1H-benzotriazole (4MBT), 5-Methyl-1H-benzotriazole (5MBT) and 5-Chlorobenzotriazole (5CBT). We investigated the acute toxicity of benzotriazoles on a rainbow trout gill cell line, RTgill-W1 for the first time. The 24h median lethal concentration (LC50) values of benzotriazoles for RTgill-W1 were estimated to be less than 500mg/L. The benzotriazoles induced DNA breaks but no DNA laddering was seen at concentrations causing a loss of viability, suggesting a non-apoptotic mechanism of death. Concurrent with cell death was a significant decrease in the level of p53, a master regulator of cellular functions. Further research is being carried out to assess the potential longer term sublethal impacts of benzotriazole on fish cells.

TP231 Combined effect of hypoxia and exposure to biodiesel B5 in biochemical and cytotoxic parameters in mussels Perna perna (Mytilidae, Bivalvia)

L. Nogueira, São Paulo State University; D. Garcia, São Paulo State University / Chemistry and Environmental Sciences; F.S. Cortez, Universidade Santa Cecilia; C.D. Pereira, Universidade Federal de São Paulo; E.A. Almeida, Chemistry and Environmental Sciences

The intertidal marine environment promotes fluctuations in the oxygen availability for its inhabitants. Hypoxia followed by reoxygenation produces reactive oxygen species (ROS), which can cause oxidative stress in organisms. Bivalve mollusks have antioxidative defenses to fight ROS formed during the reoxygenation. However, it is unknown if the increase in antioxidant system generated by hypoxia also contributes to neutralize ROS generated by exposure to pollutants and if contaminant exposure can affect the hypoxia metabolism. In Brazil B5 blend (5% biodiesel and 95% diesel oil) has been adopted as mandatory fuel for automotive vehicles and could become mandatory for marine fleet in the future. So it becomes necessary to assess whether this fuel can generate disturbance in aquatic biota. Thus, this study aimed to assess in mussels *Perna perna* the combined effect of hypoxia and exposure to B5 in biochemical and cytotoxic parameters. Mussels were exposed for 48 h to filtered seawater as control and to air for 24 h followed by reoxygenation in clean seawater and seawater with B5 0.01 mL/L for 48 h. Further, mussels were exposed to B5 0.01 mL/L for 48 h followed by air exposure for 24 h. Then we evaluated the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST) and glutathione reductase (GR) as well as glutathione concentration (GSH) and lipid peroxidation by measure the malondiadehyde concentration (MDA) in gills and digestive glands. Also we conducted the neutral red retention time assay (NR) in hemocytes. We found reduction in activity of GST, GR and CAT in gills of groups exposed first to hypoxia when compared with groups exposed first to seawater or B5. These results should be more related to the fact that the animals have gone through a prolonged period of hypoxia and not to the presence of B5, because those groups which were exposed first to B5 or clean water followed by hypoxia were not different when compared to the control. Also, GSH content was decreased in digestive gland in mussels from hypoxia followed by B5. Lysosomal membrane stability was decayed when mussels were undergone hypoxia followed by B5 exposure compared with group which was reoxygenated in clean water and peroxidation levels also increased compared to the control group. Taking together, these results shows that B5 can interfere in hypoxia antioxidative metabolism and vive-versa. (Financial support: FAPESP-2010/20365-5).

TP232 Effects of a Short-term Exposure to Fadrozole on Steroid Production and Gene Expression in the Ovary of Female Fathead Minnows (Pimephales promelas)

A. Schroeder, G. Ankley, E. Durhan, USEPA / Mid-Continent Ecology Division; N. Garcia-Reyero, Mississippi State University / Institute for Genomics Biocomputing and Biotechnology; T. Habib, US Army Engineer Research and Development / Environmental Laboratory; K. Jensen, M. Kahl, E. Makynen, USEPA / Mid-Continent Ecology Division; D. Martinovic, University of Saint Thomas / Dept. of Biology, USEPA, Mid-Continent Ecology Division; E.J. Perkins, US Army Engineering Research & Development / Environmental Laboratory; D.L. Villeneuve, USEPA / Mid-Continent Ecology Division

Cytochrome P450 aromatase is a steriodogenic enzyme that converts C19 androgens to C18 estrogens and is critical for normal reproduction in females. Fadrozole is a well-studied aromatase inhibitor that has been shown to suppress estrogen production in the ovaries of fish. However, little is known about the early impacts of aromatase inhibition on steroid production and gene expression in fish. Adult female fathead minnows (*Pimephales promelas*) were exposed to 0, 5, or 50 µg/L fadrozole for a time-course of 0, 0.5, 1, 2, 4, and 6 hours or exposed to 0 or 50 µg/L fadrozole for a time-course of 6, 12, and 24 h. We examined *ex vivo* 17β-estradiol (E2) and testosterone (T) production and plasma E2 and T concentrations from each study. Expression profiles of genes known to be impacted by fadrozole including aromatase (*Cyp19a1a*), steriodogenic acute regulatory protein (*StAR*), *Cyp11*, *Cyp17*, and follicle stimulating hormone receptor (*Fshr*) were measured in the ovaries by quantitative real-time polymerase chain reaction (QPCR). In addition, ovarian gene expression profiles were examined using a 15k fathead minnow microarray. *Ex vivo* E2 production was significantly reduced by the 5 µg/L exposures after 6h. In the 50 µg/L exposures ex vivo E2 was significantly reduced after just 2 hours of exposure and remained depressed at all

time-points examined through 24 h. Plasma E2 was significantly reduced as early as 4 hours for both fadrozole concentrations and remained depressed throughout the 24h for the 50 µg/L exposure. *Ex vivo* and plasma T remained unchanged by either fadrozole concentrations throughout the time-course. Transcripts examined by QPCR showed an initial significant increase in expression followed by a significant decrease in expression by 6h. *Star*, *Cyp17*, and *Fshr* showed a significant increase in expression again by 12h and *Cyp191a1* showed a significant increase in expression by 24h. Microarray results showed concentration- and time-dependent changes in gene expression profiles associated with the chemical exposure. These results provide an indication of the early effects of aromatase inhibition on steroid production and gene expression in the fathead minnow ovary. The contents of this abstract neither constitute, nor necessarily reflect, official USEPA policy.

TP233 Effects of Estrogenic Compounds on Cardiac Development in Zebrafish

G. Diamante, University of California, Riverside; D. Schlenk, University of California-Riverside / Department of Environmental Sciences

Several studies have shown that exposure to estrogenic compounds like 17β-estradiol (E2) and bisphenol A (BPA) can cause cardiac edemas in developing vertebrates including zebrafish. In mice, exposure to BPA disrupts calcium signaling leading to leakage via the RYR receptor. Exposure to endocrine disruptors could lead to a disruption in the of signal transduction events required for normal heart development via activation of G protein-coupled estrogen receptor 1 (GPER). Potential targets include, GATA4, Hand2, Lrrc10, ERK, PKA and NFAT. GATA4 regulates the expression of Hand2 and Lrrc10, which are involved in heart cell differentiation and development. GATA4 can be activated by several kinases, such as ERK and PKA, both of which are downstream targets of the estrogen signaling pathway. GATA4 has also been proposed to interact with NFAT, whose activation is regulated by calcium influxes. We hypothesize that exposure to E2 would alter the expression of important heart determinant genes such as Hand2 and Lrrc10, as well as calcium homeostasis in zebrafish. To explore potential molecular mechanisms of cardiac dysfunction, expression of genes involved in signal transduction and calcium homeostasis were examined in zebrafish embryos following exposure to E2. Embryos were exposed to 2.5µM of E2 for 28 hrs and RNA was extracted at 30hpf. Preliminary qPCR results suggest that E2 exposure during early embryogenesis causes an increase in the expression of Lrrc10, although more experiments are needed to confirm this trend. Additional studies will not only measure the expression of other genes related to GPER activation by estrogen, but also measure intracellular calcium to better understand how endocrine disruptors alters cardiac development.

TP234 Fish embryo-toxicity test to establish safety levels of Estericide® solution to aquatic organisms

O.A. Aguilar, Universidad Nacional Autonoma de Mexico / Medicina Genómica y Toxicología Ambiental, Institute for Biomedical Research. Universidad Nacional Autónoma de México / Department of Environmental Toxicology; S. Solis-Angeles, R. Montero Montoya, Institute for Biomedical Research. Universidad Nacional Autónoma de México / Department of Environmental Toxicology

Electrolyzed solutions of sodium chloride are used extensively due to their disinfection potential. Currently, diverse products and novel applications have popularized around the World. Estericide® solution is a Mexican product manufactured by Esteri*pH*arma México Company. It is an electrolyzed solution with pH close to neutrality with antiseptic activity which is attributed to the proportion of hypochlorous and hypochlorite acids in a hypotonic concentration. A possible application of Estericide® solution is for controlling microbial pathogens in fish and shrimp farms. However, this use could affect wildlife fish as a consequence of residual discharges in the environment. In order to determine the safety of Estericide® levels for use in aquatic animals, we performed, according to OCDE 2006, the fish embryo-toxicity test. Embryos of zebrafish (*Danio rerio*) were exposed (n=8) in 40L tanks in triplicate, at 27°C, to a gradient of 11 dilutions (0.2-100 %) of Estericide®. We performed a toxicity test to obtain the LC50, using the Trimmed-Sperman- Karber Test. Then after, embryos (*n*=4 per six replicates) were exposed to a gradient of 10 Estericide® dilutions (0.9-50%); embryos in clean aquarium water were used ascontrol group. Malformations and heart frequency at 24, 48 and 72 hours post-fertilization were registered. Water quality parameters such as ammonium, nitrite, nitrate, dissolved oxygen, ORP, pH, temperature and conductivity were registered at the beginning and at the end of treatments. LC50 was found at 24.35 ± 1.3 % dilution of Estericide®. Yolk sac edema and changes of embryonic development(advance of 2 hours) in exposed embryosbetween 1.8 and 15%of dilutions of Estericide® were found. In addition, the average heart rate in exposed embryos presented a reduction without compromising egg hatching when the dilutions were above 1.8%. The physicochemical parameters showed a slight change in the exposure tanks. Estericide® is an oxidative solution and its negative effects on embryo development were observed above of 7.5% of dilution. Therefore, Estericide® solution should be used in dilutions below 2% in order to reduce a possible impact on fish embryo development.

TP235 Fly ash and cenosphere leaching characteristics as a function of pH: application of proposed USEPA Method 1313

P.A. Pier, P.D. Enlow, V.J. Van Pelt, J.G. Broder, Tennessee Valley Authority

The US Environmental Protection Agency (USEPA) has proposed a suite of four new methods (Methods 1313 through 1316), called the Leaching Environmental Assessment Framework (LEAF), for determining the leaching characteristics from coal ash and other landfilled materials. *Method 1313: Liquid-Solid Partitioning as a Function of Extract pH in Solid Materials using a Parallel Batch Procedure* is designed to provide an estimate of the liquid-solid partitioning (solubility and release) of inorganic constituents under conditions that approach liquid-solid chemical equilibrium and as a function of pH through the range of 2 to 13. Leaching tests were performed in accordance with Method 1313 on two composite ash samples from the Kingston ash cell and on cenospheres collected from the TVA Johnsonville Fossil Plant. Site-specific pH conditions at the Kingston site do not vary over a large pH range, therefore leaching characteristics were not tested below pH 5. Cenospheres were tested over the pH range from 2 to 13. Arsenic concentrations in the sample leachate for fly ash were below 0.1 mg/L between pH 5 and 8, and the response for both the fly ash and cenospheres were similar, although the concentrations for the fly ash samples were 2 to 4 times greater than for the cenospheres. At the more basic pH levels of 9 and above, however, arsenic concentrations for fly ash increased rapidly to a maximum of 4.57 mg/L at pH 13, while concentrations for the cenospheres increased relatively slightly to a maximum of 0.173 mg/L at pH 13. Selenium leaching concentrations from fly ash were similar or slightly higher than cenospheres concentrations at pH 5, with concentrations between 0.015 and 0.049 mg/L. For the cenospheres, leachate concentrations decreased with increasing pH to a minimum concentration of 0.0042 mg/L at pH 9, with concentrations then increasing to a maximum of 0.0403 mg/L at pH 13. Concentrations increased with increasing pH much more rapidly for fly ash, with a maximum concentration at pH 13 of 0.186 mg/L. Cenosphere leaching results were also compared to fly ash leaching results obtained from the LeachXS™Lite database developed by Vanderbilt University, which contains leaching results from CCPs using Method 1313. This comparison indicates that cenospheres and fly ash produced from burning the same type of coal would be expected to leach approximately the same amount of arsenic and selenium.

TP236 Gene expression in Japanese medaka (Oryzias latipes) response to surfactants at a sub-lethal concentration

J. Tasaki, Kao Corporation; K. Nakayama, Ehime University / Center for Marine Env. Studies; Y. Honda, Kao Corporation; M. Yamane, N. Nishiyama, Kao Corporation / Safety Science

Linear alkylbenzen sulfonate (LAS) and Alcohol ethoxylate (AE) are high productive chemicals used in many consumer products. These surfactants are finally released into river water via wastewater treatment plants. An acute and chronic toxicity of these surfactants were examined in several aquatic organisms. However, a molecular mechanism of toxicity of these surfactants is still unknown. Nowadays molecular biological techniques such as microarray and quantitative reverse transcription-polymerase chain reaction (RT-PCR) are utilized to examine the molecular mechanism involved in a toxicity of many chemicals in ecotoxicological field. In this study, we analyzed a mechanism of toxicity of C12-LAS homologue and AE (alkyl chain length: C12 ethylene oxide length: ave. 10) in Japanese medaka (*Oryzias latipes*) commonly used in ecotoxicity tests. A microarray analysis to investigate gene expression profile after exposure of these surfactants to Japanese medaka was performed. Japanese medaka was exposed for 24 hrs to nominal concentrations (2.5 mg/L and 1.25 mg/L) under static condition. LAS and AE did not affect obvious morphology in liver and gill and animal behavior when Japanese medaka was treated with these surfactants for 96 hrs. LAS-exposed animals showed expression alteration of 567 and 566 genes at 2.5 mg/L and 1.25 mg/L,

respectably. AE-exposed animals displayed expression alteration of 912 and 910 genes at 2.5 mg/L and 1.25 mg/L, respectably. Based on this analysis, hypoxia-inducible genes were up-regulated. We then analyzed a dose- and time-dependent expression of these genes by RT-PCR. These results may suggest that these surfactants induce hypoxia without morphological defects in liver and gill. We will discuss a toxicity mechanism of these surfactants.

TP237 How does temperature affect the toxicokinetics of PCB and PBDE in Rana pipiens?

Y. Tsai, University of Wisconsin – Madison / Molecular and Environmental Toxicology Center

Climate change will have far-reaching consequences on natural systems. One of its important consequences is its potential to alter the biological effects of environmental contaminants. Studies have investigated how different temperatures affect the acute toxicity of anthropogenic toxicants; however, temperature can affect multiple factors that contribute to this overall toxicity. These multiple factors, and their possible varying responses to temperature change, make it hard to predict how climate change will affect species' abilities to cope with environmental toxins. In our study, we investigated alterations in toxicokinetic rates in frogs challenged with temperature changes. Polybrominated diphenyl ether (PBDE) and polychlorinated biphenyl (PCB) are persistent organic pollutants found in the Great Lakes region. In the past, our lab has found that these pollutants affect the immune functions in Northern Leopard frog (*Rana pipiens*). In this study, *R. pipiens* were exposed to PCB and PBDE at two different temperatures, 18 and 28 degrees Celsius, representing the current temperature distribution of *R. pipiens* and the future projected temperature of the Great Lakes Region. Two circulating water systems were set up at 18 and 28 degrees Celsius were *R. pipiens* tadpoles were reared from hatch. The tadpoles were raised to an asymptotic size and then exposed to either 100ng/g PCB or 1000ng/g PBDE for two. The tadpoles were then allowed to depurate for two weeks. Body burden data along with growth and digestive efficiency data were collected weekly. The specific aims in this study were (1) Establish the temperature dependent PCB and PBDE toxicokinetic rates in *R. pipiens*, (2) Test for temperature-dependent PCB and PBDE toxicity in *R. pipiens*, and the results will be presented in this meeting. There is a paucity of information for ectothermic vertebrates on how temperature affects the chronic toxicity of environmental contaminants, but we feel that understanding this is key to anticipating the ecological impacts of warming climates.

TP238 Insights from Chemical Fingerprinting of Produced Water from Coalbed Methane Operations in the Central Raton Basin, Colorado

M.J. Benotti, Analytical and Environmental Chemistry, Battelle; A.D. Levine, Battelle

Significant quantities of produced water are associated with unconventional oil and gas production, particularly coalbed methane (CBM) development. In arid regions, produced water can serve as a water resource to support livestock operations, irrigation, and to augment in-stream flows. Typically, management of produced water is regulated at the state level and includes beneficial reuse, permitted surface water discharge, or subsurface injection using permitted Class II underground injection control (UIC) wells. However, due to the complex biogeochemistry of produced water, the ability to use traditional monitoring approaches to evaluate environmental fate and transport is limited. Interpretation of data on individual chemical constituents detected in produced water can be challenging due to differences in detection limits, solubility, and other water quality factors that affect environmental fate and transport (e.g., pH, oxidation potential, ionic strength, temperature, colloidal particles, microbially mediated reactions, etc.). Sample collection protocols, the presence or absence of coal-fines, the effectiveness of well purging, and potential ingress of drilling muds or soils may also influence the analytical results. For example, hydrocarbons, when detected in produced water, may have been introduced from fuel, coal, or anthropogenic sources, depending on the integrity of the well, site management practices, and dominant weathering reactions. Advances in hydrocarbon fingerprinting techniques provide more sensitive detection limits for individual compounds and offer a tool for comparing groups of chemicals, such as PAHs, from different sources. This paper will present results from exploratory chemical fingerprinting of produced water, groundwater, and surface water discharges in the central Raton Basin of Colorado in the context of CBM well characteristics and local hydrogeology. Complementary monitoring data on dissolved solids, dissolved gases, stable isotopes, metals, and radionuclides are used to interpret the hydrocarbon fingerprinting results. The value of a forensics approach to hydrocarbon identification is highlighted, particularly to provide sound evidence about the presence or absence of contamination from diesel fuel or other hydrocarbon sources. Research needs relevant to hydrocarbon fate and transport in unconventional oil and gas development will be discussed with an emphasis on coalbed methane applications.

TP239 Irgarol, Diuron and Its Metabolites in Seawaters Samples at the Port Of Itaqui, Maranhão, Brazil

L. Diniz, São Paulo State University- Institute of Chemistry; T.C. Franco; I.A. Ribeiro, M.S. de Jesus, Maranhao Federal University; T.M. Oliveira, São Paulo State University- Institute of Chemistry; O.P. Amarante Jr, Federal Institute of Maranhao

Antifouling paints are used on surfaces exposed to the aquatic environment with aim to combat the formation and establishment of biofouling communities. Irgarol and diuron are biocides regularly found in these paints. However, these compounds, including their degradation products are of considerable toxicity to non-target organisms and can be environmentally cumulative. This study established and validated analytical conditions for the determination of irgarol, diuron and the principal degradation products of diuron (3,4-dichloroaniline, DCA, 3,4-dichlorophenyl-urea, DCPU, and 3,4 – dichlorophenylmethyl-urea, DCPMU) the extraction procedure, the biocides in the extract were directly determined by high performance liquid chromatography with diode array detector (HPLC-DAD). The flows were tested between 1.0 and 1.7 mL min^{-1}, checking up better signal separation and analytical flow 1.0 mL min^{-1}. It was determined the best wavelengths for determination of compounds checking for better analytical signal in irgarol 226 nm, DCPU and DCA in 245 nm and DCPMU and diuron in 250 nm. For determination of sensitivity and linearity of the method have been established parameters of the calibration curve for concentrations between 0.05 and 8 μg L^{-1}. The correlation coefficients (R^2) obtained, were between 0.9931 and 0.9995 and quantification limits between 0.02 to 0.04 μg L^{-1}, with a preconcentration 500 mL samples of sea water. Monitoring studies will be made in the same area that were identified in the years 2010 and 2011, residues of biocides at concentrations ranging 0.05 to 7.8 mg L^{-1} for irgarol and 0.012 to 4.8 μg L^{-1} for diuron.

TP240 Measuring the acute and chronic toxicity to aquatic and sediment dwelling organisms for a series of cyclic and linear volatile methyl siloxanes

C.A. Staples, Assessment Technologies, Inc.; E. Mihaich, Environmental and Regulatory Resources; G. Kozerski, K. Woodburn, Dow Corning Corporation

The cyclic volatile methyl siloxanes (VMS) include hexamethyl cyclotrisiloxane (D3), octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5),and dodecamethylcyclohexasiloxane (D6). The linear VMS include hexamethyldisiloxane (L2 or HMDS), octamethyltrisiloxane (L3 or MDM), and decamethyltetrasiloxane (L4). Both cyclic and linear VMS are used as intermediates or in a variety of industrial applications and consumer/personal care products. Due to their uses, these VMS may enter wastewater treatment plants, with trace residues subsequently entering surface water and sediments via effluent discharges. VMS entering surface waters are subject to various loss processes including pH dependent hydrolysis and sorption to particulate organic matter. Some VMS also volatilize to the atmosphere, where they are subject to sorption to aerosols or other air-borne particulates and photo-oxidation via hydroxyl-radical mediated degradation. The assessment of potential risks of the VMS to aquatic and sediment organisms requires appropriate toxicity data. To support the risk assessment of these materials, acute and chronic aquatic and sediment toxicity testing following international guidance have been conducted. The results of the toxicity testing show that the higher solubility VMS do pose toxicity, while the lower solubility materials do not. The aquatic toxicity data for the VMS will be discussed in terms of their physical-chemical properties and critical body burden. The sediment toxicity data for the VMS will be discussed in terms of their properties, including organic carbon solubility limits. The available aquatic toxicity for these cyclic and linear VMS have been used in regulatory-driven screening risk assessments in North America, Europe and Japan. The toxicity data will be discussed in context with the results of those assessments.

TP241 Microplastics in Waters of the Chesapeake Bay and Coastal Mid-Atlantic, USA

L.T. Yonkos, University of Maryland / Environmental Science and Technology; E.A. Friedel, University of Maryland, Wye Research and Education Center / Wye Research and Education Center

Discovery of plastics during the last century has led to the manufacture of numerous inexpensive and disposable commodities now in common use. Once believed to degrade into simple compounds and eventually "disappear" in our environment, increasing evidence suggests otherwise. Plastics entering the environment are mechanically and/or photochemically degraded to the extent that they may become imperceptible to the naked eye yet in total mass are not significantly reduced. Thus, more and smaller plastics particles occupy various environmental compartments. Microplastics, defined operationally as measuring between 0.3 mm and 5.0 mm, are now a contaminant category of concern for aquatic ecosystems. These materials may be toxic themselves or allow adsorption and transport of other toxic substances. There also exists the possibility of ingestion by aquatic organisms introducing microplastics to aquatic food webs. For the current study surface waters from four estuarine tributaries within the Chesapeake Bay, USA and two mid-Atlantic coastal regions (i.e., Maryland and Delaware, USA) were sampled by 1 km trawls with a manta net to collect appropriately sized microplastics (i.e., 0.3 mm to 5.0 mm). Triplicate samples were collected on up to five occasions between early summer and late fall 2011 from the various estuarine and coastal systems. Samples were digested in 30% hydrogen peroxide followed by hyper-saline density separation to isolate plastics particles from other marine debris. Results are reported as mass of microplastics per volume of water sampled (a surrogate for concentration). Of 60 estuarine samples only one contained no microplastics. Similarly, only two of 54 coastal samples were without idetifiable microplastics. Mass, size, and variety of plastics constituents varied substantially among and between coastal and estuarine systems and across sample dates. Samples with the highest microplastics concentrations were in the low ng/L (ppb) range.

TP242 Natural attenuation of arsenic and selenium in sediments impacted by coal combustion waste

J.T. Markwiese, Environmental Standards Inc. / Environmental Assessment Department; B.J. Rogers, Tennessee Valley Authority; N. Carriker, TVA; R.J. Vitale, Environmental Standards; E. Rodgers, Environmental Standards / Chemistry; J. Gruzalski, Environmental Standards, Inc. / Geosciences

This research describes the weathering and decreasing bioavailability of coal combustion waste in a lotic aquatic environment following the Tennessee Valley Authority ash spill of 2008 in Kingston, TN. Heavy metal bioavailability in sediments can be assessed with the sequential extraction procedure, which speciates metals in decreasing order of availability. Sequential extraction usually starts with water to extract the most mobile/labile metals and ends with solvents that can liberate immobile metals. Each solvent is selected to attack a specific phase of the sample, such as carbonate or manganese-iron oxide. Relative to total metals, sequentially-extracted metals (SEM) represent a more ecologically-realistic exposure for sedimentary organisms. A sequential extraction procedure developed specifically for coal ash was used to measure arsenic (As) and selenium (Se) bioavailability in the ash-impacted sediments. Heavy metals were extracted from water soluble (distilled water), ion exchangeable (ammonium acetate, pH 7), carbonate-bound (ammonium acetate, pH 5) fractions followed by a step which solubilizes the reducible (hydroxylamine hydrochloride) manganese and iron oxide fraction. Total metals were also measured. In newly-formed ash, As resides in the carbonate (60%) and manganese-iron oxide (90%) bound fractions, cumulatively, and Se is predominantly associated with the ion exchangeable (40%) and to a lesser extent carbonate (30%) and manganese-iron oxide (20%), respectively. Sediment samples collected in the river system soon after the spill had As concentrations roughly equivalent to proportions in newly-formed ash, while Se was more evenly distributed among the fractions. Almost three years later, As and Se are detected significantly less frequently and at significantly lower concentrations in the more bioavailable water soluble, ion exchangeable and carbonate-bound fractions. Virtually all of the As and Se is currently associated with the manganese-iron oxide bound fraction, the least bioavailable fraction of those measured. Concentrations of Se have remained stable in this fraction over time while As has decreased significantly. Concentrations of As and Se in the bioavailable fractions are one to several orders of magnitude lower than total As and Se. These observations illustrate the decreased bioavailability of sedimentary As and Se over time in a flowing water system.

TP243 Passive detection of arsenic using iron-doped hydrogel beads

D.J. Vesper, West Virginia University; H.M. Edenborn, US Department of Energy

The passive accumulation of pollutants from aqueous systems onto selective absorbents allows the determination of elements that are normally present in concentrations too low to detect, or those that are present sporadically and may be missed by conventional grab sampling. Because many commonly-used absorbents and complexing agents occur as fine particles or are soluble, their incorporation into a matrix that allows the rapid diffusion of pollutants is one potential approach to their use in the field. We have experimented with the use of hydrogel beads as high-water-content (>96%) matrices for the inclusion of agents to adsorb or trap specific pollutants. In this study, iron (III)-doped spherical beads were prepared from calcium alginate or agarose: 2% alginate was dropped into a 100 mM calcium chloride solution to form calcium alginate beads, and 4% dissolved agarose was dropped into mineral oil to form agarose beads. Both bead types were equilibrated for 3 days in 100 mM ferric chloride solution and rinsed in 1 mM HCl for an additional 3 days prior to deployment. Field experiments testing As adsorption were carried out by placing beads in bags made of plankton netting into the discharge of a coal-fired power plant ash pond for 2 weeks and in a small stream that bisected an abandoned sulfuric acid recycling plant site for 3 months. At both locations, As was concentrated in iron-doped beads versus control beads lacking iron by up to 64-fold. The power plant discharge contained ca. 30 ppb As, but it was undetectable by ICP-MS analysis in the acid plant stream. Calcium alginate beads did not maintain their integrity over the course of the long-term experiment, presumably due to chelation of calcium from the gel matrix, but agarose beads remained stable in both systems.

TP244 Perfluorooctanesulfonate (PFOS) Developmental Toxicity in the Proposed EDSP Tier II Larval Amphibian Growth and Development Assay (LAGDA)

D.J. Fort, Fort Environmental Laboratories, Inc.; M. Mathis, C.E. Fort, H.M. Fort, Fort Environmental Laboatories; P.D. Guiney, J.A. Weeks, S.C. Johnson & Son, Inc. / Environmental Safety

Exposure of *Silurana tropicalis* to perfluorooctanesulfonate (PFOS) during development was previously found to increase the proportion of phenotypic males, alter steroidogenesis during sexual differentiation, and induce abnormal ovary development. To further evaluate the proposed USEPA Endocrine Disruptor Screening Program (EDSP) Tier II Larval Amphibian Growth and Development Assay (LAGDA), the advanced developmental toxicity of PFOS was evaluated (< 0.0 [control], 0.13, 0.25, 0.5, and 1mg PFOS/L) using the LAGDA model under flow-through exposure conditions to 10 weeks post-metamorphosis. Metamorphic study endpoints included survival, median time to metamorphosis (MMT), growth at metamorphosis, plasma Vitellogenin (VTG) and T4, and thyroid histopathology. Post-metamorphic endpoints included growth, plasma VTG, plasma T4, genotypic and phenotypic sex ratios, nuptial pad development, and gonad histopathology. Results indicated that none of the metamorphic endpoints associated with development or morphology were affected by PFOS exposure. Plasma levels of T4 were not affected by PFOS exposure. However, plasma VTG levels were significantly reduced in the 0.5 and 1.0 mg PFOS/L treatments. A similar trend was noted in the post-metamorphic endpoints including a more dramatic decrease in the plasma VTG in juvenile frogs exposed to 0.5 and 1.0 mg PFOS/L. Interestingly, an increase in number of genetic females displaying a male phenotype was observed in 0.5, and more notably in the 1.0 mg PFOS/L treatments. As with the historical studies in *Silurana*, these results demonstrate the anti-estrogenic effects of PFOS on the development of *X. laevis*.

TP245 Persistent organic pollutants in yearlings of Magellanic penguins (Spheniscus magellanicus) found on the southern and southeastern coast of Brazil

P. Baldassin, Instituto Oceanografico; S. Taniguchi, University of São Paulo; H. Gallo, Aquário de Ubatuba; A. Maranho, GREMAR; P. Serafini, National Center for Bird Conservation Research (CEMAVE); C. Kolesnikovas, Associação R3 Animal; M. Tavares, Centro de Estudos Costeiros, Limnológicos e Marinhos, Instituto de Biociências, Universidade Federal do Rio Grande do Sul (CECLIMAR/IB/UFRGS) e Grupo de Estudos de Mamíferos Aquáticos do Rio Grande do Sul (GEMARS); R.C. Montone, Instituto Oceanografico – Universidade de Sao Paulo

The Magellanic penguin, *Spheniscus magellanicus,* is the most abundant of the penguins that live in temperate regions of the Southwestern Atlantic Ocean. Its breeding season runs from October to March, when it feeds off the coast of Argentina and southern Chile. In its pelagic phase, the species

migrates north and winters on the continental shelf off Uruguay and Brazil. Juvenile individuals are frequently found dead on the beaches of southern and southeastern coast of Brazil in the austral winter. Living specimens were found debilitated (i.e., hypothermic, cachectic and apathetic) and sent to rehabilitation centers. The present study assessed the occurrence of persistent organic pollutants (POPs) in Magellanic penguins found debilitated on the beaches of the states of Rio de Janeiro, São Paulo, Santa Catarina and Rio Grande do Sul, Brazil, between 2008 – 2011. Liver samples of fifty six yearlings were analyzed, due to the body state, as the birds were found virtually without adipose tissue. The following POPs concentrations in wet weight were found: ∑ PCBs (3.2 to 2794 ng g^{-1}), ∑ DDTs (2.3 to 275 ng g^{-1}), ∑ HCHs (1.0 to 19.8 ng g^{-1}) and HCB (2.2 to 108 ng g^{-1}). Among the PCBs, there was a predominance of hexachlorobiphenyls (138 and 153) and heptachlorobiphenyls (180 and 187). Among the organochlorinated pesticides, DDT predominated, mainly in the *p,p*'-DDE form. In a general way, the concentrations of POPs found in the specimens of *S. magellanicus* reached levels (10^2 to 10^3 ng g^{-1}) similar than those found in *Pygoscelis adelie* and *Pygoscelis papua* from Antarctica. Although penguins appeared as good biomonitors since they show the distribution and fate of POPs in the Southwestern Atlantic Ocean, ecological factors (e.g., accumulation and biomagnification of pollutants through the food chain) and/or physiological/biological aspects (e.g., mobilization of lipids-pollutants in cachectic individuals) should also be considered.

TP246 Planning for Natural Resource Damage Assessment in the Alaskan Arctic: Exposure pathways, effects endpoints and ephemeral data collection

S.E. Allan, Environmental and Molecular Toxicology, National Oceanic and Atmospheric Administration / Office of Response and Restoration

The Alaskan Arctic is facing changing conditions that increase the likelihood of an oil spill in the region, including the loss of sea ice and increased offshore oil and gas development, ship traffic and other human activities. Natural Resource Damage Assessment (NRDA) is a legal process that is conducted by state and federal natural resource trustees, in the event of an oil spill that impacts trust resources. It aims to determine resource injuries and the type and amount of restoration required to restore those resources and compensate the public for interim losses. During the first phase of the NRDA process, the trustees assess the extent and severity of resources injuries. This generally includes collecting time-sensitive data to assess exposure to oil chemicals and effects of those exposures. The rapid changes that are taking place in the physical and biological systems in the Arctic exacerbate the challenges of evaluating injuries to natural resources in the event of an oil spill. The Assessment and Restoration Division of the National Oceanic and Atmospheric Administration's (NOAA) Office of Response and Restoration is developing new science and methods, using existing information, incorporating traditional ecological knowledge and integrating lessons learned from other spill events to improve preparedness to conduct NRDA in the Arctic. A detailed conceptual model was developed to describe potential oil and oil chemical exposure pathways and effects in the Arctic. Based on this model, high priority exposure and effects endpoints that are relevant to NOAA trust resources, scientifically valid and logistically feasible to assess during the ephemeral data collection phase were identified. Protocols for collecting this data were developed and tested in the Alaska Arctic. Data collected from the field, including baseline conditions and natural variability in endpoints of interest, were used to support and refine plans for ephemeral data collection for NRDA in the event of an oil spill in the Arctic. This information significantly improves preparedness to respond to an oil spill in the Arctic and helps ensure that appropriate data, of sufficient quality and quantity to support NRDA, is collected, especially immediately during or after a spill.

TP247 Porewater Studies Subsequent to the Kingston Ash Event

B.J. Rogers, Tennessee Valley Authority; N. Carriker, TVA; R.J. Vitale, Environmental Standards; J.N. Gable, E.E. Rodgers, J.P. Kraycik, Environmental Standards, Inc.

he 2008 TVA Kingston Fossil Plant fly ash release into the Emory River near Kingston, Tennessee, provided an opportunity to study the impacts of coal ash on the environment. TVA, in conjunction with university researchers, sampled porewater from the top few inches of sediment at various locations on the Emory and Clinch Rivers from November 2009 through 2010. Three sampling techniques were used to collect porewater samples. Early sampling events utilized on-boat filtration of well-mixed sediments using a battery-operated vacuum pump and 0.45 micron filters. Also in 2010, porewater samples were collected from the relic ash cell and active ash processing areas by using a hydraulic push tool to insert a well screen into the top meter of the groundwater. Porewater samples were collected through a similar filter via a peristaltic pump. Thirdly, sediment was sampled in a box corer, brought near the surface of the water and sub-sampled into sealed tubes to protect the sediment from exposure to air. Samples were delivered to a laboratory for centrifugation and filtration in an inert atmosphere. Porewater concentrations were substantially lower for arsenic and selenium than those for those filtered while exposed to the atmosphere. It is not known whether this represents an effect due to oxidation of arsenic and selenium species during sampling or whether it represents natural attenuation following completion of dredging in May 2010. Temporal and spatial information for arsenic, selenium, iron and various other elements in the porewater are presented and compared to solid phase concentrations.

TP248 Preliminary environmental fate and toxicity assessment of Navy tactical fuels produced from alternative sources

G.H. Rosen, SPAWAR Systems Center Pacific / Environmental; R.D. George, US Navy, SPAWAR Systems Center Pacific / Environmental; R.D. George, US Navy, Spawar Syst Systems Center 2361; R. Dolecal, San Diego State University; M.A. Colvin, SDSURF/SPAWAR

The United States (US) Navy is currently qualifying for use fuels produced from alternative sources to help power its aircraft and ships in what is often referred to as the 'Great Green Fleet'. The environmental fate and effects of these alternative sourced fuels, potentially introduced to marine environments through transport, storage, and spills, however, is unknown. Concurrent laboratory-based experiments were conducted with both conventional (petroleum-based) and recently procured alternative sourced fuels under consideration by the US Navy's Fuels Program. Conventional fuels included petroleum-based jet fuel (JP-5) and ship diesel (F-76), while alternative equivalents were derived from *Camelina* seeds (Hydroprocessed Renewable Jet; HRJ-5) and algae (Hydroprocessed Renewable Diesel; HRD-76), respectively. The Navy is conducting performance testing as 50/50 blends; therefore, toxicity tests were conducted both with each fuel type separately, and as 50/50 mixtures of the relevant conventional and alternative fuels. Uncontaminated seawater was saturated with neat fuel and then isolated prior to chemical analysis and toxicity evaluations performed on the fuel-saturated seawater phase. Toxicity tests were conducted with common marine test organisms and endpoints, including mysid shrimp (*Americamysis bahia*) and topsmelt (*Atherinops affinis*) larvae in acute tests, and mussel (*Mytilus gallprovincialis*) and purple sea urchin (*Strongylocentrotus purpuratus*) embryos in chronic/sublethal tests. Initial results indicated marked differences between conventional fuels (highly toxic) and alternative sourced fuels (generally non-toxic). Initial chemical analysis of seawater extracts supported the toxicity results, with substantially lower concentrations of volatile organic compounds and other hydrocarbons (e.g., alkanes) in alternative sourced fuel extracts relative to conventional extracts. Initial and pending toxicity studies will be used to help the Navy identify sustainable solutions towards energy sustainability and security. Pending experiments will investigate the effects of natural environmental processes including microbial- and photo-degradation on the bioavailability and toxicity of alternative fuels on marine life, as well as quantification of other traditionally important fuel components, such as polycyclic aromatic hyrdocarbons, in seawater extracts used for toxicity testing.

TP250 Three Novel Brominated Flame Retardants: Potential Effects on Fecundity, Reproductive Fitness, and Maternal Transfers in Japanese Medaka

D.M. Saunders, University of Saskatchewan / Department of Toxicology; S. Wiseman, R. Mankidy, J.P. Giesy, University of Saskatchewan / Toxicology Centre

The novel brominated flame retardants (NBFRs), Bis(2-ethylhexyl)-2,3,4,5-tetrabromophtalate (TBPH), 2-ethylhexyl-2,3,4,5 tetrabromobenzoate (TBB), and 1,2,5,6 tetrabromocyclooctane (TBCO) are components of flame retardant mixtures including Firemaster 550 and Saytex BC-48. Despite the detection of these NBFRs in environmental abiotic and biotic matrices, little is known about the toxic effects of these compounds to aquatic organisms. Results of previous *in vitro* screening-level assays have reported potentials of these three NBFRs to modulate endocrine signaling. As demonstrated using the H295R assay, the three compounds caused greater concentrations of 17-β-estradiol in assay media by a maximal 2.82

fold change (TBB), 3.29 fold change (TBCO), and 5.29 fold change (TBPH) compared to solvent controls. The present *in vivo* study is a logical progression in an effects-directed investigation of these three NBFRs. The OECD 21-day short term fish reproductive assay (229) has been adapted to use Japanese Medaka (*Oryzias latipes*). Twenty fish, 10 male and 10 female, were fed greater (15 µg/g food) or lesser (1.5 µg/g food) doses of TBCO, and greater (1500:1500 µg/g food) or lesser (150:150 µg/g food) doses of a TBPH/TBB mixture. Production of eggs, a direct measure of fecundity and reproductive fitness, was measured and contrasted between control-, and greater/lesser doses of both dosing mixtures for the duration of the 21-day exposure. Secondary sexual characteristics and behavioural abnormalities were also characterized for the duration of exposure. Molecular endpoints associated with fecundity and endocrine functioning were examined using a real-time PCR array of 36 genes along the hypothalamus-pituitary-gonadal-liver (HPGL) axis. This study is the first to investigate the potential endocrine modulation in fish exposed to TBPH/TBB and TBCO, and their effects on fecundity.

TP251 Toxicity of 1-alkyl-3-methylimidazolium chlorides to wetland and agricultural plants

H.D. Sutton, Kennesaw State University / Department of Bio. & Phys. Sciences; J.L. Wilson, S.L. Falcon, H.M. Craytor, J. Silvius, B.M. Brown, Kennesaw State University

Ionic liquids (ILs) are nonvolatile, liquid organic salts that are increasingly being used as an alternative to volatile organic compounds (VOCs) in a variety of applications. ILs have low volatility, low melting points, lack of flammability, and are easily manipulated by altering their cation and anion components. ILs are not only being considered as a safer, potentially more environmental friendly alternative to VOCs, but have the ability to be used for a wide array of industrial uses. Although ILs have not yet been released into aquatic systems, as the use of ILs increases, so does the potential for their release. As many of them are miscible with water and may be able to disrupt membranes, the potential for harm to organisms in aquatic ecosystems exists. In this study, the effects of four 1-alkyl-3-methylimidazolium chlorides of differing alkyl chain length (1-ethyl-, 1-butyl-, 1-hexyl-, and 1-octyl-), were assessed with respect to the growth rates of native wetland plants (*Asclepias incarnata, (*swamp milkweed), *Actinomeris alternifolia*, (wingstem) and *Lemna gibba*, (duckweed)), and two agricultural species (*Raphanus sativus* (radish), and *Lactuca sativa* (lettuce)). Agricultural plants were included because these are most often used as surrogates for native species in phytotoxicity testing. The response endpoints measured for the species grown from seed were shoot and root length, and shoot and root mass (wet and dry); the endpoint measured for *L. gibba* was frond growth. Alkyl chain length was found to affect toxicity. The lowest toxicity based on IC50 values occurred with the shortest alkyl chain (1-ethyl-3-methylimidazolium chloride), and toxicity increased with increasing alkyl chain length. For example, in *L. gibba*, IC50s were: 45.5mg/L, 6.05mg/L, 0.225mg/L, and 0.117mg/L, after exposure to 1-ethyl- , 1-butyl-, 1-hexyl-, and 1-octyl-3-methylimidazolium chloride respectively. Results suggest variation in sensitivity of endpoints between the agricultural species and the native wetland species.

TP252 Toxicity of the azo dyes Acid Red 97 and Bismarck Brown Y to Western clawed frog (Silurana tropicalis)

V.S. Langlois, J.J. Soriano, Royal Military College of Canada / Chemistry & Chemical Engineering; J. Mathieu-Denoncourt, Royal Military College of Canada; G. Norman, Royal Military College of Canada / Chemistry & Chemical Engineering; S.R. de Solla, Environment Canada / Wildlife Toxicology Research Section

Azo compounds are used in a variety of industrial applications, such as textile colorant. Azo dyes have been found to contaminate aquatic environments and it has been shown that these compounds could potentially be toxic or cause endocrine disruption in aquatic organisms. However, there are few data available on the toxicity of these dyes, specifically Acid Red 97 (AR97) and Bismarck Brown Y (BBY). The aim of this study was to determine the toxicity and the endocrine disrupting properties of AR97 and BBY in frogs. As fugacity modeling predicted that both compounds would sorb to sediment, sediment exposures were performed using a geometric range of concentrations (0, 1, 10, 100 and 1000 ppm). Both AR97 and BBY dyes were not lethal to *Silurana tropicalis* embryos; however BBY significantly induced malformations. Gene expression analysis of oxidative-stress and mutagen-related genes was performed in BBY treated larvae. There were significant 2-fold increases of the tumor suppressing protein p53 and heat shock protein 70 mRNA at 1000 ppm suggesting that BBY induces cellular stress in early *S. tropicalis* development. Transcripts of the heat shock protein 90 did not change. Furthermore, reproductive-related genes were assessed and a 2.1-fold change was observed in the mRNA of the steroidogenic acute regulatory protein while steroid 5 alpha-reductase type 2 and androgen receptor transcript levels did not vary among treatments. In conclusion, high concentrations of BBY lead to increased developmental defects in frog embryogenesis and early larval development.

TP253 Toxicity of Vascular Disrupting Chemicals to Developing Zebrafish

M.J. Hemmer, USEPA / Gulf Ecology Division; T.L. Tal, USEPA / Integrated Systems Toxicology Division; P.S. Harris, USEPA / Gulf Ecology Division; S. Padilla, USEPA / Integrated Systems Toxicology Division

There are ~80,000 commercially available chemicals with incomplete toxicity profiles. This highlights the need for new methods to assess compound toxicity, particularly during development. This study focuses on the vascular system, development of which is both critical to embryogenesis and sensitive to perturbations by chemical exposures. We developed a quantitative assay of vascular development in transgenic TG(kdr:EGFP)^{s843} zebrafish (*Danio rerio*) embryos and evaluated the assay using inhibitors of angiogenesis that target the VEGFR2 (PTK787) or EGFR (AG1478). PTK787 exposure resulted in specific impairment of intersegmental vessel (ISV) sprouting. In contrast, AG1478 exposure produced caudal and pectoral fin defects at concentrations below those necessary to blunt ISV morphogenesis. A grow-out study was performed to examine the functional consequence of vessel toxicity during development. We performed static exposures from 24-72 hours post fertilization (hpf) with PTK787 and AG1478 followed by a 28 day grow-out period in clean untreated water. Exposures were conducted at nominal concentrations of 0.07, 0.12, 0.22, 0.7 µM PTK787, 0.17, 0.98, 1.76, 3.14 µM AG1478 or a 4% DMSO solvent control with 3 replicates per exposure concentration and 15 fish per replicate. Embryos and larval fish were assessed for mortality, morbidity and normal development daily with growth parameters determined on day 30. Moderate to severe pericardial and yolk sac edema were observed in fish exposed to 0.22 and 0.7 µM PTK787, with significantly higher larval mortality occurring in the 0.7 µM treatment. Fish standard length and body weight were not affected by exposure to PTK787. Moderate to severe edema of the pericardium and yolk sac were also evident in fish treated with 1.76 and 3.14 µM AG1478 as well as structural abnormalities of the pectoral fins and slight vertebral curvature. Exposure AG1478 reduced survival while significantly increasing body weight at higher exposure levels while significantly decreasing standard length at the lowest concentration tested. Information provided by these studies will assist in anchoring specific toxicity pathways to vertebrate development for integration into predictive virtual tissue models.

TP254 Toxicology Pathways of Dechlorane Plus Based on Hepatic Proteome Profile of Juvenile Chinese Sturgeon (Acipenser Sinensis)

X. Liang; J. Zha, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences; G. Cheng, South Central University for Nationalities / Key Lab for Biotechnology of National Commission for Nationalities, College of Life Science; Z. Wang, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences

Dechlorane Plus (DP) is a widely used chlorinated flame retardant frequently detected in water and aquatic organisms. Previous study has revealed that DP is able to accumulate in chinese sturgeon (Acipenser Sinensis) liver and in eggs. However, the toxicology pathways of DP in aquatic vertebrates keep unknown. To improve our understanding of mechanisms of action and identify the potential protein biomarkers for DP exposure, two-dimensional electrophoresis coupled with mass spectrometry has been applied to identify proteins differentially expressed in the livers of juvenile Chinese sturgeon (*Acipenser Sinensis*) following DP exposure of 1, 10, and 100 mg/kg. After analyzing and comparing the protein profiles of treated and control groups, 35 protein spots were found significantly altered in abundance (>2-fold) and 25 spots of them were successfully identified using matrix-assisted laser desorption/ ionization (MALDI) tandem time-of-flight mass spectrometry (TOF/TOF). These proteins were mainly involved in carbohydrate metabolism, signal transduction, calcium ion binding and protein folding. The expression level of heat shock cognate protein 70 was significantly up-regulated while T-complex protein 1, Ras-related protein Rab-6B, GDP

dissociation inhibitor, and Annexin A4 were down-regulated in abundance ranged from 2 to 5 fold in 10mg/kg exposure group. Our results suggested DP significantly affected the small G-protein signal cascades, Ca2+ signaling pathway and carbohydrate metabolic process and probably had the carcinogenic potential.

TP255 Vitamins as Confounding Factors when Measuring PAHs by FACS

E.A. Pena, Seton Hall University / Biological Sciences; C.S. Bentivegna, Seton Hall University / Department of Biological Sciences

Crude oil contamination has commonly been tracked by measuring polycyclic aromatic hydrocarbons (PAHs) in fish bile and or tissues using fluorescence. Research on the planktivorous fish species, menhaden, raised questions about other types of fluorescent compounds measured in these assays- particularly vitamins A and E, which are obtained from phytoplankton. Vitamins are fluorescent compounds, and it is possible that these vitamins are confounding factors when trying to measure PAHs using fluorescence. In order to evaluate this, scanning fluorescence spectroscopy was used to detect PAH and vitamin standards alone and when combined with fish oil. Fish oil was obtained from an "over the counter brand" (Nature's Bounty), a prescription brand (Lovaza), a commercial product (menhaden oil from DayBrook Industries) and wild menhaden collected from the Delaware Bay, NJ and from Barataria Bay, LA in 2010. Vitamins and PAH standards were bought commercially. Fish oil and standards were analyzed in 75% ETOH using a SpectraMax M5. Results showed that fluorescence was a sensitive detection tool: PAH-like substances were quantified in wild fish at the ppb level. Major peaks in wild fish samples were at Em350/Ex280 and Em450/Ex350. For vitamin standards: peaks were at Em350/Ex290 for vitamin E and at Em350/Ex280 and Em450/Ex320 for vitamin A. Vitamin A and E peaks appeared to be present in wild fish oil and commercial fish oil products. However, commercial fish oil products also contained a major peak at Em450/Ex350. This peak was found in many 4-6 rings PAH standards but not vitamins. Results indicated that vitamins in fish may be confounding factors when detecting PAHs using fluorescence technologies.

TP256 Zebrafish embryo as an in vivo model for assessment of adverse effects of bisphenol A and nonylphenol

M. Yang, H. Xu, W. Qiu, C. Pan, M. Wu, Shanghai University / School of Environmental and Chemical Engineering

Bisphenol A (BPA) and nonylphenol (NP) are well-known endocrine-disrupting chemicals ubiquitous in the aquatic environment, and present an ecotoxicological risk for the health of aquatic organisms. Bisphenol A is widely employed as a plastic monomer and plasticizer in the production of epoxy resins and polycarbonate plastic. Although BPA has a relatively short half-life for bioaccumulation, it still exerts detrimental effects on the aquatic ecosystem as a consequence of large-scale production and extensive application. Nonylphenol in the environment results from the degradation of nonylphenol ethoxylates, which are highly cost-effective surfactants commonly used in industrial, institutional, commercial, and household applications. Nonylphenol is highly resistant to biodegradation and consequently can be taken up and bioaccumulated by aquatic organisms. We employed zebrafish embryos and examined the oxidative stress indices and antioxidant parameters, as well as transcription of genes related to immune response and neuron-related genes reported as neurotoxicity biomarkers in zebrafish embryos after a short-term exposure to various concentrations of BPA, NP and their mixture (BPA-NP) from 4 h post-fertilization (hpf) to 168 hpf. The exposure enhanced the production of hydroxyl radicals and lipid peroxidation in a concentration-dependent manner. However, the content of total glutathione, reduced glutathione, and oxidized glutathione, as well as the activity of antioxidant enzymes were all significantly inhibited after exposure to BPA, NP and BPA-NP, indicating the occurrence of oxidative stress. It was also revealed a concentration-dependent increase of reactive oxygen species content and an induced expression of redox-sensitive transcription factors in zebrafish embryos after exposure. Furthermore, exposure to BPA and NP significantly affected the expression of genes related to immune response and lead to transcriptional changes in neuron-related genes in fish embryos even at relatively low and environmentally relevant concentrations in zebrafish embryos following oxidative stress. Our results indicated adverse effects of BPA and NP on fish and that zebrafish embryos could be as an attractive model for studies aimed at understanding toxic mechanisms and environmental risk assessment of chemicals.

TP257 Acute and chronic toxicity of Perfluorooctane sulfonate (PFOS) to Physa pomilia

M.A. Funkhouser, Texas Tech University / TIEHH; A.D. Olson; C.J. Salice, Texas Tech University / Environmental Toxicology

Perfluorinated compounds (PFCs) are anthropogenic pollutants that persist for years in the environment and have been measured in a wide variety of biota. One of the most common PFCs is Perfluorooctane sulfonate (PFOS), which was used in fire-fighting foam formulations that were in turn used throughout Canada and the United States. Perfluorooctane sulfonate has been detected in a number of waterways and studies show PFOS can bioaccumulate and biomagnify in aquatic organisms such as macrophytes, fish, macroinvetebrates, and freshwater snails. Despite the prevalence of PFOS, toxicity data for a wide variety of taxa are not available. The purpose of this study was to characterize the acute and chronic toxicity of PFOS to the freshwater gastropod Physa pomilia. We first conducted an extensive assessment of the behavior of PFOS in our experimental system that involved static renewal replacement of treatment solutions (twice per week) in polypropylene beakers. We could reliably measure PFOS down to 10µg/L and found it to be stable in our system between water changes. Estimating PFOS toxicity to P. pomilia was achieved through three separate experiments: a 96-hour acute study on physid adults, 2-week sub-chronic study on adult snails, and a full life cycle study starting with exposure to egg masses that was continuous up through reproduction. For the adult acute study, concentrations ranged from 100-375mg/L, with a 96-h LC50 of 161.77mg/L. This is comparable to a previous report of 178mg/L for Physa acuta. In the sub-chronic exposure to adults, concentrations ranged from 0-175mg/L, with a 2-week LC50 of 94.99mg/L. The life cycle study exposure concentrations ranged from 0-90mg/L and results indicated that P. pomilia to be most sensitive when exposed as egg masses although there were apparent effects on reproductive endpoints as well. The increased sensitivity of developing life stages is consistent with other research on gastropods and may emerge as a general rule. Overall, we found Physa pomilia to be less sensitive to PFOS compared to taxa previously reported, such as Chironomus tentans. Nonetheless, the data we generated are useful in developing an improved perspective on the potential risk of PFOS to aquatic taxa.

Toxicological Mechanisms at the Cellular Level: Expanding In Vitro Techniques beyond Overt Cytotoxicity

WP001 Gap junctional intercellular communication – biomarker for mechanistic assessment of non-genotoxic effects of chemicals

P. Babica, D. Jancula, Z. Lencesova, Institute of Botany, ASCR / Department of experimental phycology and ecotoxicology; J. Trosko, B. Upham, Michigan State University / Department of Pediatrics and Human Development

Environmental contaminants can induce variety of effects at cellular level, including cytotoxicity or genotoxicity. However, alterations of biochemical and signaling pathways by non-genotoxic (epigenetic) mechanisms at non-cytotoxic doses of chemicals are also known to result in changes of cell physiology, gene expression and cell behavior that contribute to adverse and often chronic effects, such as developmental and reproductive toxicity, inflammatory diseases or cancer. Gap junctional intercellular communication (GJIC) represents a key mechanism involved in the maintenance of tissue homeostasis and it is a central regulator of cell signaling and gene expression. On the other hand, GJIC is controlled by multiple signaling pathways, whose alteration may result in dysregulation of GJIC and subsequent changes in cellular phenotype. In fact, many toxicants inducing endocrine-disrupting, inflammatory or tumor promotional effects have been also demonstrated as potent in inhibitors of GJIC, whereas chemopreventive or anticancer compounds usually upregulate or induce GJIC. Thus, GJIC can be used as excellent marker for the assessment of non-genotoxic effects of various chemicals and for further elucidation of their molecular and cellular mechanisms. *In vitro* evaluation of GJIC can be effectively employed for mechanistic understanding not only using hypothesis-driven approach but also applied in exploratory techniques as an endpoint to anchor functional genomics and proteomics analyses to relevant non-genotoxic and non-cytotoxic cellular events. This will be demonstrated on examples of several environmental toxicants (PAHs, pesticides, phthalates) and chemopreventive phytochemicals, including an *in vitro* study determining early cell responses to lower molecular weight PAHs, which linked the observed effects on GJIC to activation of signal transduction pathways and used phosphoproteomics approach to identify proteins involved either upstream or downstream of chemical-induced GJIC inhibition. Support: NIEHS grant #R01 ES013268-01A2 to Upham and MSMT grant #LH12034 "CHEMO-PREV" to Babica.

WP002 Polycyclic aromatic hydrocarbons and male reproductive system: the role of junctional intercellular communication

P. Babica, Institute of Botany, ASCR / Department of experimental phycology and ecotoxicology; P. Kubincova, A. Basu, I. Sovadinova, Research Centre for Toxic Compounds in the Environment – RECETOX, Masaryk University

Male reproductive health has become a worldwide issue in the last decades. Many disturbing trends have been observed in male fertility, such as decreasing sperm counts, deteriorating semen quality and increasing frequencies of malformations of testis and incidence of testicular cancer and an apparently growing demand for assisted reproduction in humans. Endocrine-Disrupting Chemicals (EDCs) are discussed as possible cause of these adverse trends in male reproductive health. In addition to estrogen- and androgen-receptor mediated processes, there is strong evidence that testicular cell-to-cell communication mediated by gap junctions, termed Gap Junctional Intercellular Communication (GJIC), is involved in testicular development, regulation of hormone release, cell differentiation, initiation, and maintenance of spermatogenesis. Thus, inhibition of GJIC during critical stages of development may result in male reproductive dysfunction leading to infertility. Indeed, many chemicals known to be EDCs modulate GJIC and/or impair connexin expression in gonadal or non-gonadal cells. However, there is limited information on the detailed role of GJIC in adverse reproductive effects caused by specific EDCs. Recent studies indicate that anthropogenic air pollutants can possibly impair reproduction of human and wildlife. It has been reported that Polycyclic Aromatic Hydrocarbons (PAHs) on airborne particulate particles compromised sperm functions and altered endocrine hormone levels in exposed animals. Our study addressed the endocrine-disrupting potential of air pollution as a source of compounds that may alter male fertility. The inhibition of GJIC by PAHs and air samples was assessed in vitro in testicular cells, in order to determine whether PAHs could cause endocrine disruptive effects by closing gap junction channels. To clarify the specific mechanism leading to closure of gap junction channels by PAHs, we determined the expression and the phosphorylation of major connexin in GJIC in testicular cells, Cx43, and the activity of mitogen-activated protein kinase (MAPK) ERK1/2 (Extracellular-Signal-Regulated Kinases 1/2). This research was supported by the SoMoPro project number 2SGA2764 (funded from the European Community within the Seventh Framework Programme (FP/2007-2013) under Grant Agreement no. 229603) and by CETOCOEN project from the European Regional Development Fund (Z.1.05/2.1.00/01.0001).

WP003 Evaluation of whole cytochrome P450 (CYP) genes in the copepod Tigriopus japonicus as indicators for water accommodated fraction of oil pollution

J. Lee, Hanyang University / Dept of Chemistry

The inflows of crude oil by accidental spillage in marine environment cause unexpected effects on ecosystem. To evaluate effect of oil pollution using whole *cytochrome P450* (*CYP*)genes from the copepod *Tigriopus japonicus*, we cloned 52 novels *CYP* genes, and measured the responses of these genes against water accommodated fraction (WAF) of crude oil. Based on the phylogenetic analysis, *CYP* genes from *T. japonicus* were apparently separated into four different clans; *CYP2*, *CYP3*, *CYP4*, mitochondrial. Of these, several *CYP* genes belonged to *CYP2* and *CYP3* clan were significantly induced in WAF with concentration-dependent manner. Particularly, *TJ-CYP23, TJ-CYP25, and TJ-CYP33* were dramatically increased upon WAF exposure. Several responsive elements such as aryl hydrocarbon responsive elements (AhRE), xenobiotic responsive elements (XREs), and metal response elements (MRE) were observed in the promoter region of these CYP genes, supporting that these genes were highly associated with the metabolism of toxicants. We also checked the correlation of ROS with antioxidative stress enzyme activities to compare with CYP gene expressions upon WAF exposure. In this paper, we demonstrated that WAF would trigger up-regulation of the *CYP* genes that is associated with the initiation of the PAHs metabolisms originated by crude oil. This finding showed potential of *CYP* genes in *T. japonicus* as an early warning signal for oil pollution.

Applications and Advances in Omics

WP004 Characterizing Metabolomic Responses of Zebrafish following Exposure to Ethinyl Estradiol and Bisphenol A

J.P. Benskin, Axys Analytical Services Ltd.; J. Liu, Axys Analytical Services Ltd. / Metabolomics; C.C. Helbing, University of Victoria / Department of Biochemistry & Microbiology; J.R. Cosgrove, AXYS Analytical Services Ltd

Ethinyl estradiol (EE2) and bisphenol A (BPA) are common contaminants of receiving waters around waste water treatment plant (WWTP) outfalls. EE2 is used in some female contraceptive pills, while bisphenol A is an industrial chemical widely used in the production of plastics, epoxy resins, coatings, and papers. Both EE2 and BPA are xenoestrogens and pose a risk to certain aquatic organisms, even at low (ng/L) concentrations. In the present work, the whole-body metabolomic response of zebrafish embryos was characterized following 24h sub-lethal exposure to EE2 or BPA. The objective was to evaluate whether exposure to BPA or EE2 could be distinguished by metabolite profiles, potentially leading to metabolomic fingerprinting of specific contaminant exposures in complex mixtures (e.g., WWTP effluent). In addition, we investigated if the metabolomic response was unique to exposure at a given developmental stage and whether a threshold dose existed for eliciting particular metabolomic responses, akin to the no observed transcriptional effect level (NOTEL) concept. Five biological replicates of 60 AB/Tubigen Zebrafish embryos were exposed at each of two developmental stages (24hpf or 96hpf) with up to three different concentrations of test substance. Following 24h exposure, embryos were flash frozen in liquid nitrogen, and then solvent-extracted using a bead blender. From the resulting extract, 186 metabolites were measured (semi-) quantitatively, including acylcarnitines (40), amino acids (21), glycerophospholipids (90), ∑hexose, sphingolipids (15), and biogenic amines (19) using a kit-based approach with analysis by flow injection- or liquid chromatography-tandem mass spectrometry (FI-MS/MS or LC-MS/MS, respectively). Statistical and pathway analyses were carried out using MetaboAnalyst 2.0. These data form the basis of a library linking contaminant exposures to metabolomic responses in zebrafish with applications in chemical toxicity testing, mode-of-action studies, early drug-development, and helping direct chemical analysis in complex environmental samples.

WP005 Plasma protein profiles of White Sucker (Catostomus commersonii) sampled from the Athabasca River, Upstream and Downstream of Oil Sands Development

D.B. Simmons, Denina Simmons Scientific Consulting / Aquatic Contaminants Research Division; J.P. Sherry, Environment Canada / Water Science & Technology Directorate

Oil extraction from the bitumen deposits in the Athabasca Oil Sands region in Northern Alberta generates large volumes of contaminant waste in the form of tailings and oil sands process-affected waters (OSPW). There are questions about whether OSPW or their components might enter the Athabasca River, which drains into the Peace-Athabasca Delta, at concentrations sufficient to cause effects in the biota. Shotgun proteomics can be used to assess the health of animals, to determine protein biomarkers that are specific to environmental exposures, and also to characterize unique mechanisms of action of contaminants. As part of a broader wild fish health assessment, we have successfully developed and applied shotgun proteomics to generate protein profiles from plasma of mature male and female white sucker taken from the various sites along the main stem of the Athabasca River in 2011 and 2012. The study sites were located within and outside of the oil sand deposit including a site downstream of Fort McMurray but above the oil sands operations, and two sites downstream of the oil sands extraction facilities. Over 6000 proteins were identified across all sampling locations for both years. On average, 376 ± 96 proteins were identified in plasma from each location in both years. Gene names corresponding to those identified proteins were analyzed using interactive pathway software (Ingenuity Systems, Inc.) to determine their core functions and to compare the datasets by location, year, and sex. There were over 500 unique proteins expressed only in fish sampled from the locations downstream of the oils sands development. Those unique proteins were significantly ($p< 0.01$) related to neurological disease, skeletal and muscular disorders, and development disorders. The data suggest that wild fish residing downstream of oil sands operations may be exhibiting unique, site-specific responses in terms of their plasma protein profiles compared to fish residing upstream of oil sands operations. We propose to use these proteomics data to elucidate mechanisms of potential health effects in the parent fish populations, or to suggest additional health metrics for assessment where applicable.

WP006 Sex differences in the biotransformation of fluoranthene in fathead minnows

E.N. Linard, Clemson University / Institute of Environmental Toxicology (EN-TOX); S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX); P. van den Hurk, Clemson University / Biological Sciences

Similar to many polynuclear aromatic hydrocarbons (PAHs), when fish are exposed to fluoranthene it is quickly metabolized in the liver and the metabolites are excreted from the body through urine and bile. The highly fluorescent nature of fluoranthene allows for it be monitored in the bile via a microplate fluorescent reader and general trends of biotransformation can be observed. Fluoranthene is phototoxic and ubiquitous in the aquatic environment and is phototoxic although there is a considerable lack of information on the biotransformation of fluoranthene, particularly in fish. Previous research has indicated that unlike most other PAHs, fluoranthene may actually be an inhibitor rather than an inducer of cytochrome P-450, CYP1A. In light of this, fluoranthene is most likely metabolized by other CYP isoforms, although no published studies have confirmed this. In hopes to gain more insight on the enzyme largely responsible for the biotransformation of fluoranthene, fathead minnows were exposed to varying concentrations of fluoranthene for 15hrs. Through preliminary exposures, a significant and unpredicted trend was observed, indicating a potential sex difference in the biotransformation of fluoranthene. Using bile as an exposure biomarker, we found that female fish had a significantly higher level of fluoranthene metabolites in their bile than male fish. Furthermore females exhibited a dose-response while males only exhibited a response to exposure although dose was not a factor. After analysis of the bile we further investigated the apparent gender differences by running EROD and CYP3A induction assays on S9 liver fractions of the exposed fish. Based off the EROD data, there is no significant difference of CYP1A activity between sexes nor was there a dose-response. The results indicated that, as hypothesized, CYP1A is not responsible for the majority of the biotransformation of fluoranthene although it may be a weak substrate for the enzyme. With the use of a CYP3A induction assay we were able to determine if it is the CYP isoform largely responsible for fluoranthene metabolism and potentially explain the apparent gender differences observed.

Ecological Consequences of Exposure to Pharmaceuticals: From the Laboratory to the Field

WP007 Dynamic Energy Budget (DEB) modeling approach for linking sublethal effects from individuals to the population level over multiple generations

K. Lamichhane, University of North Texas / Environmental Science; D. DeAngelis, University of Miami; T.W. La Point, University of North Texas / Institute of Applied Science; T. Jager, Vrije Universiteit / Dept. of Theoretical Biology

Trace amounts of pharmaceutical chemicals are ubiquitous in environmental matrices, and pose potential risks to aquatic ecosystems. To fully understand ecological risk of selective serotonin reuptake inhibitor (SSRI-sertraline) exposure to *Ceriodaphnia dubia,* we implemented a process-based dynamic energy budget (DEB) model of life history traits to link individual-level stress to population level effects over three successive generations. We observed sertraline exposure to *C. dubia* delayed reproduction with significantly fewer and smaller-sized offspring per female across three generations. Model output shows energy allocation has been shifted from growth to reproduction for sertraline exposures of ≥0.47 μg/L. Therefore, the DEBtox model enables one to detect sub-lethal effects of sertraline exposure on the allocation of energy to the life history processes of the organism. Furthermore, the DEBtox model demonstrates that multigenerational effects should be considered in chronic exposure studies, because standard toxicity test do not take account of the increases in sensitivity and loss of tolerance in successive generations to toxicants.

WP008 The development of a model to predict the adverse effects of SSRI antidepressants on fish brain chemistry and predation behavior

L.E. Sweet, Institute of Environmental Toxicology, Clemson University; J.H. Bisesi, Clemson University / Institute of Environmental Toxicology (CU-ENTOX), University of Florida / Department of Environmental and Global Health; K.M. Beckhorn, US Food & Drug Administration / Center for Veterinary Medicine; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX)

Widespread use of antidepressant pharmaceuticals and their incomplete removal from waste streams has led to the detection of these compounds in the aquatic environment. Our previous research has shown that aqueous exposure to serotonin reuptake transporter targeting antidepressants decreases brain serotonin levels in hybrid striped bass (*Morone saxatilis x. M. chrysops*). Exposure to these antidepressants also increases the time it takes hybrid striped bass to capture four prey (*P. promelas*). Finally, we have demonstrated that this negative relationship between brain serotonin levels and time to capture prey is quantitative and provides evidence of additivity with an antidepressant mixture. In the current study the receptor binding affinity (K_i) of the selective serotonin reuptake inhibitor (SSRIs) antidepressants fluoxetine, sertraline, citalopram, and venlafaxine were quantified using radioligand binding assays on a hybrid striped bass brain homogenate. Radioligand transport assays were also performed on a mammalian cell line transfected with the zebrafish serotonin reuptake transporter. The ultimate goal of this work was to develop a model to predict changes in brain serotonin levels and time to capture prey upon exposure to an environmentally relevant mixture of antidepressants. To develop the model, previous data from exposures to venlafaxine and fluoxetine were used. The independent variables for the multiple regression model were each antidepressant's binding affinity, plasma concentration, and binding affinity*plasma concentration. A preliminary model using the environmentally predicted plasma concentrations predicted changes in serotonin levels (R^2=.71) and time to capture prey one (R^2=.97). Plasma concentrations were confirmed with exposure to venlafaxine, fluoxetine, sertraline, and citalopram. The resulting model was used to predict the effects of sertraline and citalopram individually, as well as environmentally relevant exposures to mixtures of several SSRIs. These predictions will be tested in future work to further validate the model. Development of this model provides a way to extrapolate data from the labratory to predict adverse effects in the field through examining the effects of antidepressants on bioavailability, binding affinity, brain chemistry and predatory behavior.

WP009 FDA CVM Environmental Risk Assessment: Case Studies for Animal Drugs

W.S. Hunter, US Food and Drug Administration / Center for Veterinary Medicine; H. Zahner, US FDA / Center for Veterinary Medicine; K.M. Beckhorn, E.M. Silberhorn, US Food & Drug Administration / Center for Veterinary Medicine

As part of the pre-market approval process for a new animal drug, the Food and Drug Administration (FDA), Center for Veterinary Medicine (CVM), must determine whether the future commercial use of the drug will result in significant environmental impacts. This is done to fulfill the requirements of the National Environmental Policy Act (NEPA), which requires all US Federal agencies to consider the environmental effects of their actions. Under FDA regulations, agency actions on drug approval applications require the preparation of an environmental assessment (EA) or a claim of categorical exclusion from the need to prepare an EA. This presentation will focus on case studies of EAs that have been prepared for the approval of animal drugs for aquaculture and terrestrial animals, including antimicrobials and antiparasitics. An EA is a document, typically prepared by the sponsor of the drug application, that summarizes the results of a science-based risk analysis. This analysis is used to determine if significant impacts to the quality of the human environment are expected as a result of the investigational or commercial use of an animal drug. The information included in an EA for animal drugs may include physico-chemical, environmental fate, and toxicity/effects data. After the approval of an animal drug, the EA becomes a publically available document. The case studies presented will demonstrate the risk assessment process used by CVM to make informed decisions regarding the potential environmental impact of animal drugs.

WP010 Nutrients, pharmaceuticals, and antibiotic resistance genes in wastewater after wetland treatment: A case study at Grand Marais, Manitoba, Canada

J. Anderson, Department of Chemistry; J.C. Carlson, University of Winnipeg / Richardson College for the Environment; J.E. Low, University of Winnipeg / MSc student; J. Challis, University of Manitoba; C.S. Wong, University of Winnipeg / Richardson College for the Environment, Richardson College for the Environment, University of Winnipeg; C.W. Knapp, University of Strathclyde; M.L. Hanson, University of Manitoba / Department of Environment and Geography

The discharge of complex mixtures of nutrients, organic micropollutants, and antibiotic resistance genes (ARGs) from treated municipal wastewater into freshwater systems are global concerns for human health and aquatic organisms. In the rural community of Grand Marais, Manitoba, Canada, wastewater is treated passively in a sewage lagoon prior to passage through a treatment wetland and subsequent release into surface waters. Using this facility as a model system for wastewater lagoon and constructed wetlands wastewater treatment, particularly in the Canadian Prairies, the two aims of this study were to assess the presence of nutrients, micropollutants (i.e., pesticides, pharmaceuticals), and ARGs in lagoon outputs; and their potential removal by the treatment wetland prior to release to surface waters in 2012. Concentrations of nitrogen and phosphorus species were greatest in the lagoon and declined with movement through the wetland treatment system. Pharmaceutical and agricultural chemicals were detected at concentrations in the ng/L range. Concentrations of these compounds spiked downstream of the lagoon following discharge and attenuation was observed as the effluent migrated through the wetland system. Results suggest that the wetland attenuated atrazine and carbamazepine significantly. Hazard quotients calculated for micropollutants of interest indicated minimal toxicological risk to aquatic biota. There was no significant targeted removal of ARGs in the wetland and our data suggest that the bacterial population in this system may have genes imparting antibiotic resistance. Overall, our results suggested that this treatment system was operating to eliminate wastewater contaminants including pharmaceuticals, despite shortcomings in operations (e.g., short-circuiting of the wetlands flow path from sediment build-up over the years). However, restoration of the original flow path would decrease anoxia and ammonia at hot-spots within the wetland.

WP011 Effects of long-term exposure to bezafibrate on steroidogenesis and reproduction of Japanese medaka (Oryzias latipes)

N. Ha, Seoul National University, Graduate School of Public Health; S. Lee, K. Choi, Seoul National University / School of Public Health

Fibrates are commonly used lipid regulator to control hypercholesterolemia in humans, and have been frequently detected in the freshwater environment. Bezafibrate is one of lipid regulators and has been detected in streams at up to 134.3 ng/L. Bezafibrate has greater tendency of bioaccumulation and persistence due to its high lipophilicity (LogKow 4.25). Since lowered plasma cholesterol will eventually affect steroidogenesis, fibrates have been suspected to cause endocrine disruption. However, the effect of fibrates on fish reproductive endocrinology remains unclear. The present study was carried out to evaluate the effect of endocrine disruption, and to investigate underlying mechanisms emplying Japanese medaka (*Oryzias latipes*) following long-term (>5 months) exposure to bezafibrate. Four replicates with 15 fertilized eggs each were exposed to various concentrations of bezafibrate (0.01, 0.1, 1, 10 and 100 mg/L), and were carried out for 165 days. The effects on development and reproduction success were measured, as well as changes in expressions of mRNAs involved in blood plasma sex hormone regulation. After 135 days exposure, adverse effects in F1 performance, e.g., a reduction in hatchability and a delay in hatching were observed. At 165 days post-hatching (dph), concentration of T in plasma displayed a decreasing trend in both male and female fish. Some steroidogenic genes in the gonads such as *CYP19* or *VTG1* were down-regulated at 100 mg/L. The observation of this study suggests that reproduction effect of bezafibrate was associated with changes of genes regulating sex steroid hormonal, steroidogenesis, and lipid regulation, but at the levels orders of magnitude greater than those observed in the ambient water.

WP012 Characterization of transcriptomic responses to cyclooxygenase inhibitors ibuprofen, indomethacin and celexocib in fathead minnow

J. Heitzman, University of Saint Thomas; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology; E.J. Durhan, US-EPA / Mid-Continent Ecology Division; A.C. Mehinto, Southern California Coastal Water Research Project / Department of Physiological Sciences and Center for Environmental and Human Toxicology; D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; D. Martinovic, University of Saint Thomas / Dept. of Biology, USEPA, Mid-Continent Ecology Division

Prostaglandins are a class of hormones important for the regulation of gonadal steroidogenesis and ovulation in vertebrates. In fish they are also used as pheromones; they are released by ovulating females and are important for the initiation and synchronization of male reproductive behaviors. Non-steroidal anti-inflammatory pharmaceuticals (e.g., aspirin) are designed to inhibit cyclooxygenase (COX) enzymes which catalyze prostaglandin synthesis. Effects of COX inhibitors on fish physiology, and molecular mechanisms of their action (MOA) are not well understood. In the present study, we examined effects of a waterborne, short-term exposure (96 h) to three COX inhibitors (indomethacin (INDO), ibuprofen (IBU) and celecoxib (CELE)) on gene transcription in the ovaries of sexually mature fathead minnows (n=6-8 per treatment), using commercially available oligonucleotide microarrays (4 x15K platform). Differentially transcribed genes were identified (t-test, $p < 0.01$), and functional analyses performed to determine enriched ontology (GO) categories (JMP Genomics v4.1) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. We found that three COX inhibitors altered similar GO processes (e.g., muscle contraction, sodium transport) suggesting common MOA of these chemicals in fish. INDO had the most prominent effects on gene transcription (circa 2000 genes were altered versus 300 in IBU and CELE treatments). KEGG pathway analyses show that INDO had extensive effects on oocyte meiosis and muscle contraction processes, which is consistent with physiological roles of prostaglandins in the ovary. Transcriptomic data was congruent with apical endpoint data – INDO caused severe suppression in plasma prostaglandin F2 alpha concentrations, and reduced ovarian COX activity, whereas IBU and CELE did not. These findings will be used for development of adverse outcome pathways for this important class of endocrine-active chemicals.

WP013 Bioaccumulation of Pharmaceutical Compounds and Endocrine Disruptors in Fish from four Mediterranean Rivers

B. Huerta Buitrago, Catalan Institute for Water Research (ICRA) / Department of Water Quality; A. Jakimska, Gdansk University of Technology / Department of Analytical Chemistry; S. Rodriguez-Mozaz, Institute for Water Research (ICRA), University of North Carolina at Chapel Hill / Environmental Science and Engineering; D. Barcelo, IIQAB-CSIC, ICRA / Environmenta Chemistry, ICRA / Catalan Institute for Water Research, IDAEA-CSIC / Environmental Chemistry Department

The presence of emerging pollutants such as pharmaceuticals and endocrine disruptors (EDCs) in the aquatic environment is an ever-increasing issue of concern. Despite their usual low concentrations in water and their very diverse physico-chemical properties, some studies have pointed out the possibility of bioaccumulation of these compounds in exposed aquatic organisms inhabiting contaminated waters. New and sensitive multi-residue analytical method methods based on specific sample pretreatments followed by ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS/MS) were developed for the determination of twenty pharmaceuticals and nineteen endocrine disruptors and related compounds belonging to different classes in various fish species. A monitoring study was performed in 4 different Mediterranean rivers inSpain (Llobregat, Xucar, Ebro and Guadalquivir), where water, sediments and biota were analyzed to determine the occurrence of the different emerging pollutants. Nine pharmaceuticals (carazolol, carbamazepine, citalopram, clopidogrel, diclofenac, propanolol, salbutamol, and venlafaxine) were determined in fish at concentrations higher than MDLs (0.03–0.50 ng/g) and lower than 15.4 ng/g (maximum concentration found for diclofenac). As regards EDCs and related compounds, 11 compounds (bisphenol A, estrone, triclosan, caffeine methylparaben, propylparaben, ethylparaben, benzylparaben, tolyltriazole, TBEP and TCEP) were found in fish between MDLs (0.005-9.26 ng/g) and 224 ng/g (maximum concentration found for bisphenol A). Overall most ubiquitous compound were diclofenac, benzylparaben, methylparaben caffeine and TBEP, which were detected in fish samples from all rivers studied in the 9%, 22%, 42% 46% and 74% of the total number of samples analyzed (xx fish samples) respectively. Llobregat was identified as the most polluted river, where high concentrations were measured in fish homogenates.

WP014 Formation of stable transformation products of pharmaceuticals in the water treatment cycle

J. Weiss, Institute for Environmental Studies, VU University; E. Baginska, C. Lutterbeck, R. Bolek, Leuphana University; V. Boireau, Veolia Environnement Recherche et Innovation SNC; B. Roig, Ecole des Hautes Etudes en Santé Publique; K. Kuemmerer, Leuphana University; M. Lamoree, Chemistry & Biology, Institute for Environmental Studies, VU University

Risk assessment of pharmaceutical products in the environment is currently covered by high uncertainties, due to, on the one hand, the lack of data (in particular of long term exposure and toxicity) and, on the other hand, the lack of consideration of additional parameters such as the exposure to mixtures and the presence of metabolite and/or transformation products (TPs). After human consumption, pharmaceutically active substances can be excreted and enter the effluent treatment facilities. Often degradation in sewage and water treatment and the environment is incomplete, resulting in the formation of transformation products. Within the Pharmas project (EU grant agreement no. 265346) three anticancer drugs were evaluated for the formation of stable transformation products. The compounds selected are 5-Fluorouracil, Imatinib and Cyclophosphamide. Treatment processes include the most commonly used ones i.e. chlorination, and ozonation for drinking water and UV and Xenon lamp disinfection together with biodegradation for sewage treatment. Our study revealed that 5-Fluorouracil was degraded by chlorination, ozonation and UV lamp treatment. No TPs were identified although their presence is likely after ozonation and UV. Imatinib was degraded by chlorination, ozonation, Xenon and UV lamp treatment. One TP was isolated after chlorination. Cyclophosphamide was partly degraded by ozonation, and the results are inconclusive regarding biodegradation. Two TPs were isolated after ozonation and biodegradation. Cyclophosphamide was not degraded fully by any treatment. The identity of the masses isolated and suspected to be TPs will be further investigated. The samples with a mismatch between the degradation degree and the measured TOC will be searched for additional masses. The knowledge on specific transformation product formation pathways, the molecular identity and (eco)toxicological behavior is expected to ultimately lead to recommendations for the targeted design of pharmaceuticals with improved degradation and elimination properties, whilst maintaining their therapeutic value.

WP015 Xenobiotic Metabolism and Toxicity Effects of Chemical Mixtures in Daphnia magna

N. Sengupta, Clemson University / Department of Biological Sciences

Daphnia are probably the most commonly used toxicity test organisms. HR96 is a promiscuous endo- and xenobiotic nuclear receptor thought to induce phase I – III detoxification enzymes, and an ortholog of CAR/PXR found in vertebrates. *Daphnia* HR96 is activated by chemicals such as atrazine, chlorpyrifos, pyriproxyfen, estradiol, and linoleic acid (LA) (n-6 fatty acid). Triclosan and the n-3 fatty acid, docosahexaenoic acid (DHA) inhibit HR96. We performed acute toxicity tests with triclosan, atrazine, DHA and LA. LC50s to the polyunsaturated fatty acids are typically about 10 mM, with DHA showing the greatest toxicity. Triclosan and atrazine have LC50's of 0.84 and 78 mM, respectively. We hypothesize that inhibitors of HR96 may block the protective responses of HR96 and in turn cause synergistic toxicity. Acute mixture toxicity tests were performed using an HR96 inhibitor (DHA or triclosan) coupled with an HR96 activator (LA or atrazine) in each assay. Surprisingly, results demonstrated that atrazine (20-40 mM) decreases the toxicity of triclosan and DHA, presumably by activating HR96 and inducing protective enzymes. However, LA, a weaker HR96 activator, did not provide protection from triclosan or DHA. We hypothesize that atrazine is protective because it activates HR96 and induces phase I-III enzymes, providing a potential mechanism by which some xenobiotics and dietary components could alter an individual's sensitivity to specific chemicals. We are currently investigating triclosan metabolism in *D. magna*, and whether atrazine pre-exposure increases triclosan metabolism. In summary, the HR96 activator, atrazine reduces the toxicity of triclosan and DHA, presumably by inducing phase I-III detoxification enzymes. HR96 inhibition or activation by individual chemicals or mixtures can elicit different responses in *Daphnia,* demonstrating the organism's sensitivity to toxicant stress. Activation of HR96 leading to induction of metabolic enzymes could be a key to providing protection from certain environmentally relevant chemicals.

What Do We Know About the Ecological Risk of Personal Care Product Ingredients?

WP016 Simultaneous Determination of Polar Pharmaceuticals and Personal Care Products (PPCPs) in Avian Liver and Kidney

R. Tanoue, Ehime University / Center for Marine Environmental Studies; K. Nomiyama, Center for Marine Environmental Studies (CMES), Ehime University; T. Hayashi, Tochigi Prefectural Museum; T. Isobe, Ehime University / Center for Marine Environmental Studies (CMES); H. Nakamura, R. Shinohara, Prefectural University of Kumamoto / Graduate School of Environmental and Symbiotic Sciences; S. Tanabe, Ehime University / Center for Marine Environmental Studies (CMES)

Because of their continuous loading to aquatic systems through effluent discharges of wastewater treatment plant (WWTP), pharmaceuticals and personal care products (PPCPs) are considered as pseudo-persistent contaminants. In addition, *in vitro* and *in vivo* studies have recently shown that certain pharmaceuticals such as antidepressants have greater potential for bioaccumulation in aquatic biota than terrestrial mammalian species and as a result, residues and adverse effects of PPCPs on fish and also fish-eating birds are becoming problems of deep concern. In the present study, a sensitive and accurate isotope dilution method was developed for the simultaneous determination of 21 hydrophilic PPCPs (log K_{ow} = 0.96–6.07); 14 pharmaceuticals and 7 personal care products (PCPs) in avian liver and kidney samples. The proposed method involved extraction after enzymatic hydrolysis, followed by clean-up on silica gel chromatography and gel permeation chromatography, and analysis by ultra-high-performance liquid chromatography with tandem mass spectrometry (UHPLC–MS/MS). The method yielded good absolute recoveries (62–98%) and internal standard-corrected recoveries (82.3–135%) for 21 PPCPs. The repeatability (intra-batch) and within-laboratory reproducibility (inter-batch) were less than 15% at three spiked levels. Method detection limits were between 0.14 and 3.3 ng/g, and limits of quantification were between 0.088 and 8.5 ng/g. The developed method was applied to determine 21 PPCPs in liver and kidney of 9 avian species collected from Japan, and also in liver of 5 crucian carps collected in receiving water area of WWTP effluent at Kumamoto, Japan. One

pharmaceutical chemical and 4 PCPs; crotamiton (anti-itch agent), methyl paraben (preservative agent), N,N-diethyl-m-toluamide (DEET; insect repellent), triclosan, and triclocarban (antibacterial agent), were detected in fish-eating birds. It should be noted that, methyl paraben with low lipophilic property showed the highest residual level, suggesting that not only chemical lipophilicity but other factors also are involved in bioaccumulation. On the other hand, 11 of the 21 compounds were detected in crucian carp liver, and the highest residual level was found for triclosan, followed by diphenhydramine and DEET. To our knowledge, this is the first report on the comprehensive analytical method for parabens, antibacterial agents, DEET and pharmaceuticals (except for NSAIDs) in avian tissues.

WP017 Titanium dioxide nanoparticles affects the next generation of the water flea Daphnia magna

M. Bundschuh, Department of Aquatic Sciences and Assessment; Swedish University of Agricultural Sciences; F. Seitz, Inst. for Environmental Sciences; R.R. Rosenfeldt, University of Koblenz-Landau, Institute for Environmental Sciences; R. Schulz, University of Koblenz-Landau / Institute for Environmental Sciences

The nanoparticle industry is expected to become a trillion dollar business in the near future. Therefore, the unintentional introduction of nanoparticles into the environment is increasingly likely. However, currently applied risk-assessment practices require further adaptation to accommodate the intrinsic nature of engineered nanoparticles. Combining a chronic flow-through exposure system with subsequent acute toxicity tests for the standard test organism *Daphnia magna*, we found that juvenile offspring of adults that were previously exposed to titanium dioxide nanoparticles exhibit a significantly increased sensitivity to titanium dioxide nanoparticles compared with the offspring of unexposed adults, as displayed by lower 96 h-EC_{50} values. This observation is particularly remarkable because adults exhibited no differences among treatments in terms of typically assessed endpoints, such as sensitivity, number of offspring, or energy reserves. Hence, the present study suggests that ecotoxicological research requires further development to include the assessment of the environmental risks of nanoparticles for the next and hence not directly exposed generation, which is currently not included in standard test protocols.

WP018 Effects of acute Triclosan exposure on the gut microbiome of the fathead minnow

M.A. Lantz, University of Colorado Denver / Integrative & Systems Biology; A.M. Vajda, University of Colorado / Integrative Biology; T. Roane, C. Miller, A. Narrowe, University of Colorado Denver / Integrative & Systems Biology

Gastrointestinal microbial communities are capable of regulating the expression of many genes essential for the normal development of their vertebrate hosts. The antimicrobial chemical Triclosan, an additive in many consumer products and recently identified as an environmental pollutant, might disrupt the colonization and assemblage of complex gut microbial communities. In this study, the gut microbiome of fathead minnow (*Pimephales promelas*) were characterized before and after Triclosan exposure. Four groups of fry were subjected to a seven day Triclosan exposure: including untreated (no chemical), and solvent only controls, a "low" Triclosan group (100ng/L Triclosan) and a "high" Triclosan group (1000ng/L Triclosan). Multiple intact gastrointestinal tracts were collected at three time points for each experimental group: prior to exposure, immediately after a seven day exposure, and after a seven day recovery period after exposure had ceased. Gut-associated microbial community DNA was extracted and 150bp paired end sequencing of the PCR-amplified V4 region of the 16S rDNA was performed on the Illumina MiSeq platform. Results indicated that temporal variation of the gut microbiome was visible over the two week experiment for all exposure groups. Exposure to Triclosan in both the high and low exposure groups altered the composition of the gut flora in reproducible ways, with the differences between exposed and unexposed fish becoming more pronounced over time. Abundances of known microbial degraders of chlorinated aromatic compounds, including *Hydrogenophaga* and *Methylophilaceae* were enriched in Triclosan-treated fish, and this enrichment persisted through the recovery phase. We hypothesize that these changes in community composition in response to acute Triclosan exposure at an early developmental stage may impact the later development and phenotype of the host animal by interfering with microbially-mediated physiological processes. Future work on this project includes characterization of the metabolic consequences of an altered microbiome on host development and physiology.

WP019 Effects of dissolved organic carbon on acute developmental toxicity of triclosan to zebrafish larvae

S. Grandstrand, N. Carmosini, University of Wisconsin – La Crosse; T. King Heiden, University of Wisconsin – La Crosse / Department of Biology

A variety of organic compounds contained in personal care products have emerged as environmental contaminants of concern. One such contaminant is triclosan (TCS), a broad-spectrum synthetic anti-microbial agent that is a widely used in antimicrobial soaps, deodorants, cosmetics, and other products. Triclosan is released in effluents from wastewater treatment plants, and has been shown to be a persistent environmental contaminant with potential for bioaccumulation. We show that waterborne exposure to TCS induces Blue Sac Syndrome in zebrafish larvae, suggesting that bioaccumulation of TCS could pose a risk to wild fish populations. Further, we have shown that TCS adsorbs to dissolved organic carbon (DOC), which may make it less bioavailable to aquatic organisms. Since sorption to particles within the water column could impact its bioavailability, we hypothesized that co-exposure to TCS in DOC will reduce it's bioavailability and therefore its toxicity to zebrafish larvae. Zebrafish were exposed to various concentrations of TCS (0 – 900 µg TCS/l), DOC (0-50 mg leonardite humic acid/l), or triclosan (0 – 900 µg TCS/l) together with either 10 or 25 mg DOC/l during early larval stages of development (8-120 hours post fertilization) via static waterborne exposure. Development, hatching, and mortality were recorded daily to qualitatively assess toxicity, and lateral images of a subset of larvae were used to quantify developmental toxicity. We found that DOC does not appear to significantly decrease bioaccumulation as it only partially protected zebrafish larvae from TCS-induced toxicity. This study furthers our understanding of the risks of triclosan to wild fish populations and suggests further studies into its fate and transport are warranted.

WP020 A weight of evidence approach to the assessment of the bioaccumulation potential of triclosan in fish

S. Pawlowski, BASF, BASF SE; M.C. Capdevielle, Colgate-Palmolive Company / Environmental Occupational Health & Safety Dept.; C. D'Ruiz, Henkel Consumer Goods, Inc.; S.E. Belanger, The Procter & Gamble Company / Environmental Stewardship and Sustainability Organization; G. Carr, The Procter & Gamble Company / Quantitative Sciences; P.C. DeLeo, American Cleaning Institute; B. Montemayor, Canadian Cosmetic, Toiletry and Fragrance Association

The anti-microbial biocide triclosan (TCS) is used in various consumer products. A primary pathway into the environment for triclosan is from products which are disposed of down the drain and are processed in wastewater treatment plants. Elimination during the wastewater treatment process (via biodegradation and sorption to sludge solids) results in removal of up to 97% of triclosan. Traces of TCS may enter surface waters such as rivers and lakes. Based on its physical-chemical properties (e.g., logPow of 4.76 at pH 7), bioaccumulation in aquatic organisms cannot be excluded. Many regulatory agencies have criteria for determining whether a chemical is bioaccumulative. Such agencies often use a criterion based on the bioconcentration factor (BCF) in fish and a BCF value as low as 1000 or, more commonly, up to 5000 may result in characterization as "bioaccumulative." Two fish bioaccumulation studies are available for TCS indicating, in one case, a BCF of below 5000 and in the other, above 5000. However, both studies, which are dated, were probably accurate at the time they were carried out but may not now be suitable for regulatory purposes based on current knowledge and state of the science. Therefore, a review of other available information on fish, mammals and humans was conducted to carry out a weight-of-evidence (WoE) assessment of bioaccumulation potential in fish. Taking into account the additional data on uptake, distribution, metabolism and excretion (ADME) in fish, along with the ADME data in rodents and humans, it can be concluded that TCS is excreted from fish via both urine and feces comparable to mammals, as indicated by half-lives of below one day. These findings are further supported by QSAR calculations indicating a BCF value of 642 (log BCF 2.8) and a biotransformation half-life of 3.29 d (SRC BCFBAF v. 3.01, 20.02.2012). Taking these data into account, it can be concluded that triclosan does not bio-accumulate in food-chains because it is metabolized and excreted by fish, animals and man.

WP021 Integration of spatially explicit aquatic exposure and ecologically relevant effects assessments for Chemicals in Personal Care Products

S. Gutsell, Unilever / SEAC; A. Franco, Unilever / Safety and Environmental Assurance Centre; S. Marshall, Unilever; I. Malcomber, Unilever R&D Colworth; T. Gouin, Unilever / Safety and Environmental Assurance Centre; G. Hodges, Unilever Research; R. van Egmond, Unilever / SEAC

Recent publications, such as the EU Scientific Committees report on 'Addressing the new challenges of risk assessment', provide a vision of future chemical risk assessments where uncertainty is reduced through integrating hazard and exposure characterisation within mechanistically-based risk assessments. Our vision is to apply these mechanistically-based risk assessment approaches to identify and characterise the environmental safety impacts of Personal Care (PC) ingredients used by consumers across the globe. PC products are marketed in most geographical regions. Challenges in assessing exposure to PC ingredients are driven largely by the variation in product disposal practices as well as in receiving environments (primarily surface waters). To take account of this variation as well as the need to assess chemical risk in countries without waste water treatment we are developing capability and tools that integrate PC product purchasing estimation, population distributions and spatially explicit infrastructure models to predict environmental concentrations with less uncertainty. To match the capabilities being developed in exposure assessment, we are also developing ways to better relate toxicity to ecologically relevant endpoints using a deeper mechanistic underpinning. This will involve: Better understanding of toxicity pathways by combining knowledge of Mode of Action with physiological characteristics within and between species thereby helping to identify sensitive taxa (or traits) Use of Toxicokinetic/Toxicodynamic models to provide a basis for explaining differences in toxicity between chemicals and across different species and life stages. The use of ecological models of population growth and food web interactions to assess the potential for effects of chemicals not only on individual populations but also on interspecies relationships within or between different trophic levels. In conclusion, we aim to build a series of models that describe and predict the fate and effects of chemicals at different levels of environmental and ecological complexity. These will be applied in a tiered approach enabling appropriate levels of assessment that account for geographical location and conditions.

WP022 Risk to Aquatic Systems from Three Alternative Insect Repellent Active Ingredients

J.A. Weeks, P.D. Guiney, S.C. Johnson & Son, Inc. / Environmental Safety; P.H. Howard, SRC, Inc.

DEET (N,N-Diethyl-m-toluamide) is the predominant active ingredient used over the past 60 years in personal insect repellents. It has often been detected in surface waters in recent years as it has become a routine analyte in large-scale programs for monitoring trace contaminants in streams and rivers. Alternative repellent active ingredients have become available and now make up a significant part of total repellent usage. The potential risk to aquatic organisms from DEET and two of these alternatives (Icaridin and p-menthane-3,8-diol) are examined. DEET has other known uses, but its observed concentrations in the environment are consistent with what should be expected from widespread repellent use. p-Menthane-3,8-diol is a naturally occurring compound that also can be used as a flavoring agent. Icaridin (also called Picaridin) is a piperidine carboxylate with excellent repellent qualities. All of these active ingredients have a similar potential pathway to surface water as a result of application to skin and clothing, as they are subsequently washed off and carried in waste water. The expected loading to waste water treatment plants (WWTPs), and the expected degree of removal by these facilities is considered based on a review of field studies and modeling of WWTPs. A recent review of DEET concentrations in surface waters world-wide and modeling of all three repellents in streams and rivers throughout the United States provides a satisfactory picture of exposures for aquatic organisms. A recent review of aquatic toxicity data showed that acute endpoints are known for several genera, and some chronic values are available for both DEET and Icaridin. The toxicity data are sparse for p-menthane-3,8-diol, but the exposure is expected to be low because of its high biodegradability. The ecological risk of the repellents is evaluated by examining the relationship of the expected dose/response to observed concentrations. The assessment concludes that substitution among these active ingredients is not expected to introduce concern of effects on aquatic biota.

WP023 IFRA Environmental Standards and RIFM program advances: Update for 2013

A. Lapczynski, Research Institute for Fragrance Materials, Inc., RIFM; D.T. Salvito, Research Institute for Fragrance Materials, Inc. / Department of Environmental Science; M. Vey, IFRA

The Research Institute for Fragrance Materials, Inc. (RIFM) in coordination with the International Fragrance Association (IFRA) has been responsible for the assurance of safety of fragrance ingredients in consumer products. The environmental risk and hazard (i.e., PBT) screening of fragrance materials has been incorporated into the RIFM testing program and the results of these assessments have formed the basis of the IFRA Environmental Standards which are part of the fragrance industry's voluntary safety program. Furthermore these assessments and the new data generated have been reported in the peer-reviewed literature and at past SETAC meetings. (RIFM provides an updated report of these studies and the associated revised safety assessments at both the North American and European annual SETAC meetings.) In order to identify materials for risk assessment refinement, fragrance materials were screened using the RIFM Environmental Framework and IFRA Volume of Use Survey as reported for both Europe and North America. The Framework for this evaluation was published in Environmental Toxicology and Chemistry. The focus has been on a 'down-the-drain' emission scenario. As a result nearly 3,000 materials were screened with preliminary risk quotients estimated to rank priority materials for risk assessment refinement. In addition, hazard assessment on these materials was also performed and reviewed. Studies to refine the risk or hazard screening assessments include persistence testing (ready biodegradation tests and die-away studies), bioaccumulation, and acute and chronic aquatic toxicity. In an effort to advance the risk and hazard assessments of fragrance materials (i.e., high volume, lipophilic materials used globally), several projects are underway: the use of higher tier studies for persistence and bioaccumulation assessment, development, for priority materials, of terrestrial and sediment risk assessments, developing approaches for hazard characterization of fragrance materials derived from natural products, and considering the potential environmental risks in regions with little or no wastewater treatment.

Advances in the Estimation and Assessment of Terrestrial Bioaccumulation

WP024 Preliminary Ecological Risk Assessment of waste wood treated with preservatives, withdrawals of railroads in Brazil

T. Furley, APLYSIA Environmental Research and Solutions; A. Campagna-Fernandes, APLYSIA Environmental Consulting; T. Costa, APLYSIA; G. Martinez, G. Melo, Vale

The present study aimed characterize the chemical and ecological impact on soil and surface water of wood residue treated with creosote-based and copper chrome arsenate preservatives, withdrawals of railroads in Brazil, was elaborated specific methodology for sampling based on national standards and international work. The area to be sampled is divided into four sub-areas (A, B, C and D), which contained intact woods (which could still be reused for another function) and non-intact (stage whose degradation is presented advanced and therefore not further intended application). The residues were collected at the base, middle and top cells arranged in each area, resulting in composite samples, which were proportional to the size of areas to be sampled. The samples were chemically classified by physical and chemical analysis and ecologically through ecotoxicological tests with aquatic organisms (*Vibrio fischeri, Daphnia similis, Ceriodaphnia dubia, Pseudokirchneriella subcapitata* and *Danio rerio*) and terrestrial (*Lactuca sativa, Zea mays* and *Eisenia fetida*), exposed in isolated systems and integrated. The exposure of the waste bodies was carried out using samples solubilised extract at a concentration of 100%. The residues were chemically classified as Class IIA, namely not hazardous – None Inert man, however, environmentally, in isolated, were toxic to all aquatic species and *L. sativa*, indicating a potential risk to the environment. Moreover, the ecotoxicity of the residue was not observed separately for terrestrial species *Zea mays* and *Eisenia fetida* and, when applied to the soil in integrated systems (microcosms), who evaluated both representatives of terrestrial environment (*E. fetida* and *L. sativa*) and water (*P. subcapitata*), also showed no waste ecotoxicity. This study concluded that the methodology developed for the sampling was representative and suitable for estimating waste wood as well, enabled us to verify the need to ally isolated and integrated evaluations to establish potential risks in such studies, as advocated methodologies Ecological Risk Assessment.

WP025 Evaluation of Food Web versus Aggregate Concentration Ratio Models for Estimating Tissue Concentrations in Terrestrial Biota

J. Tang, D.R. Hart, G. Bird, A. Burt, EcoMetrix Incorporated

The concentration of a chemical in the tissue of an organism is used in environmental risk assessment to assess risk to the organism and risk to the people and animals that consume that organism. Modelling the bioaccumulation of a chemical is a challenging task because of the need to understand chemical properties, biogeochemical conditions and complex food webs. There are substantial data gaps in our knowledge of the transfer of chemicals from the environment to organism tissues. Aggregate concentration ratios, while widely used in estimating bioaccumulation in aquatic biota and soil-dwelling organisms, may not be the best approach for birds and mammals that consume food from both aquatic and terrestrial ecosystems. Two alternative approaches to estimating the bioaccumulation of chemicals in terrestrial wildlife are compared: (i) the aggregate concentration ratio or one-step model, and (ii) the food web or multi-step model. In the aggregate concentration ratio approach, the tissue concentration is estimated from the chemical concentration in physical media, such as water or soil, without explicit consideration of the food web. In the food web approach, the tissue concentration is estimated using a diet-to-tissue transfer factor and reflects the estimated intake of a chemical through ingestion of food, water, soil and/or sediment. Which of these two approaches is more appropriate for terrestrial wildlife? To respond to this question, the advantages and disadvantages of applying each approach are examined, uncertainties and data gaps in using each approach are explored, and some examples comparing the approaches are presented.

WP026 Bioaccumulation of Persistent Organochlorine Pesticides in Riparian Birds, Spiders, Mosquitoes, Oysters and Sediments of Southeastern Mexico

J.R. Herrera-Herrera, Miami University / Biology; J.T. Oris, Miami University / Department of Biology; D.E. Russell, Miami University / Biology; J. Rendon, Universidad Autonoma De Campeche; A. Tabares-Alavez, Universidad Autonoma De Campeche / Instituto de Ecologia, Pesquerias y Oceanografia del Golfo de Mexico

Assessments of the bioaccumulation of organochlorine pesticide (OCP) residues in aquatic and terrestrial biota and sediments are important to increase our understanding of potential pathways of transport through riparian food webs. We assessed OCP body residues for two species of riparian warblers, the Mangrove Warbler (a year-round resident) and Northern Waterthrush (a migrant), wolf spiders (Family Lycosidae), mosquitoes (Family Culicidae), and oysters (*Crassostrea virginica*) in the Laguna of Términos region in southeastern Mexico during the winter of 2012. OCP residues were also determined in sediments during summer 2011 and winter 2012. In decreasing order of predominance on a wet weight (ww) basis, the ranges of OCP residues for Mangrove Warbler were from below detection limit (BDL) to 160 ppm for ∑HCH, from BDL-516 ppm for ∑Endrin, from BDL-398 ppm for Methoxychlor, and from BDL-27 ppm for p,p'-DDT and for the Northern Waterthrush were from BDL-517 ppm for Methoxychlor, from BDL-505 ppm for ∑ENDRIN, and from BDL-44 ppm for ∑HCH. Wolf spiders' residues ranges were from 18.7-39 ppm for ∑HCH, from BDL-2.2 ppm for ∑Endrin, from BDL-2.12 ppm for ∑Heptachlor, and from BDL-0.5 ppm for ∑Chlordane in the mangrove habitats and from 0.1-83.4 ppm for ∑HCH, from BDL-12.6 ppm for ∑Endosulfan, from 1.1-1.5 ppm for ∑Endrin, and from 0.3-1.1 ppm for ∑Heptachlor in the tropical forest. Mosquitoes' residues ranged from 10-391 ppm ww for ∑HCH, from 59-377 ppm for Endosulfan, from 34.2-507 ppm for ∑Endrin, from BDL-477 ppm for Methoxychlor, from BDL-52 ppm for ∑DDT, from BDL-39.4 ppm for ∑Heptachlor, and from BDL-9.4 ppm for ∑Chlordane. In oysters, ranges varied from 55.2-117 ppm dry weight (dw) for ∑HCH, from BDL-41.2 ppm for ∑Chlordane, from BDL-17.2 ppm for ∑Endrin, and from 0.5-1.8 ppm for ∑Heptachlor. In sediments, ranges were from BDL-223 ppm dw for ∑Chlordane, from BDL-48.8 ppm for Methoxychlor, from BDL-25.4 ppm for ∑Endosulfan in the winter and from BDL-92.1 ppm for ∑HCH, from BDL-39 ppm for ∑Endrin, and from BDL-52.5 ppm for Methoxychlor in the summer. OCP residues varied across study categories, trophic levels and habitats. These baseline level assessments are perhaps the first measurements of OCP residues in riparian terrestrial organisms in this region and this information will support environmental management decisions.

Occurrence, Fate, and Behavior of Per- and Polyfluoroalkyl Substances in the Global Environment

WP027 A global survey of perfluoroalkyl and polyfluoroalkyl substances (PFASs) in surface soils in urban and remote locations

K. Rankin, S.A. Mabury, University of Toronto / Department of Chemistry; J. Washington, USEPA; T. Jenkins, Senior Servie America, USEPA

The heightened attention placed on perfluoroalkyl and polyfluoroalkyl substances (PFASs) over the past decades has led to their detection in many environmental and biological compartments. A detailed understanding of PFAS levels in these compartments is an important step towards elucidating the precise relationship on their transport, fate and exposure. In the present study, 64 surface soils (depth of ~10 cm) were collected from 21 different countries on all continents (North America n = 35, Europe n = 10, Asia n = 6, Africa n = 5, Australia n = 4, South America n = 3 and Antartica n = 1). Collection of the soils was performed with the help of colleagues worldwide, who were instructed to follow a specific sampling protocol to ensure homogeneity amongst the samples and minimize contaminations. Sampling sites included both urban and rural environments giving a diverse range of soil types. Soil samples were promptly shipped to the USEPA's Ecosystems Research Division laboratory in Athens, GA where they were exhaustively extracted and analyzed by LC-MS/MS. Each soil was analyzed for a suite of PFASs that included perfluoroalkyl carboxylates and sulfonates, fluorotelomer saturated and unsaturated carboxylic acids, and perfluoroalkane sulfonamides and sulfoamidoacetic acids. Analysis revealed detectable levels of PFASs ranging from pg/g to low ng/g with perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) observed to being the dominant up to ~3 and 1 ng/g, respectively. Locations nearest urban and industrial centers showed the highest levels of PFASs when compared to more rural locations. The detection of PFASs in rural locations led us to postulate about possible sources and modes of transportation. For example, a location in Conyers, GA (~30 miles east of Atlanta, GA) known to have very minimal modern activity post 1930s had detectable PFOA and PFOS of ~1.3 and 1 ng/g respectively, and implies deposition of atmospheric sources. This study provides new insight into the global distribution of PFASs in soils and considered locations not previously examined.

WP028 Aerobic biotransformation of 6:2 fluorotelomer phosphates in soil and activated sludge from a WWTP

N. Wang, DuPont / Haskell Global Centers for Health and Environmental Sciences; P.W. Folsom, DuPont; B. Wolstenholme, E.I. du Pont De Nemours & Co., Inc.; R.C. Buck, E. I. duPont de Nemours & Co., Inc. / DuPont Chemicals & Fluoroproducts

6:2 Fluorotelomer phosphates (e.g., 6:2 mono-, di-, tri-PAPs and other analogs) are analogs of 6:2 fluorotelomer alcohol [6:2 FTOH, $F(CF_2)_6CH_2CH_2OH$], and are used as surfactants for consumer and industrial applications. 6:2 FTOH-based products can enter the environment in soil and in WWTPs. Although 6:2 FTOH microbial biotransformation pathways have been elucidated, limited information is available on 6:2 fluorotelomer phosphates biotransformation potential in different environmental matrices. Hydrolysis of the phosphoester bond of 6:2 fluorotelomer phosphates may be inhibited by inorganic phosphate in the environment, and so 6:2 fluorotelomer phosphates may not biodegrade via analogous pathways similar to 6:2 FTOH. The objectives of this study were to investigate the biotransformation potential of 6:2 fluorotelomer phosphates, and to identify and quantify biotransformation products in soil and activated sludge in the presence of inorganic phosphate. 6:2 fluorotelomer phosphate mixture containing 6:2 mono-, di-, tri-PAPs and other analogs were introduced into aerobic soil and activated sludge flow-through systems (2-3 mg 6:2 FTOH equivalent kg^{-1}or L^{-1} final concentrations), respectively, to monitor their biotransformation to downstream products. The study details such as experiment setup, conditions, and LC/MS/MS analytical methods will be presented. The results show that 6:2 fluorotelomer phosphates were readily hydrolyzed to 6:2 FTOH in both soil and activated sludge, which was then biotransformed to PFBA (perfluorobutanoic acid), PFPeA (perfluoropentanoic acid), PFHxA (perfluorohexanoic acid), 4:3 and 5:3 acids [$F(CF_2)_xCH_2CH_2COOH$, x = 4, 5]. Only negligible levels (< 0.3 mol%) of PFHpA (perfluoroheptanoic acid) were observed during 6:2 fluorotelomer phosphates biotransformation in soil and activated sludge. Detailed results including time trends and molar yields of observed biotransformation

products will be presented. The data suggest that 6:2 fluorotelomer phosphates hydrolyzed readily in the presence of inorganic phosphate and then followed microbial biotransformation pathways that are similar to those of 6:2 FTOH.

WP029 Analysis for Perflourinated Compounds in Great Lake Foodwebs

D.L. Delach, Clarkson University / Environmental Toxicology; B. Crimmins, T.M. Holsen, Clarkson University

Perfloroalkyl compounds (PFCs) have a variety of properties that make them a valuable component of commercial and consumer applications, as well as persistent environmental contaminants. Since their first reported presence in biota in 2001, PFCs have been identified as bioaccumulative and found worldwide in environmental media; fish have also been identified as a source of PFCs to humans. While their sources are still being characterized, these compounds have been detected in many components of the Great Lake food webs. Kiyi, cisco, and other fish of the food web were collected from Lake Superior in 2011, from Lake Huron in 2012, and from Lake Ontario in 2013; lake trout were collected from all Great Lakes in all three years and analyzed for PFC content. Homogenates were extracted with MeOH:CAN (1:1 with 0.1% NaOH) and analyzed for a suite of 15 perfluoronated compounds. PFCs were detected in all samples, with PFOS consistently measured at the highest concentration relative to other compounds. The food web samples from Lake Superior show a general pattern in PFOS concentration where kiyi < bloater < siscowet < sculpin, and increases by an order of magnitude between bloater (0.58 ± 0 .04 ng/g w.w.) and siscowet (5.9 ± 2.0 ng/g w.w.). PFOS was detected in lake trout at concentrations as high as 94.4 ± 3.3 ng/g w.w. in Lake Superior.

WP030 Are precursors an important contributor to perfluoroalkyl acid concentrations in San Francisco Estuary? Occurrence in sediment and effluent

J.P. Benskin, Axys Analytical Services Ltd.; M.D. Sedlak, San Francisco Estuary Institute; R.D. Grace, AXYS Analytical Services Ltd; M.B. Woudneh, AXYS Analytical Services Ltd; M. Connor, East Bay Dischargers Authority; K. North, Palo Alto Public Works Department; E.G. Dunlavey, City of San Jose

Perfluoroalkyl acids (PFAAs) are ubiquitous environmental contaminants that are of increasing international concern due to their persistence, links to adverse health effects, and long-range transport potential. Sources of environmental PFAAs include a variety of commercial products and processes used over the last sixty years. In addition, PFAAs can arise from the transformation of precursor substances (i.e., PFAA-precursors) incorporated into a number of current-use consumer products. Concentrations of perfluorooctane sulfonate (PFOS) in San FranciscoBayseal serum and cormorant eggs are among the highest in the world. Source of PFOS in Bay area wildlife are unclear, but there is reason to suggest precursors may be an important source of exposure. For example, several studies have reported concentrations of ∑PFAA-precursors which are similar to, or exceed, the concentration of ∑PFAAs in samples of sediment, waste water treatment plant sludge, and storm water runoff in the Bay area. In the present work, we examined the occurrence of a variety of historical and emerging PFAAs and PFAA-precursors in effluent from three wastewater treatment facilities (WWTPs; n=3 samples/site) from the Bay Area along with sediments proximal to the WWTP effluent dispersion area (n=3 samples/site). The objective of this work was to gain a better understanding of PFAA sources in the Bay area, not only in terms of geographic location, but also the identity of substances which have the potential to degrade to PFAAs following release into the environment. Among the targets monitored were 3 perfluoroalkyl sulfonates, 9 perfluoroalkyl carboxylates, 3 perfluoroalkyl phosphonic acids, 3 perfluoroalkyl phosphinic acids, 4 (N-alkyl-substituted) perfluorooctane sulfonamide/sulfonamido acetates, 3 fluorotelomer suflonates, 2 polyfluoroalkyl phosphate monoesters, 2 polyfluoroalkyl phosphate diesters, and a perfluorooctane sulfonamide-ethanol based phosphate diester. Analysis of samples utilized 4 separate methods, each based on an acid/base extraction followed by SPE cleanup and LC-MS/MS analysis, which were validated through spike/recovery experiments. These data form the basis for a larger study examining the behavior of PFAAs and PFAA precursors in theSan FranciscoBay foodweb.

WP031 Characterizing Residuals in New and Aged Fluorotelomer Polymers in Soil

J.E. Naile, USEPA / Ecosystems Research Division; T. Jenkins, SEEP/EPA; J. Washington, USEPA

Fluorotelomer polymers (FTPs) comprise some of the major products of the fluorotelomer industry. FTPs impart anti-wetting and anti-staining properties which are invaluable to wide range of consumer products including clothing, upholstery, food packaging, and carpeting. FTPs retain monomer fluorotelomer residuals including fluorotelomer alcohols (FTOHs), which are known to degrade to perfluorocompounds (PFCs), such as perfluorooctanoic acid (PFOA). PFOA and other PFCs have been shown to be recalcitrant in the environment, bio-accumulative, and toxic. Because of the residual monomer content of FTPs, and because FTPs have been shown to degrade to form fluorotelomer monomers, FTPs constitute a source of PFCs to the environment and, consequently, there is a need to characterize the species and concentrations of fluorotelomer and perfluoro-compounds in new and aged FTPs. Methods were developed and rigorously tested to ensure sufficient extraction and cleanup for gas chromatography/mass spectrometry (GC/MS) and liquid chromatography tandem-mass spectrometry (LC/MS/MS) analyses. To evaluate possible FTP degradation, we incubated a commercially available FTP in soil microcosms and monitored the microcosms for fluorotelomer and PFC degradation products using GC/MS and LC/MS/MS. Preliminary data suggests that aged microcosms accumulated more fluorotelomer and PFCs over time than were present in the original un-aged polymer.

WP032 Elimination kinetics of perfluorohexanoic acid in humans and comparison with mouse, rat and monkey

M.H. Russell, DuPont Haskell Global Centers for Health and Environmental Sciences; H. Nilsson, Orebro University; R.C. Buck, E. I. duPont de Nemours & Co., Inc. / DuPont Chemicals & Fluoroproducts

Major fluorochemical manufacturers have developed new short-chain per- and polyfluorinated substances with more favorable environmental, health and safety profiles. This study provides the first evaluation of the elimination half-life of perfluorohexanoic acid (PFHxA) from human blood. PFHxA biomonitoring data were obtained from a recently published study of professional ski wax technicians. These data were analyzed to provide estimates of the apparent half-life of PFHxA from humans, and comparisons were made with similar kinetic results obtained from published studies of PFHxA elimination from mice, rats and monkeys. The apparent elimination half-life of PFHxA in highly exposed humans ranged between 14 and 49 days with a geometric mean of 32 days. The half-lives of PFHxA in mice, rats, monkeys and humans were found to be proportional to body weight with no differences observed between genders, indicating similar volumes of distribution and similar elimination mechanisms among mammalian species. Compared to long-chain perfluoroalkyl acid analogs, PFHxA is rapidly eliminated from biota. The consistent weight-normalized elimination half-lives for PFHxA in mammalian species indicates that results obtained from animal models are suitable for establishment of PFHxA benchmark dose and reference dose hazard endpoints for use in human risk assessments.

WP033 Is drinking water a major source of perfluoroalkyl acids to waste water treatment plants?

M. Filipovic, Stockholm University / Dept. of Applied Environmental Science (ITM); U. Berger, Stockholm University

Perfluoroalkyl acids (PFAAs) are ubiquitous in the environment including surface water and groundwater. In Sweden both types of water bodies are used for drinking water production, and the removal efficiency of PFAAs is usually very poor in this process. PFAAs or their precursors are also used in commercial household products and have been detected in dust from the indoor environment. Thus, both the drinking water itself (environmental recirculation) and contamination from indoor environments (new release) contribute to PFAA levels in the influent of waste water treatment plants (WWTPs) receiving municipal waste water. The main aim of this study was to quantify the contribution of drinking water to the total PFAA load entering a WWTP by analyzing drinking water and influent water. Furthermore, a mass balance in the WWTP was conducted by additionally quantifying the PFAAs in effluent water and sludge. The three Swedish cities Bollebygd, Stockholm and Umeå were selected in this study due to variation in the source water for drinking water production, population density and geographical location. Bollebygd and Umeå have only one major drinking

water source and one WWTP is serving each city. In Stockholm the second largest WWTP (Bromma) was selected. The Stockholm municipalities that are served by the Bromma WWTP include two major drinking water producers, both using Lake Mälaren as drinking water production source. Sampling was done during dry/cold weather conditions to avoid potential influence of street runoff. The results showed that PFAAs with 4 to 11 carbon atoms were present in all drinking water samples. For Umeå and Bollebygd drinking water accounted for a minor part of PFAAs in the influents to the respective WWTP. However, the concentrations of PFAAs in drinking water in Stockholm were higher compared to Umeå and Bollebygd, indicating pollution of the drinking water source Lake Mälaren. In such cases where the drinking water comes from contaminated surface waters, it may contribute significantly to PFAA levels in WWTP influents. WWTP effluent concentrations need to be corrected for this contribution if they are to be considered as new inputs of PFAAs to the aquatic environment. The mass balance results showed that concentrations of short chain PFAAs were lower in the WWTP influents compared to the effluents. This indicates formation of short chain PFAAs. In contrast, long chain PFAAs were removed in the WWTPs by sorption to the sludge.

WP034 Occurrence of commercial fluorosurfactants in Hong Kong marine environment

L.W. Yeung, University of Toronto / Chemistry; E. Loi, P. Lam, City University of Hong Kong / State key Laboratory in Marine Pollution, Department of Biology and Chemistry

Recent studies have reported detection of a new class of PFASs, the polyfluoroalkyl phosphoric acid diesters (diPAPs), in paper fiber, sewage sludge, and human blood sera at up to part-per-billion (ppb) levels, comparable to those of widely-used compounds like PFOA. DiPAPs are used in food contact paper and as wetting agents, and have been reported to degrade into the well-known persistent PFCAs. Mass balance analysis of our previous work on samples (e.g., gastropods, shrimps, and worms) from Mai Po Marsh Nature Reserve (MPMNR), Hong Kong showed that a relatively large portion (24 – 99.6%) of unidentified organofluorine (OF) was found in these environmental samples. Perfluoroalkyl phosphonates (PFPAs), perfluorinated phosphinates (PFPiAs), and fluorotelomer sulfonates (FTSAs) are commercial fluorosurfactants that have been newly identified and reported in different environmental matrices. It is hypothesized that these chemicals might account for a portion of the unidentified OF. To test this hypothesis, surface water, sediment, and worms were collected from the same tidal shrimp pond in MPMNR in our previous study for analysis. In order to further understand the occurrence of these commercial fluorochemicals in Hong Kong, surface water and marine sediment were collected from three locations in Victoria Harbour, the major waterway in Hong Kong, and sewage sludge was also collected from two wastewater treatment plants (WWTPs). In MPMNR, PFPiA (C6/C8: 51.6 – 1900 pg/g w.w., C8/C8: 30.8 – 295 pg/g w.w.) and 6:2 and 8:2 FTSAs were found in worm samples but not in shrimp, water, and sediment samples. In Victoria Harbour, the mean concentrations of surface water were 0.016 ± 0.012 ng/L for 6:2 diPAP, and 0.036 ± 0.045 ng/L for 8:2 diPAPs, while 6:2/8:2 diPAP was seldom detected (< 10% of samples). For sediment, the mean concentrations were 19.5 ± 20.5 pg/g d.w. for 6:2 diPAP, and 84.4 ± 192 pg/g d.w. for 8:2 diPAP. The level of diPAPs detected in Hong Kong sludge was at ppb levels, suggesting that there is extensive usage of these commercial fluorinated materials in Hong Kong. Paper products, including food contact paper (n = 21) such as French fries bag, paper bag, bread wrapping paper and burger box; and non food contact paper (p = 3) such as receipt paper from local markets were collected and analysis is being undertaken to confirm whether paper products were the sources of these newly identified commercial fluorosurfactants in Hong Kong.

WP035 Perfluoroalkyl bioaccumulation: What can we learn from fatty acids?

C.A. Ng, ETH, ETH Zürich / Institute for Chemical and Bioengeneering; K. Hungerbuehler, ETH Zürich / Institute for Chemical and Bioengineering

Fatty acids are essential to energy conversion and storage in organisms, and serve as important substrates for many bioactive compounds. Long-chain fatty acids also have very low aqueous solubility and the ability to disrupt biological membranes. Their transport and concentration are therefore tightly controlled through interactions with a number of proteins, key among them: serum albumin; membrane transport proteins such as organic anion transporters (OATs); and intracellular lipid-binding proteins such as fatty acid binding proteins (FABPs), which are ubiquitous in tissue cells. Linear perfluoroalkyl carboxylic acids (PFCAs) are essentially saturated fatty acids where the hydrogen atoms on the hydrocarbon chain have been replaced with fluorine atoms. They have been shown to interact with many of the proteins active in fatty acid metabolism, including albumin, OATs and FABPs, in a number of different species, from mammals to birds to fish. Fatty acid research may therefore help us to understand the bioaccumulation, disposition and likely targets of effects for linear PFCAs. We have recently developed a successful model for bioaccumulation of linear PFCAs and PFSAs based on these principles. However, this model has not yet been tested for non-linear PFCAs and other perfluorinated substances whose structures differ from those of fatty acids. Proteins like albumins interact with PFCAs and with fatty acids via both polar and hydrophobic interactions. Fatty acid binding sites are composed of hydrophobic 'pockets' that accommodate the hydrocarbon tail, while polar residues at the entrance of these pockets interact with the carboxylate head group. The presence of fluorine atoms on the carbon chain of PFCAs are much more hydrophobic than their fatty acid counterparts, and the chain is also much stiffer. This already changes the interaction of PFCAs with proteins, leading to different albumin conformations around these substrates. How then will the interactions change when the perfluorinated structure differs even more from that of a fatty acid—for example, for branched isomers? Here, we explore how PFAS structures, and their parallels to and dissimilarities from fatty acid structures, can inform us about their tendency to bioaccumulate in organisms and what implications this may have for the proliferation of new perfluorinated structures emerging on the industrial market.

Canadian Oil Sands

WP036 Toxicogenomic Investigation of naphthenic acids and Oil Sand Processed Water in Rainbow Trout Hepatocytes

F. Gagne, Environment Canada / Emerging Methods; C. Andre, Environment Canada; J.P. Sherry, Environment Canada / Water Science & Technology Directorate

The purpose of this study was to examine the hepatotoxicity and changes in selected gene transcripts involved in toxic stress in rainbow trout hepatocytes exposed to oil sands compounds. It is hypothesized that naphthenic acids (NAs) account for the toxicity of oil sand processed water (OSPW) in tailing ponds. To test this, primary culture of rainbow trout were exposed to different sizes of NAs (z-0 to z-10), 2 commercial mixtures of NAs, solid phase extracts (C18) of simulated OSPW and Athabasca river surface water samples upstream and downstream the oil sands development area for 48 h at 15°C. Cell viability was assessed by loss of membrane permeability, total RNA levels, and gene expression using an array of 15 genes involved in xenobiotic biotransformation (GST, CYP1A1, CYP3A4, MDR), and oxidative stress (SOD and CAT), estrogenicity (VTG, ERβ), DNA repair (LIG, APEX, UNG, and OGG), cell growth (GADD45 and PCNA), and glycolysis (GAPDH). The results revealed that individual NAs were toxic to hepatocytes at < 2 mg/L for z-0, z-4, z-6 and z-10 NAs and all increased total RNA levels below 2 mg/L. The commercial NAs were cytotoxic at 70 mg/L cells but RNA levels were decreased at this concentration. The data on the transcriptomics responses showed that the individuals NAs differed from the commercial mixture of NAs, OSPW and river water samples. OSPW toxicity was more similar to a downstream river water sample at the confluence of Ells River. OPSW most strongly affected the following gene expression changes: GST (decrease), CYP3A4 and 1A1 and DNA repair genes (APEX, LIG and ONG). The water samples at Ells River, an area of natural oil sands exhibited a similar response pattern albeit at less intensity. It is concluded that effects observed in cells exposed to OSPW could be found in river samples and the observed effects are seemingly not explained by NAs. Strong expression of CYP3A4 suggests for the presence of neutral polycyclic aliphatic hydrocarbons or heterocycles.

WP037 Genotoxicity and Mutagenicity of Oil Sands Process Water Acid Extractable Organics

N. Zetouni, J.W. Martin, University of Alberta / Laboratory Medicine and Pathology

Oil sands process water (OSPW) is a by-product of the hot alkaline water extraction process used in the surface mining oil sands industry in northern Alberta. Its constituents include residual bitumen, silts, clays, inorganic ions, and a complex mixture of toxic organic compounds that includes naphthenic acids (NA). Toxicological effects of OSPW include liver inflammation in

fish and birds, metamorphosis delay in amphibians and endocrine disruption, but the genotoxic and mutagenic effects of OSPW are not well studied. The objective of this study was to evaluate the genotoxicity of OSPW acid extractable organics, and of a commercial NA mixture, by the SOS Chromotest™ (*E. coli PQ37*) and mutagenicity by the AMES™ test(*S. Typhimurium* TA 98). In both tests, toxicity was evaluated with and without S9 fraction, and cytotoxicity was evaluated by the CellTox™ assay. For genotoxicity, a dose-dependent increasing toxicity was observed betweem 150% and 200% (% full-strength) for OSPW organics and in the range of 20-60 ppm for commercial Merichem NAs. Metabolic activation by S9 fraction increased the genotoxicity in both mixtures, implying that bio-activation was important. Results from cytotoxicity and mutagenicity assays continue to be collected, and will be presented.

WP038 Assessing the toxicity of Oil Sands region groundwaters using early life stage freshwater mussels: An examination of suitability

P.L. Gillis, Environment Canada / Aquatic Contaminants Research Division; R. McInnis, G. Bickerton, G. Bickerton, Environment Canada; J.L. Parrott, Environment Canada / Water Science and Technology Directorate

As part of the Joint Canada-Alberta Oil Sands Monitoring Plan (JOSMP), the toxicology of natural and oil sands-related environmental samples was examined. One option for monitoring potential migration of groundwater affected by oil sands process waters (OSPW) to nearby rivers is to conduct standard lab-based toxicity tests on field-collected samples of discharging groundwater. Early life stage freshwater mussels were used to assess the toxicity of groundwater collected from sites near bitumen processing facilities and tailings impoundments, compared to groundwater from sites located far from mining and processing facilities, but still within the oil sands region. Larval mussels (glochidia) were exposed to groundwater both undiluted and through a dilution series (to roughly model surface water mixing). Toxicity tests followed standard methods where the viability (ability to close valves) of the exposed organisms was determined after 24 and 48 h. Groundwater from the Ells River (far from industry facilities) and two sites on the Athabasca River (both far and near industry facilities) were acutely toxic to mussel larvae (survival < 50%). Serial dilutions revealed that diluted groundwater can also be toxic to larval mussels (approximate LC50s, 10-25% groundwater). Larval mussel toxicity was observed both in groundwater samples that originated from sites in close proximity to upgrading facilities and in some samples collected from sites far from oil sands industry. Mussel larval toxicity appears to be driven predominantly by the high salt and possibly ammonium concentrations of these groundwater samples and thus is thought to be a function of groundwater residence times and aquifer materials rather than proximity to mining and industry facilities. Because freshwater mussel larvae have a heightened sensitivity to salt, the toxicity related to naturally elevated (i.e., background) salt concentrations of some groundwaters from this region cannot be differentiated from possible effects of oil sands-related compounds (PAH, naphthenic acids). Therefore mussel larvae were deemed not to be appropriate organisms for identifying potential leaching of OSPW-affected groundwater to rivers because of their sensitivity to salts. Preliminary chronic (28 d) exposures with oil sands region river sediments and juvenile (6 month old) mussels indicate that sediment tests with juveniles are not compromised by high salt and thus are promising for employment in oil sands monitoring.

WP039 Changes in Fish Community Composition and Fish health in the lower Athabasca region: a Review of Historical and Current Data

A.N. Schwalb, University of Lethbridge / Biological Sciences; J. Rasmussen, University of Lethbridge / Deparment of Biological Sciences

There has been much debate about the environmental impact of the Athabasca oil sands operations. Fish are good indicator species for changes in aquatic ecosystems. Their community composition and their health can be used to assess responses of higher trophic levels of the food web and changes over a long time period. A considerable amount of data on the Athabasca oil sands has been accumulated in the past decades. However, a comparison of the current status with historical data is hindered by the information being scattered among numerous reports by different agencies and companies, and by differences in sampling techniques, timing of sampling, and sampling locations. We gathered and analyzed comparable data from historical reports (Alberta Oil Sands Environmental Research Program, late 1970s) and more recent reports (Regional Aquatics Monitoring Program (RAMP) and various consulting companies, since the 1990s). Preliminary results show massive declines and changes in the community composition of larger migrating species in the Muskeg River watershed, the watershed with the highest rate of land change. The highest rates of tumor-like growths in RAMP data were detected near the mouth of the Muskeg River. These rates were considerably higher compared to historical reports and background levels based on other studies in northern rivers in Alberta that examined external abnormalities of fish. An initial analysis suggests that in more recent years walleye are weighing less than expected for their length compared to the late 1970s. New data from fish fence operations will be needed to assess changes of migratory fish for watersheds for which historical data are available.

WP040 Depositional Patterns of Polyaromatic Compounds in Snow in the Athabasca Oil Sands area of Alberta, Canada

D.C. Muir, Environment Canada / Aquatic Ecosystem Protection Research Division; J.L. Kirk, Environment Canada; C. Teixeira, Environment Canada; S. May, Environment Canada / Air Quality Research Division; X. Wang, Environment Canada / Aquatic Contaminants Research Division; G. Poole, J. Charland, Environment Canada / Air Quality Research Division; S. Backus, L. Bradley, Environment Canada / Water Quality Monitoring and Surveillance Division; C. Mihele, Environment Canada / Air Quality Research Division

Atmospheric deposition has been identified as an important source of polyaromatic compounds (PACs) in the Oil Sands (OS) Region. PAC sources include OS bitumen upgraders, dust from open pit mining and land disturbances. This study was designed to confirm previous measurements, examine deposition up to 200 km from upgraders and mines, and assess sources using characteristic PAC patterns and ratios. Snow fluxes were also compared with results from monthly collections in 3 wet-only precipitation samplers installed within 10 km of the upgraders and mines. Snow was collected in March 2011 and 2012 at maximum snowpack depth and stored in sealed polypropylene buckets. Melted snow (4-5 L water) was filtered and dissolved phase was XAD-2 extracted. Filters were extracted using pressurized solvent extraction with dichloromethane. Samples were analysed for 64 PACs (29 unsubstituted (unPACs), 21 alkylated (aPAC), 6 dibenzothiophenes (DBTs)) by GC-MS, as well as for particulate organic carbon. Results were assessed by distance from a near-field site designated "AR6" by Kelly et al. located on the Athabasca River. Total(Σ) PAC fluxes ranged from 4-25 ug m^{-2} at distances of 60-85 km from AR6 but were more elevated within 0.1-22 km (450-7500 ug m^{-2}). Profiles in both snow and precipitation were dominated by C1-C4 aPACs, which represented 50-85% of ΣPAC. The proportions of DBTs in snow where higher within 22 km of AR6 (12-21%) than at 60-85 km (4-9%) while aPACs did not change significantly. Snow particulates at sites within 22 km of AR6 had >95% of the mass of ΣPACs but only 40-74% at 60-85 km. Higher methylphenanthrene /phenanthrene (PHE) (2.6-3.2) and C2-DBT/C2-PHE (0.9-1.3) ratios were found within 30 km of AR6 indicating greater petrogenic sources at near field sites. Snow fluxes of ΣPACs within 10 km of AR6 were generally higher than total fluxes of ΣPACs in Dec-Mar 2011 and 2012 precipitation (800-1250 ug m^{-2}). Greater dryfall/dust inputs in the snow likely account for the higher flux than in wet-only samplers and also explain the higher proportion of PACs on particulates at near field sites. UnPAC concentrations and fluxes in snow and precipitation within 10 km of AR6 were similar to results for urban areas in the Great Lakes region. The results were in general agreement with spatial trends in deposition of ΣPACs observed in sediment cores from the same area.

WP041 Using Naphthenic Acids and Poly Aromatic Hydrocarbons for Fingerprinting Environmental Impacts of Oil Sands Processing

M.B. Woudneh, AXYS Analytical Services Ltd / R&D Chemist; J.P. Benskin, Axys Analytical Services Ltd.; C. Hamilton, Axys Analytical Services Ltd. / Client Services; G. Wang, Axys Analytical Services; E. Krogh, Vancouver Island University / Department of Chemistry; C. Gill, Vancouver Island University; J.R. Cosgrove, AXYS Analytical Services Ltd

One of the major concerns around the rapidly growing oil sand industry in Alberta is contamination of the environment by petroleum derived contaminants such as Naphthenic Acids (NAs) and Polycylic Aromatic Hydrocarbons (PAHs). PAHs are petroleum derived toxic contaminants and NAs are a complex mixture of naturally occurring carboxylic acids which are natural constituents of oil sands bitumen. In this work analytical methodologies were developed to quantify NAs and PAHs from water and sediment samples. The environmental concentrations of NAs and PAHs were measured in paired water and sediment samples collected from Northern Alberta. The measured concentrations were used to estimate sediment-water partition

coefficients that may be used to predict the environmental behaviour and fate of these contaminants. To assist in environmental fingerprinting, principal components analysis results for both classes of compounds are generated and compared for significance. Diagnostic ratios, using paired NAs/PAHs, that may be used in fingerprinting are discussed.

WP042 Fractionation and characterization of polar organic compounds in OSPW using mixed-mode solid phase extraction coupled to mass spectrometry (Orbitrap)

H. Alharbi, Carleton University / Department of Biology, University of Saskatchewan / Toxicology Centre; S.B. Wiseman, University of Saskatchewan / Toxicology Centre; A. dos Santos Pereira, University of Alberta / Division of Analytical and Environmental Toxicology; J.W. Martin, University of Alberta / Laboratory Medicine and Pathology; J.P. Giesy, University of Saskatchewan / Toxicology Centre and Department of Veterinary Biomedical Sciences

In the surface-mining oil sands industry a byproduct of the extraction of bitumen is oil sands process-affected water (OSPW). Currently, greater than 1 billion m^3 of OSPW is held in tailings ponds. It is commonly stated that the toxicity of OSPW is caused by naphthenic acids, however this is not proven, and evidence to date only allows for the conclusion that the toxicity is caused by the complex mixture of dissolved organic chemicals. An effect directed analysis of the toxicity of OSPW is important to identify chemicals responsible for effects of OSPW on aquatic organisms. Advances in mass spectrometry techniques allow for better characterization of chemicals dissolved in OSPW, but little attention has been given to methods of sample preparation to improve effect-directed fractionation. Although liquid-liquid extraction at various pH can be used to fractionate OSPW, it is time consuming and uses large volumes of solvent. The objective of this study was to use mixed-mode sorbents (solid-phase extraction-SPE) to enhance the capacity of extraction of polar organic compounds from OSPW because they can extract analytes (charged or uncharged) selectively. OSPW was fractionated into acidic (strong and weak), basic (strong and weak), and neutral fractions using strong cation exchange, weak cation exchange, strong anion exchange, and weak anion exchange based on mixed-mode chromatography that uses polymeric sorbents/multi-model phases that combine an anionic or cationic ion-exchange with reverse phase chromatography. The five fractions were analysed by Orbitrap in negative and positive modes. Preliminary results indicated that the majority of organic compounds were in greatest abundance in negative mode in the neutral and acidic (strong and weak) fractions. These compounds matched the formulae: $C_xH_yO_z$ (z = 1 to 6); $C_xH_yO_zN$ (z = 0 to 4); $C_xH_yO_zS$ (z = 1 to 4) and $C_xH_yO_zNS$ (z = 1 to 2).The O_2 species ($C_xH_yO_2$; naphthenic acids) were the most abundant species detected in both the neutral and acidic fractions under negative ionization mode. Acute toxicity of fractions was determined by use of the Microtox® assay. Results indicated that the neutral fraction had the greatest toxicity following by acidic fractions, and basic fractions had the least toxicity.

WP043 The Effect of Different Solvents and pHs on the Extractability of Oil Sands Process-Affected Water Organic Contents

N. Sun, University of Alberta / Department of Civil and Environmental Engineering; P. Drzewicz, University of Alberta / Civil and Environmental Engineering; L. Perez-Estrada, Y. Chen, Y. Chen, University of Alberta / Department of Civil and Environmental Engineering; M. Gamal El-Din, University of Alberta / Department of Cival and Environmental Engineering; N. Klamerth, University of Alberta / Department of Civil and Environmental Engineering

The oil sand industry in Alberta has been one of the fast growing industries in the past decade in Canada. To extract crude oil from the oil sand, huge amount of water is needed, and as a result large amounts of oil sands process-affected water (OSPW) are generated and stored on site. There are concerns about the impact of the release of OSPW to the environment due to the high content of toxic constituents in OSPW such as naphthenic acids (NAs) and polycyclic aromatic hydrocarbons (PAHs). From a remediation point of view, it is required to degrade the toxic constituents in OSPW and monitor the degradation process by estimating the level of NAs or PAHs in treated OSPW. This has raised analytical challenges since OSPW is a very complex matrix associated with suspended solids, high concentration of inorganic salts, and bicarbonates. Extraction and fractionation of OSPW can sufficiently reduce the OSPW complexity by separating organic species into different portions based on their physical and structural properties. In the work presented here, various organic solvents were used to extract OSPW at different pH levels. Each organic fraction and its counterpart water residue were analyzed by UPLC coupled with SYNAPT G2 High Resolution Mass Spectrometer (HRMS) and the concentrations (mg/L) of both classic NAs and oxidized NAs were estimated for each sample. Fluorescence spectrometry was used to check the presence of PAHs. It was found that both solvent polarity and sample pH levels had significant impacts on how the analytes were extracted from raw OSPW and fractioned into small portions. pH adjusted fractionation of OSPW with dichloromethane (DCM) or hexane was also investigated along the study. The amount and type of total organic content extracted was found to be dependent on both pH values and type of solvent used.

WP044 Liquid chromatographic resolution of various isomers in oil sands process water by extended column length and elevated temperature

Z. Kun, University of Alberta; A. dos Santos Pereira, University of Alberta / Division of Analytical and Environmental Toxicology; J.W. Martin, University of Alberta / Laboratory Medicine and Pathology

Qualitative and quantitative analyses of surface mining OSPW from the Canadian Athabasca region poses a great technical challenge due to the wide variety of isomers (i.e. >200,000 individual compounds are estimated) that have proven difficult to separate by current LC-based methods. In this study, we established a high separation efficiency method by coupling several columns at high temperature, and by using the ultra-high resolution Orbitrap detector to better understand the structure of isomers that are resolved. Results showed that the theoretical plates on each column were approximately 26000 at 30°C at a flow rate of 0.5ml/min, and that plate counts remained constant at different temperatures (30°C and 60°C). At 60°C, four XSELECT CSH C18 XP columns (130Å, 2.5µm, 3mm×150mm) could be coupled to generate 105,000 effective plates at a back pressure of 718 bar. The method was tested with commercial Refined Merichem naphthenic acid oil using an ACQUITY UPLC PDA detector, and the Trennzahl (TZ) for adjacent unidentified peaks increased from 0.1 to 1.12, while the retention time increased from 16min to 64min, thus providing improved resolution of isomers for various chemical groups, albeit at the expense of increasing analysis time. Further examples will be presented from coupling of the method to Orbitrap mass spectrometry.

Ecotoxicology and Risk Assessment of Soils

WP045 Impact of agricultural management on chemical and physical soil properties

C. Parelho, University of Azores / Department of Biology; CITA-A – Azorean Biodiversity Group, Departamento de Ciências Agrárias, University of the Azores; A.S. Rodrigues, University of Azores / Department of Biology, (CVARG) Center of Volcanology and Geological Risks Assessment; J.V. Cruz, University of Azores / Department of Geosciences, University of the Azores; Center of Volcanology and Geological Risks Assessment, Ponta Delgada, Portugal; P.V. Garcia, University of Azores / Department of Biology, CITA-A – Azorean Biodiversity Group

The widespread use of agrochemicals in agricultural ecosystems has led to a growing concern about the adverse effects on human health and environment. The present study was designed to evaluate the impact of different management practices [conventional (CF), traditional (TF) and organic farming (OF)] on soil chemical and physical properties. Comparisons between farming management practices for organic matter, pH, electrical conductivity, humidity, clay-silt and trace elements (As, Ba, Cd, Cr, Cu, Li, K, Mn, Mo, Ni, P, Pb, U, Zn) contents were established under CF, TF and OF vegetable farms, using a soil with no historic activity of agriculture as background [reference soil (RF)]. Organic matter fraction of RF was significantly higher when compared to all the managed soils, while no significant differences were observed between the former. In general there was a significant increase in the loads of Li (3 to 13 mg kg^{-1}), Cd (0,16 to 0,44 mg kg^{-1}), Mo (0,74 to 1,69 mg kg^{-1}), U (1,03 to 3,68 mg kg^{-1}) and P (1046,67 to 1985,83 mg kg^{-1}) from RF to CF soils, reflecting the chronic and repeated use of phosphate based fertilizers and pesticides. Traditional farming had the highest values of Cr (59,42 mg kg^{-1}), Ni (108,12 mg kg^{-1}) and Cu (287,17 mg kg^{-1}) differing significantly from RF soils (12,60, 17,87 and 13,60 mg kg^{-1}, respectively), indicating the use of pesticides and/or sludge as fertilizers. These differences in metal soil loads between conventional and traditional farming practices is clearly highlighted by the Principal Component Analysis, where biplot evidenced that samples from CF soils had higher amounts of Li, Mo and P (38,8% of the variance), while TF soils are associated to large amounts of Cr, Ni and Cu (47,3 % of variance).

Metal loads of Pb (57,47 mg kg^{-1}), Zn (323,50 mg kg^{-1}) and P (2046,67 mg kg^{-1}) where significantly higher in OF soils in comparison to RF soils. The compost used in the organic farm had high values of Pb, P and Zn, revealing that even organic soil amendments are considerable sources of heavy metals pollution in soils. Our results showed that agriculture is an anthropogenic source of metals in agricultural soils, particularly in traditional management. This study shows the relevance of soil monitoring for metals and, provides support information for future assessment purposes for bioindicators and biomarker studies regarding agricultural and public health interests.

WP046 Toxicity of Soils and Waters Contaminated by Explosives and Heavy Metals at Firing Ranges

M. Kim, Soil Quality Laboratory; J. Jung, Department of Civil and Environmental Engineering; K. Nam, Seoul National University / Dept. of Civil and Environmental Engineering; H. Moon, Korea Institute of Geoscience and Mineral Resources

Soil contamination with explosives at firing ranges could be influential on the surrounding ecosystem. At highly active firing ranges, active soil remediation is impractical. Instead, it is more reasonable to manage the toxic effect of explosives on surrounding ecosystem. This study was performed to determine the effects of soil and water contaminated by explosives and heavy metals on the surrounding water systems and to estimate the risk of explosives and heavy metals on surrounding ecosystem, and the results can be used to set site-specific ecological guidelines. Among explosives and heavy metals, 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), lead (Pb) and copper(Cu) were selected as target pollutants. For test organisms, *Lemna minor* (i.e., duckweed) and *Vibrio fisheri* (i.e., bacteria) were used for water toxicity test and *Hordeum vulgare* (i.e., barley) was used for soil toxicity test. Toxicity tests were performed according to the ISO 15799 (2003) A.1.2.1 and A.2.3 method. The changes in the number of frond of *L. minor*, the changes on the luminescence emitted by *V. fisheri*; and the changes in the germination rate of *H. vulgare* were observed to determine the toxicity. The EC50 values for *V. fisheri* after 5 min exposure to TNT, RDX, Pb and Cu were 0.6294, 0.0356, 1.554, and 0.8675 mg L-1, while the EC50 values after 15 min exposure to TNT, RDX, Pb and Cu were 0.2723, 0.0384, 1.254, and 0.394 mg L-1, respectively, indicating the highest acute toxicity of RDX. The effect of exposure time on toxicity was different depending on pollutants. The EC50 values of TNT and Cu were much lower at 15-min exposure than at 5-min exposure while EC50 value of RDX at 15- min exposure was similar to that at -5 min exposure. Similarly, the acute toxicity values for other test organisms are to be determined and compared to the chronic toxicity values from literatures in order to derive site-specific standards. These ecological toxicity studies at firing ranges can be used to quantify the risk and the effects of explosives and heavy metals on surrounding ecosystem.

WP047 Investigations into the Use of Waste Rock Pile Soils for Mine Reclamation Activities

N. Love, GEI Consultants, Inc.; S. Skigen, GEI Consultants; E. Redente, Ecological Consultants

Determining the ideal materials to use for a mine reclamation project is never an easy process. Ideally soils from the site would be used to decrease waste materials, utilize easily accessible naturally occurring materials, and reduce costs. Through soil toxicity testing, Chevron Mining Inc. has been investigating the viability of waste rock pile soils for use during the reclamation process. The soil toxicity testing was designed to help determine the best ratio of site soils and soil amendments to use for reclamation activities, as well as the most appropriate grass species for establishment and soil stability. Six plant species were tested in a variety of soil types and molybdenum (Mo) concentrations ranging from roughly 250 – 2400 mg/kg. Some species exhibited poor germination throughout all soil types, including control soils. For the plants with the best germination, the potential toxicant of concern for the site, molybdenum, proved to have little impact, even with concentrations more than nine times the expected concentrations at the site (approximately 250 mg/kg). In some cases, increased Mo concentrations actually resulted in an increase in germination, shoot and root length. For some species, the largest inhibiting factor for germination was determined to be copper (Cu) concentrations in the biosolids from a wastewater treatment plant being tested as an organic soil amendment. The site-specific and species-specific toxicity of Mo and Cu will be discussed as well as lessons learned will be presented.

WP048 Dose-dependent reactions of Aporrectodea caliginosa to perfluorooctanoic acid and perfluorooctanesulfonic acid in soil

P. Zareitalabad, Department of Soil Science; J. Siemens, W. Amelung, University of Bonn; R. Joergensen, University of Kassel

To evaluate the ecotoxicity of PFCs on soil organisms, a microcosm experiment was set up with PFOA and PFOS at three concentration levels (1, 100, and 500 mg/kg). The soils were subjected to the activity of endogeic geophagous earthworms of the species *Aporrectodea caliginosa* for 40 days, using labeled with oats straw *(Avena sativa L.)* as carbon source. Microbial biomass C increased in the presence of the PFOA, PFOS in all of the treatments, irrespective of the applied PFOA and PFOS concentrations. In contrast, the basal respiration followed the inverse trend and produced scattered data. Also the fate of the labeled oat carbon source was not significantly influenced by the presence of PFCs, whereas soil $\delta^{15}N$ values clearly differed among the treatments. We conclude that PFCs affect the fate of soil N more sensitively than the fate of soil C. Besides, they displayed a significant toxicity on the earthworms itself. The earthworms lost between 29 and 78% of their weight in the soils contaminated with lower PFC concentrations, but in the treatments with the highest concentration of 500mg/kg PFOA or PFOS, no earthworms survived. We conclude that faunal activity may be strongly hampered by PFC contamination, whereas overall microbial activity may even be enhanced, possibly because some organisms benefited from the death of other community members.

WP049 Potential cytotoxicity and genotoxicity of wastewater from alcoholic beverage industry in Southeastern Nigeria

A.C. Udebuani, Federal University of Technology / Department of Biotechnology; A.C. J, Dept of Biotechnology, Federal University of Technology, Owerri / Dept of Biotechnology; I.N. Onwurah, University of Nigeria / Dept of Biochemistry

The potential cytotoxic and genotoxic effects of wastewater from alcoholic beverage industry in Southeastern Nigeria was evaluated using *Allium cepa* Linn assay in a soil slurry microcosm experiment and standard routine analysis of water properties. The soil slurry microcosms were prepared using different dilutions (0.1 1.0, 10.0 and 20.0 % v/v) of the effluents with distilled/deionised water. Onion seedlings (four) were planted in each soil slurry microcosm and allowed to grow for four weeks and measurements taken at weekly intervals. The experiment was repeated with different soil types and the mean root growths (± SD) of the onion seedling evaluated. The least growth was observed in soil slurry-microcosms treated with 20.0% dilution. The decrease in mitotic index relative to control ranged from 69.00 to 58.00 and the differences in growth of the seedlings were significant ($p < 0.5$). Genotoxicity of the effluent was based on the diverse chromosomal aberrations observed in the exposed root tips of onion and they include stickiness, vagrant, bridges, multi polar deviation, and laggard. Stickiness and bridges were most frequent and the polar deviation was observed in microcosms having higher concentrations of the effluent. Since effects at higher levels of organization (i.e., populations and communities) represent the net sum of effects on individuals that resulted from alterations in cellular and molecular responses, the corollary holds for the result of this experiment. Therefore this model has the potential to enhance ecological risk assessment. It also reduced some uncertainties usually extrapolated from individuals to populations.

WP050 Evaluation of Soil and Sediment Toxicity at Former Sawmill

N.L. Bonnevie, ARCADIS / Environmental Sciences, Assessment, and Planning; M.P. Bartee, S. Dunn, ARCADIS; M. Butcher, ARCADIS

An ecological risk assessment (ERA) was conducted to evaluate potential risks associated with chemicals of potential ecological concern (COPEC) in soil and sediment at a former sawmill in the northwest US. These investigations identified petroleum hydrocarbons, including polycyclic aromatic hydrocarbons (PAHs), and metals as constituents of interest detected in soil, groundwater, and sediment at the site. The lines of evidence evaluated in the ERA suggest that risks to ecological receptors at the site are negligible with the possible exception of exposure to invertebrates in a small portion of the site near a former powerhouse. The state agency noted that the potential for soil and sediment toxicity to invertebrates in the vicinity of the former powerhouse was the primary uncertainty preventing concurrence with the conclusions presented in the ERA that no further action was required at the site. To address this uncertainty, bioassay testing was conducted to directly measure associated toxic endpoints in site soils and sediment. The results of these soil and sediment bioassays confirm that risks to terrestrial and aquatic

invertebrates at the site are negligible or absent. None of the sediment samples evaluated indicated statistically significant reductions in either survival or growth compared to reference, and only one soil sample was found to have statistically significant affects. However, the absence of a toxic response at any of the surrounding locations confirmed that any potential risks to ecological receptors at the site are localized to a very small portion of the site that is unlikely to have an impact on the overall population. Based on these results, the state agency issued a No Further Action Determination for the site.

WP051 Derivation of Wildlife Toxicity Reference Values: Why the Theory of Similarity of Doses Is Not Supported

A. Fairbrother, A.A. Kaiser, Exponent, Inc. / EcoSciences; B.E. Sample, Ecological Risk, Inc; B.J. Adams, Rio Tinto / Product Stewardship

Development of wildlife TRVs for use in screening-level risk assessments requires some method of determining the most sensitive species to select a value sufficiently protective of all possible wildlife exposures. While the preferred approach would be to array a range of toxicity threshold values representative of a broad phylogeny of wildlife species and select a value at or below the 5th percentile of the distribution, the lack of such data necessitates the development of models to extrapolate values from relatively few species. These models assume that adsorption, metabolism, and excretion mechanisms are functionally the same across species, so all responses should be the same on a per-kg-body-weight basis. This assumption has been criticized as too simplistic, unvalidated, and most likely inaccurate. A further complication is that animal toxicity studies generally report data based on food concentrations, which then are converted to average daily doses (mg/kg-body weight/day) for extrapolation to target species. The dose for the target species then is converted back to a food concentration to calculate a protective dietary exposure concentration. We critically examined such extrapolations using toxicity data for copper from tests with 1-day-old chicks and 21 to 28 days duration and discovered that the underlying assumption that body-weight-normalized no-effect levels can be used to derive protective TRVs for any species is not supported by the data. Furthermore, because detailed information on food consumption rates as a function of body weight or toxicity frequently is not provided in study reports, extrapolations to and from dose-based metrics add significant uncertainty and variability to the results. Therefore, the theory of similarity of doses is not supported. Selection of an appropriate TRV is highly dependent on test species, age of test animals, duration of study, and type of metal salt. We suggest extrapolating toxicity thresholds from one test species to the species of concern based on a meta-analysis of all available toxicity data for the two species (e.g., EPA's Interspecies Correlation Estimation [ICE]). The interspecies correlation coefficient can be used to estimate the toxicity of a new chemical, as long as data are available for one of the species in the pair. ICE is available only for acute exposures; however, this type of meta-analysis of an entire set to toxicity data for one species is a promising direction for future work.

WP052 How to develop and use mechanistic effect models for assessing combined effects of chemicals and environmental stressors: case study for springtails

N.T. Hamda, Jagiellonian Univeristy / Institute of Environmental Sciences; R. Laskowski, Jagiellonian University; T. Jager, Vrije Universiteit / Dept. of Theoretical Biology

With the current focus on predicting population-level effects of chemicals and integrating population dynamics into risk assessment process, the need for developing appropriate models has become more important than ever. The intent of these models is to extrapolate organism-level effects observed in a laboratory to population-level impacts. However, extrapolating these effects to the population level is neither direct not easy because typical bioassays do not take into account the factor of time and natural environmental stressors that can influence effects of chemicals on life history traits and, in consequence, on populations. Formulating mechanistic effect models to interpret organism-level effects of chemicals helps to understand the interactions between individuals and the environment. The models can also help to make an educated extrapolation to the population level. These models must be conceptualized scientifically and grounded in sound theoretical base. Furthermore, their development and implementation process should follow principles of good modelling practices. In our study, we implemented a metabolic theory-based energetic model for the mechanistic interpretation of the effects of chemicals on individuals and integrated it with different population dynamics models to extrapolate bioassay-derived data to the population level. We developed and implemented the model in accordance with the principles of good modelling practices, which follow cyclic and iterative processes of parameterization, calibration, testing and uncertainty analysis. The suitability of the model as a tool for ecological risk assessment is demonstrated by assessing the combined effects of chemicals and environmental stressors on population dynamics of *Folsomia candida*, the standard OECD and ISO bioassay species.

WP053 Upper bound TRVs for ecological risk assessment: Are LOAELs identified in eco SSLs the way to go?

S.M. Jones, Conestoga-Rovers & Associates

Development of ecological soil screening levels (Eco SSLs) for avian and mammalian wildlife has produced a dilemma for ecological risk assessment. The original intent of the Eco SSLs is identification of screening level concentrations in soil based on some metric of no observed adverse effect levels (NOAELs) reported in studies vetted by the Eco SSL development teams. In some cases (e.g., lead and vanadium), the Eco SSLs are below naturally occurring concentrations. One of the factors contributing to the unrealistically low Eco SSLs is selection of a NOAEL. Although use of Eco SSLs that are below ambient concentrations is problematic, the real dilemma is manifested in the appropriate selection and use of lowest observed adverse effect levels (LOAELs), which are identified along with the NOAELs in the source documents for the Eco SSLs. As the risk assessment process advances to the baseline ecological risk assessment (BERA), consideration of only NOAELs is no longer appropriate. The LOAELs identified in the Eco SSL source documents are increasingly being used to develop upper bound toxicity reference values (TRVs) for BERAs. Herein lies the problem. Test parameters for the LOAELs vetted in the Eco SSL source documents are highly variable. As a result, LOAELs typically span several orders of magnitude. Parameters that vary among the vetted studies include test organisms, which range from laboratory stock to free ranging birds and mammals; age, body weight, and sex of organisms tested; test duration; chemical form and method of exposure of a contaminant (e.g., gavage, water, food); and measurement endpoint. This talk will discuss the how LOAELs have been used to develop upper bound TRVs (e.g, bounded vs. unbounded LOAELs for growth and reproduction), as well as present a sensitivity analysis of the factors that contribute to wide range of vetted LOAELs. Recommendations for selection of the most ecologically realistic LOAELs will also be presented.

WP054 Fixing the Eco-SSLs One Step At a Time: High Molecular Weight PAHs

D.W. Smith, CRA, Inc.; A.V. Pawlisz, Conestoga-Rovers&Associates

As should be evident from the name, ecological soil screening levels (Eco-SSLs) for avian and mammalian wildlife were developed by USEPA to screen out those chemicals that have minimal potential to cause ecologically significant risk. EcoSSLs were developed with very conservative methods that, unfortunately, did not include potential utility, often resulting in screening levels that do not screen. Worst of these are EcoSSLs for lead and vanadium, which are well below many and almost all, respectively, naturally occurring soil concentrations. However, the EcoSSL for high molecular weight PAHs (e.g., 4 or more rings), 1.1 mg/kg, is also too low to be very useful for screening. This utility of this already low screening value is further reduced by the typical screening methods, which compare the maximum detected concentrations of many HMW PAHs from many, oftentimes biased soil samples. A closer look at the toxicological basis for this EcoSSL suggests that it can be significantly improved, as a screening tool, without posing unacceptable risk to ecological receptors. Notably, the EcoSSL for HMW PAHs is based on a low outlier NOAEL, reductions in mouse survival noted in cancer bioassays with benzo(a)pyrene (BaP), from Culp et al. However, the Culp et al. bioassay results are inappropriate for the EcoSSL dataset in general, much less as the sole basis for an EcoSSL. For one, the strain of mouse used, B6C3F1, has abnormally high cancer rates. Secondly, the NOAEL was defined based on LOAEL that pertains to moderate reductions in late-life survival, effects which have limited impacts on population growth. Arguably, then, the identified LOAEL is an ecological NOAEL. Eliminating the Culp et al. NOAEL from EcoSSL calculation would increase both the magnitude (about 30 fold) and the scientific legitimacy (the TRV and EcoSSL would based on the median of 13 NOAELs rather than one NOAEL) of the EcoSSL. Further inspection of the 13 other NOAELs suggests that mechanisms of HMW non-cancer toxicity are due to the known effects on these HMW PAHs on DNA-binding and mutagenic effects. Given this likely

mechanism of non-cancer toxicity, EcoSSLs for other HMW PAHs should be based on their cancer/mutagenic potency compared to BaP. Since pyrene is not a likely mutagen/carcinogen, it should be screened with the EcoSSL for LMW PAH. Other HMW PAHs should be screened as the BaP EcoSSL divided by their BaP toxicity equivalence factor.

Contaminant Accumulation in Plants: Mechanisms, Models, and Potential Risk

WP055 Analysis of the uptake of triclosan and triclocarban in plants grown in biosolids-amended soil

R.S. Prosser, School of Environmental Sciences; L. Lissemore, University of Guelph / Laboratory Services Division; L. Sabourin, E. Topp, Agriculture and Agri-Food Canada / Southern Crop Protection and Food Research Centre; P.K. Sibley, University of Guelph / School of Environmental Sciences

Triclosan (TCS) and triclocarban (TCC) are antimicrobial chemical that are present in a variety of personal care products. Due to the relatively high persistence and hydrophobicity of TCS and TCC, the chemicals partition into the solid portion of municipal sewage. TCS and TCC continue to persist after the solid sewage is processed into biosolids. There is concern about whether TCS and TCC are taken up in roots and translocated throughout plants that are grown in biosolids-amended soil. In order to answer this question, a modified method was developed to analyze triclosan and triclocarban residues in biosolids, soil, and plant tissue. Soybean (*G. max*), radish (*R. sativus*), and carrot (*D. carota*) were then grown in soil amended with dewatered anaerobically-digested biosolids. Amendment rates followed the best management practices employed in the province of Ontario in Canada. Biosolids were spiked with increasing quantities of TCS to produce a range of environmentally relevant exposures. Plants were harvested midway through their life cycle and at maturity. Root, stem, edible portion of the plant, and soil were analyzed. In addition to the plants grown in growth chambers, carrot, green bell pepper (*C. annuum*), cucumber (*C. sativus*), and tomato (*S. lycopersicum*) were grown in a field amended with dewatered anaerobically-digested biosolids in 2011 and radish, carrot, and tomato were grown in 2012. The soil and edible portion of these plants were harvested when the plant reached maturity for the analysis of TCS and TCC residues. Residues of TCS up to 61 ng/g dry weight (dw) were found in plant tissue grown under controlled conditions. Residues of TCS and TCC were found in edible portions of plants grown in field up to 5.6 and 5.7 ng/g dw, respectively.

WP056 Uptake of human pharmaceuticals and personal care products by plants grown in fortified and biosolids-amended soils

C.S. Holling, Colorado State University-Pueblo / Biology; J.L. Bailey, Colorado State Unviersity-Pueblo / Biology and Chemistry; B. Vanden Heuvel, Colorado State University-Pueblo / Biology; C.A. Kinney, Coloraodo State University-Pueblo / Chemistry

Pharmaceuticals and personal care products (PPCPs) are commonly present in wastewater biosolids and subsequently present in soil amended with biosolids. A series of greenhouse experiments were used to study the accumulation of PPCPs in Chinese cabbage (*Brassica campestris*) and alfalfa (*Medicago sativa*). To measure the potential for PPCPs to accumulate in crops, two sets of studies using Chinese cabbage grown in either soil fortified with environmentally relevant concentrations of pharmaceuticals or soil amended with an agronomic rate of biosolids were conducted. After reaching maturity all four human pharmaceuticals added to the soil, carbamazepine, sulfamethoxazole, salbutamol, and trimethoprim, were detected in both the aerial and root tissues of the cabbage. Although all study compounds were present in the biosolids-amended soil, only carbamazepine, salbutamol, and triclosan were detected in both the aerials and roots of the cabbage. Sulfamethoxazole was also detected in the roots of a single plant grown in the biosolids-amended soil. Sulfamethoxazole and trimethoprim were not detected in any of the above ground biomass of the cabbage from the biosolids-amended soil. To better understand factors that might influence the uptake and accumulation of PPCPs alfalfa was grown in the presence or absence of vesicular arbuscular mycorrhizae (VAM) in soil fortified with carbamazepine or amended with biosolids. VAM forms a symbiotic relationship with vascular plants and assists plants in retrieving nutrients from the soil. Alfalfa inoculated with VAM accumulated significantly less carbamazepine than alfalfa that had not been inoculated with VAM. This was true of alfalfa grown in soil fortified with carbamazepine and alfalfa grown in soil amended with biosolids. Interestingly, significantly less carbamazepine accumulated in VAM inoculated alfalfa grown in the soil directly fortified with carbamazepine compared with the biosolids amended soil. The results of these studies demonstrate that PPCPs can accumulate in plants exposed to environmentally relevant concentrations of PPCPs, and that factors such as the symbiotic colonization of roots with VAM may play a role in PPCP accumulation in plants.

WP057 Phytoforensic Tools for Assessing Fate and Potential Exposure via Plant Sampling

J.G. Burken, Missouri University of Science and Technology / Civil, Architectural and Environmental Engineering; M. Limmer, A.J. Holmes, Missouri University of Science and Technology / Environmental Engineering; H. Shi, Missouri University of Science & Technology / Chemistry

Vascular plants concurrently collect and store chemicals and elements from the water, air, and soil in the surrounding environment. Chemical uptake from groundwater is active, not passive, driven by evapotranspiration. The methods discussed herein take advantage of this natural energy transfer which brings contaminants from the subsurface to above ground where they can be sampled easily, rapidly, inexpensively and with little impact. Fugitive contaminants in groundwater are difficult to assess regarding fate and potential risk assessment. Using novel techniques in phytoforensics, we can gather this data on contaminants in the subsurface environment to help in contaminated-site investigations, risk assessments and plume delineations that are too often costly and inaccurate, thereby aiding in human health protection. The talk will overview recent breakthroughs in the field of phytoforensics. Advances to be covered include sampling and analytical methods that have led to breakthroughs in: directional analysis from a single tree, non-volatile compound detection, advances in solid phase sampler development and long term monitoring advances. Compounds that have been verified to date include a wide range of chlorinated solvents, explosive compounds RDX and HMX, 1,4-Dioxane, perchlorate and BTEX contaminants. These methods are extremely rapid and have almost no noticeable impact on ecology or private property. The low impacts are a stark difference from traditional methods of using drill rigs and energy intensive machinery for each sample collected. With a team of two, over 100 tree core samples can be collected in a day with analysis complete in one to three days. All equipment can fit in one toolkit. *In-planta* solid phase microextraction (SPME) analysis can be completed in similar timeframe, with data in under 10 minutes using a portable GC.

WP058 Bioaccumulation of Endocrine-Disrupting Chemicals in Food Crops through Reclaimed Water Irrigation

J. Lu, University of Florida / Indian River Research and Education Center; J. Wu, University of Florida; P.C. Wilson, University of Florida / IFAS / IRREC-Soil & Water Science; P.J. Stoffella, University of Florida

The bioaccumulation of bisphenol A (BPA) and nonylphenol (NP) (from reclaimed irrigation water) in lettuce and tomato was investigated. Plants, grown hydroponically, were exposed to BPA and NP via the roots only (simulating subsurface irrigation) or foliage only (simulating overhead irrigation). Bioaccumulation of these endocrine-disrupting chemicals (EDCs) in vegetables and fruit was observed. Concentrations in tomato fruit were 31.41 ± 13.37 (root exposure) and 46.09±16.27 (shoot exposure) μg/kg, respectively, while those in lettuce leaves were 124.97 ± 44.24 (root exposure) and 137.08 ±27.73 (shoot exposure) μg/kg, respectively. The total daily intake of BPA and NP, through consumption of vegetables and fruits (assuming a person eats 534 g d^{-1} fresh vegetables and 383 g d^{-1} fruit per day) would be 78.76 and 90.85 μg, respectively, based on the data from the root exposure treatment. The total daily intake of BPA and NP (4-n-NP) through consumption of vegetables and fruits would be 100.12, and 103.49 μg, respectively, based on the data from the foliar exposure treatment. About 50% of BPA accumulated in lettuce leaves while about 20% of BPA accumulated in tomato fruit in both exposure treatments. About 40% of NP accumulated in tomato fruit in both exposure treatments. In the root exposure system, the bioconcentration factors (BCF) for NP were much higher than for BPA. BCFs for lettuce in the root exposure treatment were 4.80±2.04 (BPA) and 119.18±15.95 (NP), while those for tomato were 4.68±2.03 (BPA) and 8.51±1.74 (NP). For the foliar exposure treatment, the BCFs for lettuce were 3.35±1.55 (BPA) and 3.52±1.25 (NP), while those for tomato were 7.92±3.40 (BPA) and 2.72±0.67 (NP). For lettuce, the translocation factors (TFs) for BPA were 0.52±0.21 and 0.64±0.12 in the root and foliar exposure treatments, respectively. TFs for NP were 0.04±0.01 (root exposure) and 0.33±0.1 (foliar exposure). More than 70% of spiked BPA and NP

accumulated in crops under the foliar exposure treatments, which was much more than from the root exposure treatment (< 0.08%), indicating that some irrigation methods can lead to a much higher uptake potential. The bioaccumulation of EDCs in vegetables and fruit from simulated reclaimed irrigation water suggests a potential exposure pathway for humans.

WP059 Bioaccumulation of Perfluoroalkyl Chemicals in Aquatic Plants: results of mesocosm experiments with Eichhornia crassipes and Echinodorus horemanii

N. Pi, J. Ng, National University of Singapore / Department of Civil and Environmental Engineering; B.C. Kelly, National University of Singapore

The environmental behavior of perfluoroalkyl chemicals (PFCs) is not fully understood. In particular, studies regarding bioaccumulation and phytoremediation of PFCs in aquatic plants are lacking. Previous studies have demonstrated that aquatic macrophytes can be effective at accumulating and/or removing a variety of waterborne contaminants including heavy metals, nutrients and organic compounds. The present study involved controlled mesocosm experiments to investigate the uptake and elimination kinetics, bioaccumulation and translocation of PFCs in aquatic plants. Two types of aquatic plants, including a floating species (*Eichhornia crassipes*)and a submerged species (*Echinodorus horemanii*), were studied. Static-renewal experiments were conducted and involved exposure of a mixture of PFCs via spiked water (20 ppb) over a 16 d uptake period, followed by a 12 d depuration phase in clean water. Water samples were collected on day 0, day 2 and day 4 to ensure stable water concentrations during the uptake phase. Plant samples separated to leaves and roots were taken at 5 time points during uptake and 4 time points during depuration. PFC concentrations, as well as the plant dry biomass were determined for the collected samples. Water and plant tissue samples were processed by solid-phase-extraction (SPE) and analyzed using liquid chromatography-electrospray ionization tandem mass spectrometry (LC/ESI-MS-MS) to determine concentrations of individual PFCs (carboxylates and sulfonates). PFCs were found to readily accumulate in these aquatic plants. Plant dry biomass for *E. Crassipes* increased throughout the experiment. Conversely, biomasss of *E. Horemanii* was found to decrease post-exposure (day 12 onward). Bioconcentration factors (BCFs) and translocation factors (TFs) varied widely among the test chemicals, as well as between the two plant species. Longer alkyl chain PFCs were more readily taken up in the plants compared to shorter chain PFCs, while for PFCs with similar chain length (PFOA vs. PFOS), the sulfonate (PFOS) demonstrated a higher bioaccumulation potential. These results highlight the importance of molecular structure and physical-chemical properties for the bioaccumulation of PFCs in aquatic plants. The role of chemical properties and other key factors influencing bioaccumulation potential of PFCs in aquatic plants will be presented. The information generated from this study may aid future modeling, risk assessment and phytoremediation initiatives.

WP060 Perfluoroalkyl acid uptake and distribution in fresh food crops grown in biosolids-amended soils

A.C. Blaine, Colorado School of Mines / Civil & Environmental Engineering; C. Rich, Colorado School of Mines; L. Hundal, Metropolitan Water Reclamation District of Greater Chicago; C. Lau, USEPA; M.A. Mills, USEPA, ORD / Remediation and Pollution Control Division, NRMRL; K. Harris, USEPA; C.P. Higgins, Colorado School of Mines / Civil & Environmental Engineering

Perfluoroalkyl acids (PFAAs) are environmentally persistent, bioaccumulative, and toxic emerging contaminants. They are synthetic chemicals used in a myriad of consumer and industrial applications. One of their significant routes into the environment is via waste water treatment plants where they are collected but not degraded. Their remaining presence in municipal biosolids after exiting the plant has been well documented. Biosolids, like animal manures, are rich in both plant nutrients and organic matter and are commonly used as a fertilizer in crop production. As a consequence, concerns have arisen about the potential uptake and subsequent bioaccumulation of PFAAs into crops grown in biosolids-amended soils. Previous studies have documented the potential for bioaccumulation of PFAAs, particularly perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) into food crops, while other field studies have documented the transfer of these and other PFAAs from industrially-contaminated biosolids-amended soils into grass. Additional literature has documented the transfer of PFAAs from spiked hydroponic systems into lettuce. Previous work has demonstrated that lettuce, tomato, and corn plants can uptake PFAAs, and that bioaccumulation is dependent on chain length, crop type, and soil properties. This study investigated the distribution pattern of PFAA accumulation in five edible greenhouse-grown crops eaten fresh (lettuce, tomato, radish, celery, and peas). Crops were grown in three different soils, an industrially-impacted biosolids-amended soil, a municipal biosolids-amended soil, and an unamended control soil. The various crops represent differing edible compartments of plants; accumulation in both edible and non-edible compartments of each crop was examined. These results elucidate some of the PFAA accumulation and distribution trends in edible crops. Disclaimer: This abstract does not necessarily reflect USEPA policy.

WP061 Investigating plant uptake and translocation of perfluoroalkyl substances in the model plant Arabidopsis thaliana

C.E. Mueller, Stanford University / Civil and Environmental Engineering; A.E. Timofte, ETH Zürich; G.H. LeFevre, Civil & Environmental Engineering; E.S. Sattely, Stanford University / Chemical Engineering; R.G. Luthy, Stanford University / Department of Civil and Environmental Engineering

Prevalence of persistent, unregulated trace organic contaminants in recycled water is of substantial concern when applied to agricultural crops for irrigation. Poly- and perfluoroakyl substances (PFAS) are prevalent wastewater contaminants that are not efficiently removed during most wastewater treatment due to their persistence, low sorption affinity, and continued production from precursor substances. In addition, PFAS have shown to be very environmentally persistent, as well as bioaccumulative, and exhibit toxic properties. As water re-use for agricultural and landscape irrigation continues to increase, understanding the fate and transport of these contaminants becomes crucial for ecosystem and human health. In laboratory experiments with the model plant *Arabidopsis thaliana*, we are studying the hydroponic uptake of PFAS and other environmentally relevant contaminants. Arabidopsis plants are first grown in clean media under sterile conditions. After 11 days of growth, media is dosed with a known amount of PFAS mix and in the subsequent days, triplicate samples are taken. For each sample, plant, media, and wall rinse are analyzed to generate a time dependent mass balance. Proof of principle experiments with a range of perfluorocarboxylates (PFCAs and perfluorosulfonates (PFSAs) showed a clear temporal and chain length dependent trend. At day 1 of exposure, almost all the PFAS were detected in the media with some sorption to the container walls. On subsequent days, a distinct increase of PFAS in plant matter was observed. PFAS amount in plant tissue increased from C6 to C10 PFCAs and C4 to C8 PFSA, which correlates with the higher sorption affinity of longer chain PFAS. However, there was a similar increase in plant affinity from C6 to C4 PFCAs, which cannot be explained by surface sorption. These short chain PFCAs are very mobile and may therefore be efficiently transported in the plant. Following these preliminary experiments, more in-depth analysis of root uptake and internal translocation into the shoots will be performed to investigate the potential for transfer into above ground plant parts. Results on time dependent root and shoot concentration factors will be shown, which will allow for a better understanding of PFAS plant processes.

WP062 From industrial sludge to fertilizer; a sustainable approach

T.H. Schlekat, ARCADIS; R.L. Heslep, Arcadis-US, Inc / Risk Assessment and Ecological Services; C.H. Day, ARCADIS; S. Sager, Arcadis JSA

A risk assessment was conducted to evaluate the potential threat to human and ecological receptors from exposure to constituents in sludge being considered for farm land application. Land application was one of three closure strategies being considered for ten acres of unlined lagoons containing approximately 15,250 dry tons of nitrogen-rich sludge at a historical chemical manufacturing facility in the mid-west. Other closure strategies considered were closure in place as a solid waste landfill and offsite disposal at a landfill. Concentrations in the sludge were below application limits set in the regulatory agency's biosolids rule, and the agency was encouraging farmland application. The risk assessment was conducted to evaluate the specific applications being considered on 586 acres of farmland and managed grassland. Predicted sludge-related constituent concentrations in soil were estimated using mass balance models, and sludge-related constituent concentrations in a nearby surface water body impacted by storm water runoff were estimated using fate and transport models. Receptors and pathways evaluated in the risk assessment for soil included: direct contact with soil for a hypothetical resident receptor and a farmer receptor, use of groundwater as a potable water source, consumer exposure to constituents in sludge through ingestion of grain grown on farm land and ingestion of dairy and beef from cattle raised on feed grown on farm land. Exposure to surface water through direct

contact and ingestion of fish and shellfish from the water body was also evaluated in the risk assessment. Finally, exposure of terrestrial and aquatic wildlife from appropriate feeding guilds was evaluated in the risk assessment. The risk assessment utilized select literature derived uptake factors after incorporating consideration of bioavailability, biotransformation, and plant uptake mechanisms to estimate concentration in food items. Biotransformation and bioavailability of constituents were also considered in selecting toxicity values. Results indicated that predicted sludge-related constituent concentrations in soil and surface water are not expected to be associated with adverse effects on human and ecological receptors for all pathways evaluated. Further land application was deemed the most sustainable solution. Based on the results of this evaluation, sludge application is planned and will be followed by monitoring of concentrations in grain.

Remedy Effectiveness Assessments and Monitoring Contaminated Sediment Remediation

WP064 Refining contaminant transport modeling at the Palos Verdes Shelf Superfund site using data from passive samplers

L.A. Fernandez, Northeastern University / Office of Research and Development; W. Lao, Southern California Coastal Water Research Project; K.A. Maruya, Southern California Coastal Water Research Project Authority; E. Adams, Massachusetts Institute of Technology / Department of Civil and Environmental Engineering; R.M. Burgess, USEPA / Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory / ORD

The Palos Verdes Shelf Superfund site is in over 50 meters of water on the continental shelf and slope off the coast of southern California (USA). The site includes over 25 km^2 of sediments contaminated over several decades by municipal treatment plant effluent discharged via outfall pipes at a depth of 60 meters. Contaminants of concern include polychlorinated biphenyls (PCBs) and the pesticide DDT and its degradation products (e.g., DDE, DDD and DDMU). Monitoring of bulk sediment concentrations indicates that the masses of hydrophobic forms of the contaminants on the shelf are decreasing, leading to reconsideration of remediation plans for the site. Questions remain, however, regarding the transformation and transport of contaminants on the site, and the risks they pose to receptor species. In this work, polymeric passive samplers (PE, POM, and SPME) were used to measure contaminant concentrations in the water column and sediment porewaters. The results were used to calculate the diffusive flux between sediments and water column. This data, along with sediment core concentrations and water current data, were combined into a larger-scale contaminant transport model for the site. In addition, water and porewater chemical activities were compared to fish tissue chemical activities to determine how closely water, sediment, and organisms are equilibrated.

WP065 Field Sampling for Porewater Mercury and Methylmercury using DGT and Redox Profiles using Cyclic Voltammetry

D.D. Reible, The University of Texas-Austin / Environmental and Water Resources / Dept. of Civil & Environmental Eng.; P. Bireta, The University of Texas at Austin / Environmental and Water Resources Engineering; P. Schierz, University of Texas at Austin / Department of Civil and Environmental Engineering, Duke University / Pratt School of Engineering / Civil and Environmental Engineering; J. Grundy, The University of Texas at Austin / Civil, Architectural and Environmental Engineering Department-EWRE; R. Landis, DuPont

Dissolved porewater concentrations of mercury have been shown to better correlate with mercury methylation rates than bulk mercury sediment loadings. When assessing a contaminated site, it is important to be able to measure the truly dissolved porewater concentrations of mercury and methylmercury. The diffusive gradient in thin film (DGT) technique is applied to determine porewater mercury and methyl mercury concentrations in both laboratory and field studies. DGT devices have been a useful indicator of dissolved metals in aqueous systems, although the technique is not as well developed for measuring mercury or for measuring concentrations in sediment porewater. Field issues such as background mercury in field blanks, sampler design, and methylmercury recovery were examined. Laboratory testing was used to evaluate mercury DGT probe uptake parameters from the sediment and to develop procedures for analysis. DGT probes were deployed in the field at a pilot pond near the South River (Virginia, USA) to evaluate the performance of a sediment amendment. Therefore, multiple disk probes and sediment depth probes were deployed in the pilot study pond in-situ to determine concentrations of mercury and methyl mercury in sediment porewater and overlying water over time. Sampling has continued over two years to determine the long-term impact of the amendment. Sampling was also performed in the South River at several locations over a four year period. The sampling showed that the DGT could be a useful tool for exploring available and mobile mercury and methyl mercury in the field. In sediments, mercury methylation has been tied to iron and sulfate reducing bacteria. Field measurement of redox conditions can be measured in porewater using cyclic voltammetry. Redox conditions were measured over depth in order to correlate mercury methylation with redox conditions.

WP066 Challenges of human health risk assessment for contaminated sediment

S.L. Fernandes, H. Phillips, L. Leon, SENES Consultants

Human health risk assessment is the evaluation of the probability of adverse health consequences to humans caused by the presence of contaminants of potential concern (COPC) at a site. Within Canada, federal guidance is available for assessing risks to human health as a result of exposure to COPC in media such as soil and surface water, but no regulatory agency has endorsed a standard approach for assessing risks from exposure to COPC in sediment. The EPA RAGS Part E provides some general guidance relating to dermal exposure to sediment. Recently, a series of contractor documents have been prepared in Canada that relate to the evaluation of exposure of humans to contaminated sediments. These are not endorsed by Canadian federal authorities but represent the best available guidance available at this time. The approach to calculate the exposure and risk to sediment will be discussed along with the information available for several of the key parameter values. The challenges in conducting human health risk assessment for sediments, which includes uncertainty in the key variables and limited applicable site characterization data, will be discussed. The implication of these assumptions will be demonstrated and areas for improvement outlined.

Policy and Litigation in an Uncertain World

WP067 Endangered Species Evaluations: Providing a Stable Platform Leading to Compliance with FIFRA and ESA Standards

B.D. McGaughey, Compliance Services International; T. Hall, Bayer CropScience / Ecotoxicology; D. Campbell, Syngenta

Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) risk assessments related to evaluating the potential risk of pesticides to species listed by the Endangered Species Act (ESA) have been subjected to close evaluation through litigation, an appeal by agencies for scientific review by the National Academy of Sciences, recent scientific forums, and stakeholder input. Each of these activities addresses the complex nature of species assessment with regard to national pesticide registration actions. The circumstances that brought about these evaluations are driven by the fact that the laws and processes were not working in harmony, both across disciplines – as the theme of this meeting – and across policies. Considered collectively, harmonization can happen and these resources, opinions and decisions can lead to a more stable scientific platform to clearly define data use and assessment methods. A stable platform will be supported by: (1) a roadmap for guiding the evaluation of direct and indirect effects; (2) relevance and reliability attributes required for data to be used in support of risk and risk management decisions; (3) uniform application of assessment methods and data collection to give an as accurate as possible depiction of risk; and (4) employment of multiple lines of evidence using a uniform and predictable process. Since 1996, the FIFRA Endangered Species Task Force (FESTF) has been collecting data and assessing means of applying those data to species evaluations. This poster will explore how those data contribute to the science and overall platform of addressing the complex intersection of FIFRA registration actions and ESA species protection.

WP068 Strategies for Registering Polymers of Low Concern in Multiple International Markets

J. Kneeland, Gradient; W.T. Mehler, Gradient, Gradient Corporation; A.S. Lewis, M. Sharma, Gradient Corporation

In a complex and dynamic regulatory environment, multinational companies face distinct challenges in maintaining compliance as manufacturers and importers of chemical substances. Most jurisdictions require some level of notification or registration for new substances, and the requirements vary widely. One category of chemical substances that can be complex from both a chemical and regulatory standpoint are non-hazardous polymers,

known in some jurisdictions as polymers of low concern (PLCs). PLCs have reduced registration requirements in many jurisdictions, but the definitions and data required to demonstrate PLC status differ widely. For example, under the European Union's REACH legislation, polymers themselves are in general not registered; rather, the constituent monomers require registration. Though the criteria to determine PLC status are broadly similar between North America, Australia, and Asia, Japan specifically requires that the polymers not change molecular weight in acids or alkali and that the polymers not be soluble in water or organic solvents. Through a detailed analysis of the data requirements for identifying and assessing PLCs, we highlight similar requirements that can be leveraged to optimize data collection and streamline the chemical registration process in multiple jurisdictions. We also identify challenges that may arise from the differing regulatory requirements, and from technical difficulties in analyzing molecular weight of complex polymers, including UVCB substances (Unknown or Variable composition, Complex reaction products and Biological materials). This detailed analysis of polymer requirements can help enable efficient registration of polymeric substances in economically important and emerging markets, such as Australia, China, and the European Union.

Fate and Effects of Metals: Aquatic Biological Perspective

WP069 Advance research on nickel toxicity in sediments: species, bioavailability and toxicity

L.T. Nguyen, Ghent University / Applied Ecology and Environmental Biology; M. Vangheluwe, ARCHE; M.B. Vandegehuchte, Ghent University / Applied Ecology & Environment Bio; J. Garrevoet, B. Vekemans, Ghent University / X-Ray Microspectroscopy and Imaging group; L. Vincze, Ghent University; E.R. Garman, NiPERA / Ecotoxicologist; C.E. Schlekat, NiPERA; C. Janssen, University of Ghent / Laboratory of Environmental Toxicology and Aquatic Ecology

Sediment toxicity is known to be affected by abiotic factors such as the concentration of total organic carbon (TOC) and acid volatile sulphide (AVS) in the sediment, but also by biotic factors such as the intrinsic sensitivity and the behaviour of (epi)benthic test species. The goal of this project is to evaluate the relative importance of species sensitivity and bioavailability for the toxicity of nickel (Ni) in freshwater sediments. In order to achieve this, Ni toxicity and uptake are being evaluated with several species representing different taxonomic groups and life styles. Three uncontaminated natural sediments with low (1-3 mmol AVS/kg and 1.5% TOC; S1), moderate (3-5 mmol AVS/kg and 2% TOC; S2) and high (25-30 mmol AVS/kg and 4.5% TOC; S3) binding capacities are used. The first results demonstrate that in sediment with low AVS and TOC concentrations (S1), a concentration-effect relation could be observed for the biomass of *Tubifex tubifex* after 28 days exposure. The EC_{10} (95% confidence limits – CL) for biomass is 1,103 (883-1,379) mg Ni/kg dry wt. For the midge *Chironomus riparius*, body mass of larvae after 12 days of exposure was affected at 1,061 mg Ni/kg dry wt. The EC10 (95% CL) for development rate is 762 (324-1,788) mg Ni/kg dry wt. For the mollusc *Sphaerium corneum*, the EC_{10} (95% CL) for biomass (soft tissue) exposed to Ni in S1, S2 and S3 sediments are 388 (123-1,229) mg Ni/kg dry wt, 736 (201-2,695) mg Ni/kg dry wt and 2,252 (1,198-42,33) mg Ni/kg dry wt, respectively. In the low AVS sediment, the difference between the molar concentrations of simultaneously extracted Ni (SEM_{Ni}) was greater than zero for all tested concentrations. Ni tissue concentrations increased as the difference between SEM and AVS increased. This supports the basis of the SEM-AVS concept, as it is reflective of the presence of Ni in exchangeable sediment phases. In ongoing and future experiments, additional species and sediments will be evaluated. By means of micro-X-ray fluorescence (XRF) analysis and based on previous research, we will test the hypothesis that Ni accumulation and the internal distribution of Ni over different tissues is affected by bioavailability modifying factors such as TOC, AVS or diet. Furthermore the test results will be used for populating a Species Sensitivity Distribution.

WP070 Arsenic biotransformation in the Western clawed frog (Silurana tropicalis) and its effects on frog development and reproduction

V.S. Langlois, Royal Military College of Canada / Chemistry & Chemical Engineering; G. Caumette, Royal Military College of Canada / Environmental Sciences Group; L. Gibson, Royal Military College of Canada / Chemistry & Chemical Engineering; I. Koch, K.J. Reimer, Royal Military College of Canada / Environmental Sciences Group; W.R. Cullen, University of British Columbia / Chemistry

Arsenic, typically as inorganic arsenate, is ubiquitous in aquatic systems. Few studies have assessed the effects of As biotransformation in frogs but these have identified a variety of methylated arsenicals, including the less common tetramethylarsonium ion. In an amphibian *ex vivo* tail metamorphosis assay, sodium arsenite was shown to significantly alter thyroid hormones. The aim of this study was to identify developmental and reproductive-related changes in frogs exposed to arsenate and to examine the arsenic compounds (species) present. *Silurana tropicalis* embryos were exposed from Nieuwkoop-Faber (NF) stage 12 to 66 to three As^V treatments: 0, 0.5 and 1 ppm As^V. A genome-wide microarray analysis was performed in early tadpole development and thyroid hormone- and steroid-related gene expression was analyzed at several NF stages. High performance liquid chromatography coupled with inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) was used to measure As biotransformation in frog tissue. Transcriptomics and As speciation data will be discussed.

WP071 Bioaccumulation and Trophic Transfer of As, Cd, Cu, Hg, Pb, and Zn in Two Contaminated Salt Marshes on the South Shore of Long Island, New York

J. Dutton, M. Record, Adelphi University / Environmental Studies Program

Metal accumulation in estuarine food webs in heavily industrialized and urbanized coastal areas is receiving increasing attention because field studies have shown that sediment and water have elevated levels of metals due to anthropogenic activities. This has resulted in an elevated body burden of metals in estuarine organisms, which can potentially impact the health of the organism themselves, their predators, and human consumers of contaminated seafood. Prior studies have determined that the diet is the dominant metal exposure pathway in estuarine organisms. In the present study, we measured the concentration of five metals (Cd, Cu, Hg, Pb, and Zn) and one metalloid (As) in sediment, water, and biota collected from two contaminated salt marshes (Bay Park and Inwood Park, Nassau County, NY) using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) to investigate the bioaccumulation and tropic transfer of each metal within the salt marsh food web. The investigated biota included: saltmarsh cordgrass (*Spartina alterniflora*), sea lettuce (*Ulva lactuca*), marsh grass shrimp (*Palaemonetes pugio*), ribbed mussel (*Geukensia demiss*), Atlantic silversides (*Menidia menidia*), killifish (*Fundulus heteroclitus*), blue crab (*Callinectes sapidus*), and the eastern mudsnail (*Ilyanassa obsoleta*), which represents a typical estuarine food web. The two field sites were chosen because they are located in bays with limited connectivity to the Atlantic Ocean (western Hempstead Bay and east Jamaica Bay) and are heavily impacted by anthropogenic activities. The concentration of each metal in biota was consistently higher in Bay Park than in Inwood Park, and Cu and Zn were found at the highest concentration in all investigated biota. For all investigated metals, the highest concentration was found in *I. obsoleta*. Every metal was shown to bioaccumulate, whereas Hg and Zn were shown to biomagnify, and Cd and Pb were shown to biodiminish with increasing trophic level. No relationship between As and Cu and increasing trophic level was observed. Understanding metal behavior in estuarine food webs enables us to better predict the impacts of metal exposure on the ecosystem and human consumers of seafood.

WP072 Cellular Biomarker Efficacy Studies – Comparisons of Different Molluscan Species to Cu Exposures

A.H. Ringwood, UNC-Charlotte / Department of Biology; R. Ubrihien, A. Taylor, Institute for Applied Ecology, University of Canberra; W.A. Maher, University of Canberra / Dept. of Health, Design & Science

Cellular biomarkers, especially lysosomal destabilization, have consistently been identified as valuable indicators of pollutant exposure. There are some uncertainties regarding the relative sensitivity of different species, especially when the use of a resident species instead of a typical test organism is desirable. The purpose of these studies was to compare the relative sensitivity of a diverse array of molluscan species from a variety of geographical locations. Copper (Cu) is a pervasive metal pollutant in coastal ecosystems, so four-day laboratory exposure studies with Cu were conducted with four species collected near Sydney, Australia – two bivalve species (Sydney rock oysters, *Saccostrea glomerata*; and mussels, *Mytilus edulis*) and two gastropod species (*Nerita melanotragus* and *Bembicium nanum*). Studies were also conducted with an Hawaiian bivalve species (*Isognomon californicum)* collected from Oahu, Hawaii. Lysosomal destabilization, and lipid peroxidation assays as well as tissue Cu concentrations were determined in the Australian and Hawaiian species and compared to previously published studies with oysters, *Crassostrea virginica* from southeastern United States. An important aspect of the Australian studies was the involvement of multiple investigators in a round-robin style for the lysosomal destabilization assays. Lysosomal

destabilization rates were significantly elevated in all species exposed to Cu, often with evidence of a dose-dependent effect, and showed remarkable similarity between species. Lipid peroxidation levels tended to be elevated in Cu-exposed bivalve species, but no differences were observed between control and Cu-exposed gastropods. This same pattern was observed for tissue Cu concentrations – with elevated metals in the bivalve species with increasing Cu concentrations, but no significant differences between control and Cu-exposed gastropods. In part, this may be due to the short-term nature of the experiments, but may also reflect that Cu exposures contributed to increased toxicity even when overall tissue Cu levels were not significantly affected. Elevated lipid peroxidation was associated with elevated tissue Cu levels. These studies further indicate that cellular biomarkers, especially lysosomal destabilization, are sensitive, fundamental responses that can be applied with a variety of species over broad, global regions to assess impacts of anthropogenic and environmental processes.

WP073 Chicago's Toxic Legacy: Trace Metal Biomonitoring Along the Southern Shoreline of Lake Michigan

W.A. Shoults-Wilson, Roosevelt University / Biological, Chemical and Physical Sciences; N. Elsayed, K. Leckrone, Roosevelt University; J.M. Unrine, University of Kentucky

The Laurentian Great Lakes are a textbook case of anthropogenic disturbance, having been systemically contaminated by a cocktail of toxicants and ecologically disrupted by a host of invasive species. Chicago and the tri-state area, the largest urban center on Lake Michigan, have undoubtedly contributed to contaminant loads within southern Lake Michigan. This is the first study to use the invasive zebra mussel (*Dreissena polymorpha*) as a biomonitor of toxic trace metals from along the Chicago-area coastline. The study also examined quagga mussels (*Dreissena bugensis*) and sediments from stations throughout the lake. Surprisingly, the present study found significantly lower concentrations of Cd, Hg and Pb at sites in the south and east. These are areas that would have been expected to show a greater impact from industrial activity. Also unexpected was the significant negative relationship between Hg and mussel length. This trend is the opposite of that found in numerous bivalve biomonitoring studies and warrants further study. Additionally, Se showed a significant positive trend with shell length and a distinct pattern of spatial distribution along the shoreline. Tissue concentrations of metals in *D. polymorpha* were only weakly linked to concentrations in fine sediments, most likely due to the fact that *D. polymorpha* were sampled from structures rather than from the sandy sediments themselves. This study demonstrates the need to expand biomonitoring throughout southern Lake Michigan in order to better understand the extent of bioavailable toxic metal contamination.

WP074 Cyprinids and Selenium – A Case Study

S.D. Baker, GEI Consultants, Inc.; S.P. Canton, GEI Consultants, Inc. / Ecological Division; C. Johnston, Public Service Company of Colorado

Selenium (Se) is an essential micronutrient that occurs in virtually all environmental media at trace concentrations. While anthropogenic activities such as irrigation are known to increase Se beyond background concentrations in many aquatic ecosystems, the Saint Charles River basin in south-central Colorado is known to have naturally elevated Se concentrations originating from geological sources. In 2011 and 2012, we collected fish population and water chemistry data from multiple sites in the Saint Charles River to determine potential sources of Se and evaluate whether Se is impairing aquatic life in this stream. Water column Se concentrations, measured monthly, varied both spatially and temporally and ranged from less than detection up to 650 µg/L. Despite this wide range in Se concentrations, all study sites supported similarly healthy and diverse fish populations primarily composed of minnows, stonerollers, and dace (Family Cyprinidae). Multiple age classes (young-of-year, juveniles, and adults) for many species were observed throughout the study area, indicating reproduction is occurring despite often extremely high Se levels. The results of this study supported adoption of ambient-based site-specific Se standards for the Saint Charles River.

WP075 Effects of copper exposure on energy metabolism in the yellow clam Mesodesma mactroides

M. Mussoi Giacomin, Instituto de Ciências Biológicas; M.B. Jorge, Universidade Federal do Rio Grande; A. Bianchini, Fundação Universidade / Instituto de Ciências Biológicas, Universidade Federal do Rio Grande / Instituto de Ciências Biológicas

Although copper (Cu) is an essential micronutrient for cell metabolism, it can be very toxic when at elevated concentrations in the water. In many freshwater species, Cu is described as an ionoregulatory toxicant, inhibiting the activity of key enzymes involved in Na^+ uptake and consequently inducing ionic and osmotic disturbances. However, the mechanism involved in the acute toxicity of Cu in estuarine and marine invertebrates remains still unclear. Results from recent studies performed in our laboratory have suggested that the mechanism of Cu toxicity in marine invertebrates is more related to a respiratory impairment rather than to ionic and osmotic disturbances. In the present study, metabolic alterations induced by waterborne Cu exposure were evaluated in the yellow clam *Mesodesma mactroides*, a bivalve species widely distributed along the South American sandy beaches. Juvenile yellow clams were maintained under control conditions (no Cu addition in the water) or acutely (96 h) exposed to Cu (96-h LC10 = 150 µg Cu/L) in artificial sea water (30 ppt). After exposure, the following endpoints were analyzed in gills, digestive gland, pedal muscle, and hemolymph: protein, lipid, glucose, glycogen, ATP, lactate and pyruvate concentration, as well as intracellular NAD^+/NADH ratio. In all tissues, Cu exposure did not affect the ATP concentration and NAD^+/NADH ratio, indicating that clams were able to keep constant the intracellular energy level. However, a significant increase in total protein content was observed in the digestive gland. This could be explained considering an increase in protein synthesis, a possible compensatory mechanism to counteract the protein oxidation observed in a previous study in our laboratory with yellow clams exposed to Cu under similar experimental conditions. Finally, increased lactate content and decreased glucose content was observed in the pedal muscle of Cu-exposed clams. Overall, these findings indicate that Cu can be acting as a respiratory toxicant in clams, leading to an increased reliance upon the anaerobic energy production.

WP076 Effects of hypoxia on metal bioavailability, antioxidant status and tissue damage in Eastern Oysters and Ribbed Mussels

B. Khan, UNC-Charlotte / Biology; A.H. Ringwood, UNC-Charlotte / Department of Biology

Estuaries support a wide variety of organisms and are exposed to multiple stressors diurnally as well as seasonally. Fluctuations in dissolved oxygen, pH, salinity and temperature as well as heavy metal pollution from anthropogenic inputs can be challenges of living in estuaries. Habitats characterized by low dissolved oxygen (hypoxia), accompanied by increased PCO_2 (hypercapnia) and reduced pH are expanding in marine coastal habitats worldwide. Hypoxia and metals can induce oxidative stress in aquatic organisms via cellular production of reactive oxygen species, and interactions between them can exacerbate oxidative stress and subsequent tissue damage. Copper and manganese are two essential metals which can be toxic if bioaccumulated in the tissues. These metals are present in industrial effluents and sewage sludge which are introduced in many estuarine ecosystems. Metal ions can exist in the environment complexed with organic or inorganic matter, and their bioavailability may be affected by water quality parameters such as dissolved oxygen, pH and salinity. The overall goal of these studies was to evaluate the effects of cyclical and continuous hypoxia on metal uptake in tissues of two bivalves – *Crassostrea virginica*, Eastern oysters and *Geukensia demissa*, ribbed mussels. Both of these organisms are bioindicators and play critical roles in maintaining ecosystem integrity. We hypothesized that hypercapnic hypoxia increases bioavailability of copper and manganese from estuarine sediments, resulting in increases in metal concentrations in digestive gland and gill tissues of *C. virginica* and *G. demissa.* Metal concentrations in the tissues of oysters and mussels were measured using atomic absorption spectrophotometry after exposure to contaminated sediments under different hypoxic conditions to assess metal uptake. Sediment samples were also analyzed for metal concentrations. A lipid peroxidation assay was used to evaluate tissue damage, and total glutathione concentrations were quantified to examine antioxidant status. Tissue metal concentrations were found to be affected by hypercapnic hypoxia which also resulted in elevated tissue damage and altered antioxidant status. Determination of the sensitivities and vulnerability of two ecologically important bivalve species to hypoxia and bioavailability of metals is critical for predicting impacts on estuarine ecosystems.

WP077 Manifestation of arsenic induced changes in zebrafish, Danio rerio

S. Sarkar, S. Mukherjee, Visva-Bharati University / Department of Zoology; A. Chattopadhayay, S. Bhattacharya, Visva-Bharati University

Arsenic (As) is a ubiquitous environmental toxicant and the risk of arsenic poisoning is a public issue worldwide. Human exposure to this metalloid mostly comes from well water contaminated soil and occupational exposure. Despite several reports on toxic response of arsenic in aquatic system, information regarding alteration of toxicological marker genes and biochemical parameters at low dose of exposure is meager. Since, liver is a major target organ of arsenic toxicity, manifestation of arsenic (As_2O_3) toxicity was studied in zebrafish liver at 0.05 mg/l [maximum permissible limit in drinking water according to BIS Standard (BIS 10500-1991,2004) in India, Bangladesh and many other countries] for 7, 15, 30 and 60 days. Histopathology showed significant alterations in cellular architecture of As-treated zebrafish liver compared to the control group of fish. Further, screening of toxicology-marker genes by Reverse Transcriptase PCR showed down regulation in the expression of Catalase, Cu/Zn SOD, Bcl2, GPX1, GAD45 and CDKN1A genes after 15-30 days As exposure against control group. However, these expressions were reversed after 60 days exposure though still remained lower than the control group and a biphasic pattern of expression was recorded over the entire exposure period. The level of reduced glutathione (GSH) was measured and generation of superoxides was also assessed by TBARS assay method. Zebrafish exposed to As showed a biphasic response of GSH level accompanied by elevation in level of lipid peroxidation by 2-3 folds against untreated sets. Concomitantly, biphasic pattern of glutathione-S-transferease (GST) and catalase activity was noted in As exposed samples. However, above findings showed highest oxidative damage after 15 days of arsenic exposure. The expression of HSP 70, Nrf2 and Nrf2 dependent proteins were checked by immunoblot analyses. Interestingly, expressions of these proteins were correlated with the gene analyses and biochemical responses. Therefore, present study surmised that even at a low dose, arsenic elicits significant oxidative damage in zebrafish. Moreover, alteration in toxicology marker genes along with biochemical responses evidenced from the present study can be extrapolated to infer possible alterations associated with liver damages that could occur in individuals exposed to arsenic where the level of arsenic in drinking water is 0.05mg/l or above.

WP078 Redox parameters in the marine clam Mesodesma mactroides acutely exposed to copper

M.B. Jorge, Universidade Federal do Rio Grande; A. Bianchini, Fundação Universidade / Instituto de Ciências Biológicas, Universidade Federal do Rio Grande / Instituto de Ciências Biológicas

Copper exposure may induce oxidative stress stimulating the production of reactive oxygen species (ROS) and/or reducting the antioxidant defense capacity, this results in oxidative damage to biomolecules such as DNA, proteins, and lipids. The aim of the present study was to evaluate the response of redox parameters and oxidative damage to biomolecules in gills and digestive gland of juveniles of the marine clam *Mesodesma mactroides* exposed (96 h) to a sublethal concentration of copper (150 µg Cu L-1) in sea water (salinity 30 ppt). Endpoints analyzed were measurements of ROS, antioxidant capacity against peroxyl radicals (ACAP), antioxidant enzyme (glutathione peroxidase, glutathione cysteine ligase and catalase) activity, non-enzymatic antioxidants (glutathione and metallothionein-like proteins) concentration, protein carbonyl concentration, and lipid peroxidation. In gills, glutathione cysteine ligase activity, metallothionein-like proteins concentration and reduced glutathione concentration increased while glutathione peroxidase activity and protein carbonyl concentration reduced after clam exposure to copper. In digestive gland, copper exposure increased ROS level, glutathione peroxidase activity and protein carbonyl concentration while a reduced lipid peroxidation was observed. These findings suggest that an increased level of non-enzymatic antioxidants proteted the organisms against ROS and oxidative damage to lipids and proteins in gills of copper-exposed *M. mactroides*. However, the digestive gland of the clams did not show the same profile of defense and evidence of oxidative stress were observed after copper exposure.

WP079 The acute and chronic toxicity of rare earth lanthanide elements to Northern aquatic species

M. Desforges, C. Johnson, Natural Resources Canada

Rare earth elements (REEs) comprise 15 elements on the lanthanide family of the periodic table. These elements are vital to a huge variety of technological applications and it is the importance of these metals and the uncertainty of supply that has increased interest in developing REE resources in Canada. The present knowledge of the eco-toxicological effects of lanthanides is limited. A study by Borgman et al. tested lanthanide toxicity to the aquatic species, *Hyalella azteca* for use in categorization for the Domestic Substances List (DSL). As well, our laboratories did similar work with *Daphnia pulex*, another commonly used species in aquatic toxicity testing of metals, for the DSL. The studies both illustrate that lanthanide elements have the potential to be acutely toxic at low or intermediate µg/L concentrations, and that there are differences in species sensitivities. Some of the most promising REE resources in Canada are in the North and it is therefore essential to consider the environmental impacts of REE development from a Northern perspective. Toxicity tests using northern species are rare and it is not known whether the sensitivity to environmental impacts differs from the standard test species used in toxicity tests (e.g., *Daphnia magna*). Therefore, using a standard test species may not provide an environmentally relevant assessment for risk. By developing and assessing toxicity of REE's to a northern aquatic species it will be possible to compare their sensitivity to the standard Environment Canada protocols currently used under the MMERs. The objective of this paper is to provide critically lacking data on the bioavailability and toxicity of selected data-poor metals in the aquatic environment.

WP080 The Effect of Sub-Lethal Heavy Metal Concentrations on a Predator-Induced Polyphenism in Daphnia pulex

S.M. Harmon, P.W. Steen, University of South Carolina Aiken / Biology and Geology

Induced polyphenisms are developmental changes that occur in an organism following specific environmental interactions. In aquatic environments, the release of water-soluble factors called kairomones, by predators, can stimulate predator-induced polyphenisms, if detected by prey. The resulting predator-induced polyphenism assists the prey in escaping the threat. *Daphnia* are a group of filter-feeding crustaceans located in ponds and lakes and have world-wide distribution. These keystone species demonstrate various predator-induced polyphenisms in the presence of different predators. Specifically, *Daphnia pulex* juveniles (neonates) emerge from their mother's brood chamber displaying a morphological structure called "neckteeth", if exposed to kairomones that are released from *Chaoborus* spp. larvae. As a result, *Chaoborus* larvae have difficulty handling the young daphnids following capture, which increases the potential for escape. In our lab, daphnids were exposed to a sub-lethal concentration of copper (5µg/L) and zinc (120µg/L) in both the brood chamber (indirect exposure) and following dissection from the brood chamber (direct exposure). Neonates were analyzed at juvenile instars 1-4 to examine body size and determine neckteeth production. Following indirect exposure to kairomone plus copper, daphnids displayed significantly lower body lengths and body depths at instars 1-4. Following exposure to kairomone, peak neckteeth production occurred at instar 1 and declined through instar 4. The addition of copper sustained neckteeth induction through instar 2; however, induction decreased to zero at instars 3 and 4. Following direct exposure to kairomone plus copper, body lengths and body depths were similar in all groups. These findings indicate the presence of a maternal effect that influences growth during juvenile development. Following exposure to kairomone, peak neckteeth induction occurred at instar 2 and declined through instar 3. The addition of copper reduced the induction level at instar 2. These findings suggest that indirect exposure to copper plus kairomone promotes neckteeth production, while direct exposure suppresses the phenotype. Currently, experiments are being conducted to examine the effect of zinc on growth and neckteeth production.

WP081 The relationship between brain chemistry and anxiety behavior in the fathead minnow, Pimephales promelas

A.L. McLeod, Clemson University / Institute of Environmental Toxicology; L.E. Sweet, Institute of Environmental Toxicology, Clemson University; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX)

The sources of anthropogenic compounds in today's society are many and can include personal care products and pharmaceuticals. Some of these compounds are not efficiently metabolized by the human body or removed from sewage before they reach streams and rivers, and so they are often detected in surface waters. Once present in the ecosystem, they have the potential to cause adverse effects to the organisms that live there. Previous work has shown that upon exposure to 150 ug/L fluoxetine, a selective serotonin reuptake inhibitor antidepressant, hybrid striped bass (*M. saxatilis* x *M. chrysops*) brain serotonin levels decreased by almost 50% over six days. Similarly,

exposure to venlafaxine, a serotonin and norepinephrine reuptake inhibitor antidepressant, also showed a significant decrease in brain serotonin levels after six days of exposure to 500 ug/L concentrations. These studies also correlated depressed brain serotonin levels with behavioral alterations that decreased an organism's ecological fitness. While these antidepressants are designed to alter brain neurotransmitter levels, other investigators have suggested that common contaminants such as pesticides and metals can also affect brain chemistry. A previous study has shown that carp exposed to sub-lethal levels of copper over the course of one week experienced decreased brain serotonin levels in three different parts of the brain. Our study characterized the effects of copper exposure on brain chemistry and behavior in the fathead minnow (*P. promelas*) using the standard light/dark anxiety behavioral assay. Based on the preliminary results of this bioassay, there was no statistical difference in behavior of exposed and unexposed fish. However, there was an apparent dose-dependent trend in fish exposed to copper that suggested they spent less time in the light. This study further elucidates the relationship between brain chemistry and anxiety-like behavior in fish.

WP082 Trophic transfer and toxic impacts of gold nanoparticles (AuNp) from periphytic biofilm to gammarus

M. Baudrimont; J. Andrei, University of Bordeaux; N. Mesmer-Dudons, P. Gonzalez, CNRS UMR EPOC; S. MORNET, CNRS, ICMCB, University of Bordeaux; R. Maury-Brachet, University of Bordeaux; O. Geffard, J. Garric, Irstea; A. Feurtet-Mazel, Univeristy of Bordeaux

Nanotechnologies and their scientific promises know a real craze in this beginning of century thanks to new and innovative properties. Their discharge in the environment and in the aquatic ecosystems becomes inevitable and the question of a likely toxic impact must be seriously studied. Even if the studies are there still at their initial stage, it was already demonstrated that gold, chemically inert, can highly react with biological component under its nanoparticle shape. In this integrated survey of the Tro-nano program (ANSES) which is interested in the transfers of nanomaterials and in their consequences on the aquatic organisms, the bioaccumulation of gold nanoparticles (AuNp) along a simplified trophic network: periphytic biofilm, crustacean *Gammarus fossarum* was demonstrated. The peculiarity of this study consisted in developing for the first time, a transcriptomic approach and microscopic observations (transmission electronic microscopy) in the crustacean. These two approaches allowed showing the existence of cellular damage caused by an oxidative stress and in particular an impact of these AuNps at the level of the mitochondrial breath. The damage due to these nanoparticles could then have vital consequences for the organisms during longer exposure periods. Many hopes exist as for the observation of the mechanisms of penetration of the AuNps inside tissues in particular at the level of the hepatopancreas. A first step was made towards these subcellular analyses, but it will be necessary from now on in the future to make a more thorough and complete work of researches for genes and for microscopic observations to support our results to understand and to know at best the mechanisms of toxicity of these emergent contaminants.

WP083 Trophic Transfer Modeling of Selenium – how much data do we need?

L. Keller, Marshall University; M.Y. Armstead, Marshall University / Integrated Science & Technology; M. Wilson, Marshall University

Trophic transfer models are proposed for determining safe site-specific water column concentrations of selenium due to lack of correlation between water column and fish tissue selenium concentrations. Previous studies have shown variability in the models ability to predict fish tissue concentrations. The current study was undertaken to define the data required to maximize cost effective predictability in model development in a lotic, coal mining influenced system in West Virginia. Selenium concentrations were measured monthly in water column, periphyton, benthic macroinvertebrates, crayfish, and fish. Samples were collected at three sites with a range selenium water column concentrations. Fish tissue sampling included whole-body and egg/ovary analyses of the three most abundant species (creek chub, green sunfish, and northern hogsucker) at the sampling sites. Trophic transfer models were developed for the dataset using monthly, quarterly (seasonal), and semi-annual sub-sets of the data. Comparison of model outcomes will be presented at the three selenium concentrations with a discussion on data requirements for maximizing accuracy in model development.

WP084 Development of q-PCR approaches to assess water quality: Effects of cadmium and pesticides on gene expression of diatoms

S. Kim Tiam, Irstea, Centre de Bordeaux; S. Moisset, A. Feurtet-Mazel, University of Bordeaux 1; N. Mazzella, Irstea, Centre de Bordeaux; A. Arini, University of Michigan; F. Delmas, S. Morin, Irstea, Centre de Bordeaux; G. Daffe, P. Gonzalez, University of Bordeaux 1, CNRS

Periphytic diatoms are valuable tools to determine freshwater rivers quality based on diatoms identifications and distribution and constitute key indices in European standards. Nevertheless, indices currently used for water quality assessment do not take in consideration the diverse nature of contaminants, are time consuming and require important taxonomic knowledge. In this context development of q-PCR approaches have been developed to enhance a better reactivity in the diagnosis of modifications due to pollution conditions and to understand the genetic impact in relation with diatoms species. Through different laboratory experiments (14 days exposure) concerning metallic (Cd: 10 and 100 µg/L) or pesticides contamination (diuron: 1 and 10 µg/L), several target genes involved in mitochondrial metabolism (*coxI*, *nad4*, 12S), oxidative stress response (*sod Mn*), detoxification (*cyp1A1*) and photosynthesis (*psaA*, *d1*) have been characterized and their expression levels have been determined. Results showed that both compounds impacted the mitochondrial and the photosynthetic metabolisms. The observed effects were closely related to the growth rate efficiency of the cultures used. Thus, these molecular biomarkers could be used to evidence early response and sensitiveness of diatoms during environmental pollutions (ou xenobiotic exposures). These first innovative results constitute a first step towards the study of periphytic diatoms through molecular biology.

WP085 Influence of substratum on the structural and functional composition of river biofilms and implications for assessment of ecological risk

A. Paule, University of Saskatchewan – Global Institute for Water Security; J.R. Lawrence, Environment Canada / Water Science and Technology Directorate; J. Roy, Water Science and Technology Directorate, Environment Canada; G. Swerhone, Water Science and Technology Directorate, Environment Canada; M.J. Waiser, University of Saskatchewan – Global Institute for Water Security; C. Maynard, E. Yergeau, C. Greer, National Research Council of Canada

Phototrophic biofilm communities provide a particularly relevant model as bio-indicators for ecological assessment in aquatic environments. To reduce inter and intra site spatial heterogeneity, increase replication, and control cultivation time, most investigations of river phototrophic biofilms use artificial substrata. However, a lack of standardization has made it difficult if not impossible to compare results between studies and to distinguish the impact of the substratum from the influence of environmental change. The aim of this study was (i) to assess microbial community structural and functional composition of river phototrophic biofilms grown *in situ* on different artificial(polycarbonate, ceramic, glass and aluminum) and natural (limestone and wood) substrata and (ii) to reveal which substratum would be preferable when using phototrophic biofilms as bio-indicators. After 5 weeks of growth, the substrata were sampled and the biofilms analysed using a multi-endpoint approachincluding functional and structural endpoints (i.e., confocal laser microscopy imaging, molecular fingerprinting methods (DGGE), dry mass, chlorophyll a, carbon utilization spectra, and metagenomic sequencing). Based on the molecular fingerprinting method, carbon source patterns and levels of chlorophyll *a*, biofilms growing on wood were signficantly different ($p < 0.05$) from other substrata. Microscopic analyses indicated bacterial biomass was greatest on wood, whereas aluminium tended to have the greatest algal biomass. Metagenomic sequencing results also reflected significant differences between substrata. Based on phylum level analyses, wood exhibited the greatest percentage of *Bacteroidetes* but the lowest percentage of *Bacillariophyta,* although they favoured aluminium and polycarbonate. The diatoms *Thalassiosira* and *Odontella* favoured aluminium and polycarbonate but were not common on wood. No difference ($p < 0.05$) between substrata was observed in terms of bacterial production or dry mass. Metagenomic analyses did not reveal any differences in the relative distribution of genera involved in bioremediation and aromatic compound degradation. Our work demonstrates the importance of choice of substratum composition (artificial *versus* natural) in investigations of phototrophic biofilms used as bio-indicators due to their impact on the biofilm structural and functional composition, and their potential influence on the ecological risk assessment.

Bioavailability Tools for Assessing Effectiveness of Contaminated Sediment Remediation

WP086 Active sampling for bioavailability using solid phase extraction – the in situ sampler for bioavailability

S. Supowit, Arizona State University; V.D. DANG, K.J. Kroll, University of Florida / Physiological Sciences; I. Roll, Arizona State University; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology; R.U. Halden, Arizona State University / Center for Environmental Security, The Biodesign Institute at Arizona State University

The bioavailability of contaminants in the environment is important from both a bioremediation and ecological perspective. A fast and reliable means of evaluating the bioavailability may be useful for assessing the hazards posed by toxins, the feasibility of bioremediation options, and the efficacy of remediation techniques like GAC amendment and deep tilling. The *In Situ* Sampler for Bioavailability (IS2B) is an automated, multi-channel sampler that performs solid-phase extraction (SPE) on large volumes of water onsite for the purpose of evaluating the bioavailability of chemicals in the environment. This device was designed to sample water from both the pore spaces and bulk water phase, providing triplicate data for each phase. Fed from a six-channel peristaltic pump, three SPE cartridges in parallel extract the water from each source. We have selected five organochlorines for the validation and testing of this device: Fipronil, dieldrin, triclosan, triclocarban, and *p,p'*-DDE. The sampler will undergo three phases of development: bench validation, field testing, and implementation. Bench validation is currently in progress, and consists of extraction recovery tests using poly(styrene) divinylbenzene resin or C-18. Preliminary validation results show extraction efficiencies for three of the chemicals to be consistent from channel to channel (< 10% variance coefficient). Also in progress are biotic uptake experiments in order to assess the actual body burden accumulation by blackworms and fathead minnows, which is to be compared with sampler uptake data in order to determine the applicability of the sampler as a surrogate for body burden studies. Initial data indicates that the maximum body burden attained by blackworms in water spiked with *p,p'*-DDE (0.09 µg/L) and dieldrin (1.02 µg/L) is achieved between two and seven days of exposure, and ranges from 30 – 50 ppb for *p,p'*-DDE and 100 – 200 ppb for dieldrin. Worms with body burdens are to be fed to fathead minnows, which are in turn also evaluated for body burden due to biomagnification. Spiked water tests at the same concentrations can show the utility of the sampling device as a bioavailability evaluation tool for of these chemicals to macrobiota.

WP087 An Isotope Dilution Method to Measure Bioavailability of Hydrophobic Organic Compounds in Marine Sediments

F. Jia, University of California, Riverside / Department of Environmental Sciences; D. Schlenk, University of California-Riverside / Department of Environmental Sciences; J. Gan, University of California Riverside / Environmental Sciences

Bioavailability is an important variable in understanding the environmental risk of sediment-borne hydrophobic organic compounds (HOCs), where chemical methods have long been sought as an alternative to bioassays due to their simplicity, robustness, and reproducibility. However, current chemical methods have their operational limitations. For example, Tenax-aided desorption is laborious and time consuming, and passive samplers such as solid-phase microextraction (SPME) need long time to reach equilibrium. In this study, a new isotope dilution method based on the use of stable isotope labeling was developed. The method derives a new variable, the exchangeable pool (*E*) that may be used as a predictor of bioavailability of HOCs. Marine sediments (e.g., namely 8C and 6C) heavily contaminated with DDT and its metabolites and PCBs from the Palos Verdes (PV) Shelf Superfund site were used in the method development, and the measured *E* values were compared with Tenax and SPME measurements. The bioaccumulation of selected DDTs and PCBs was simultaneously measured using the marine polychaete worm *Neanthes arenaceodentata*. By introducing a small amount of ^{13}C- and/or deuterium – labeled DDTs and PCBs into a sediment sample, partitioning of the added isotope-labeled analogs reached an apparent equilibrium within 1d under mixing conditions, allowing the measurement of *E*. Results indicate that the derived *E* values correlated closely with the rapid desorption fraction given by the Tenax desorption method or the freely dissolved concentration by the SPME technique. In addition, the isotope dilution method was also a good indicator of bioaccumulation potential of HOCs from sediments into the polychaete.

WP088 Combining Bioavailability Assays with Modeling to Predict PCBs in Fish after Remediation

H. Fadaei Khoei, University of Maryland Baltimore County / Chemical, biochemical and environmental engineering; A. Watson, A. Place, Institute of Marine and Environmental Technology, UMCES; U. Ghosh, University of Maryland Baltimore County / Department of Chemical, Biochemical, and Environmental Engineering

Sediment amendment with carbonaceous materials such as activated carbon has been introduced as an effective approach to sequester hydrophobic organic chemicals such as PCBs. The effectiveness of treatment on biouptake reduction in the food chain can be evaluated by changes in PCB concentrations in sediment porewater and surface waters and assessing PCB uptake pathways to fish. Traditional food chain models that are used for risk assessment purposes rely on bulk sediment concentrations to predict uptake in the food chain. However, freely dissolved concentrations, measured by passive sampling techniques, can be used as input to bioaccumulation models, and lead to more precise predictions of PCB concentrations in aquatic species. Preliminary results from the first set of aquaria experiments showed that porewater PCB concentration in PCB impacted sediment was reduced by two orders of magnitude upon amendment with 5% AC measured after 1.5 and 3 months. Zebrafish body burdens reflected the differences in porewater concentrations among sediment types. Activated carbon amendment did not change PCB concentration in sediment, but reduced the PCB concentrations in zebrafish by a factor of 8 to 9. Ongoing work is focused on incorporating passive sampler measurements in PCB biouptake models and evaluating exposure pathways by using mummichog as a model pelagic feeding and catfish as a model benthic feeding fish in laboratory experiments. In addition, dietary uptake effect on PCB bioaccumulation in the above species are being investigated in ongoing experiments.

WP089 Development of fundamental understanding of passive sampler uptake of PAHs and PCBs

M. Jalalizadeh, Chemical, biochemical and environmental engineering; U. Ghosh, University of Maryland Baltimore County / Department of Chemical, Biochemical, and Environmental Engineering

While field deployment of passive samplers for measuring low concentration of freely dissolved hydrophobic organic compounds (HOC) in overlying water and pore water concentration in sediments has become more common, there is a little knowledge of fundamental diffusive process of HOCs in passive samplers. One approach is to use fluorescence microscopy and thin sectioning techniques to directly measure the amount of HOCs that diffuse in passive samplers over time to evaluate whether the uptake process is pure linear partitioning, is governed by Fick's law, or it is truly reversible. Adsorption and desorption profiles of a compound into a polymer was modeled using numerical integration of Fickian diffusion. This model is adjustable for different boundary conditions including variations in water concentration of the field, where the polymer is deployed. The model calibration is dependent on the direct measurement and profile observation of the diffused compound inside a polymer. Experimental measurements of diffusion are being conducted using fluorescence imaging. The initial attempts of fluorescence measurement were done by exposing a PE rod to a saturated aqueous solution of pyrene from one end. The curved side of the rod was blocked from diffusion using with aluminum tape. The rod was cut along with its axis after exposure and the interior was imaged under a fluorescence microscope. This talk will present the initial experimental results and numerical modeling of adsorption and desorption of a PAH and PCB molecule into a polymer. Also, some results from sectioning and fluorescence measurements of diffused pyrene into polyethylene (PE) will be illustrated.

WP090 Evaluation of distribution of p,p'-DDE and dieldrin in largemouth bass exposed to the chemicals by gavage

K.J. Kroll, V.D. DANG, University of Florida / Physiological Sciences; S.D. Supowit, Arizona State University / Department of Civil and Environmental Engineering; C.J. Martyniuk, University of New Brunswick; D.S. Barber, University of Florida / Dept. of Physiological Sciences; R. Conrow, St. Johns River Water Management District; R.U. Halden, Arizona State University / Center

for Environmental Security, The Biodesign Institute at Arizona State University; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology

The north shore of Lake Apopka, FL is heavily contaminated with organochlorine pesticides (OCPs) from years of heavy agricultural applications. These contaminants are readily bioavailable to largemouth bass introduced for 2 to 4 months into ponds built on site in the muck farms. Exposure studies demonstrated that, relative to control animals raised under ambient background conditions, OCP concentrations in exposed fish were 2 to 800 times higher for 4,4'-DDE, 4,4'-DDT, dieldrin, methoxychlor, and toxaphene. Female largemouth bass introduced into the ponds during peak reproduction had similar gonadosomatic indeces and plasma vitellogenin levels as control fish but plasma levels of 17beta-estradiol and testosterone were significantly decreased. Laboratory studies with largemouth bass using spiked diets for equivalent times of exposure showed equivalent tissue burdens for *p,p'*-DDE, dieldrin, toxaphene and methoxychlor, suggesting the diet as a major exposure route for these contaminants. To better understand how OCPs are distributed through internal organs in bass, we conducted gavage experiments with 10 and 1 ug/Kg fish weight of p,p'-DDE and dieldrin, respectively, for 96 hours. Fish organs (e.g., liver, gonad, bile, muscle, intestine, brain, and plasma) were collected and immediately stored at -20 ^{0}C preceding chemical analysis. Collected samples were then freeze-dried, solvent extracted with acetonitrile, and analyzed on a Hewlett Packard (HP) 6890 GC/MS. These data allowed us to determine the bioavailability and bioconcentration of OCPs introduced through the diet compared to other routes of exposure in wild fish collected from three different lakes in Florida.

WP091 Internal and External Improvements in the Sediment Quality Triad for Metals-Impacted Sediments

S.R. Clough, Haley & Aldrich, Inc / Risk Assessment; R.A. Schuck, Haley & Aldrich, Inc. / Industry & Environment

Designing a Sediment Quality Triad always looks good on paper but, more often than not, yields nebulous results. Confounding variables include variation in TOC and grain size (affecting both bioavailability and organism response), variable habitat with different benthic communities, and the presence of non-site/nonpoint pollutants. These studies are costly and often times a dose-response curve is not observed, especially when the contaminant concentrations are not distributed across a wide enough range of concentrations. At a Massachusetts site historically impacted by lead chromate, our conceptual model indicated that both lead and chromium would be sparingly soluble, so low bioavailability and low toxicity were anticipated (even well above the PEC). We developed a robust field and laboratory design to attempt to minimize methodological and regulatory uncertainty. Lead concentrations in the sediment ranged from low ppm in the reference brook to low percent levels in the impacted brook. Recognizing that randomly collecting sediment samples from impacted reaches would not necessarily provide a proper range of "doses" we 1) scrutinized historical data to determine locations that could provide a *wide* range of contaminant concentrations 2) collected samples at more locations than we intended to test in the bioassay 3) used additional 'chelation' replicates as an internal TIE control (to identify site-specific effects) 4) instructed the toxicity laboratory to store all the samples until the analytical results provided us with an optimal range of "doses" and 5) collected benthic macroinvertebrates immediately adjacent to samples obtained for sediments for toxicity testing. Although these improvements required more field effort, the improvements only added 15% to the total cost of the project and, more importantly, allowed us to choose the proper sediment levels to test, ultimately yielding a robust range of dose-response concentrations. The results validated the conceptual model, resulting in high confidence in the risk management strategy and greatly narrowing down the range of remedial options.

WP092 Summary of SETAC Pellston Workshop on Passive Sampling: A practical Guidance for Bioavailability Assessment

U. Ghosh, University of Maryland Baltimore County / Department of Chemical, Biochemical, and Environmental Engineering; S.B. Kane Driscoll, Exponent, Inc.

Freely dissolved concentrations (C_{free}) of hydrophobic organic chemicals in sediment porewater drive uptake in benthic organisms and flux into overlying water. When used in appropriate models of risk assessment, C_{free} can provide critical site-specific input on chemical bioavailability. Despite significant advances in the development and application of passive sampling methods for C_{free} over the past decade, incorporation of these tools in contaminated sediment management decisions has been limited. On November 7-9, 2012, 45 experts from academia, government and industry, representing Passive Sampling Method (PSM) developers, users and decision-makers, participated in a SETAC Technical Workshop in Costa Mesa, CA, USA. Participants reviewed the state of science to gain consensus on PSM use in assessing and supporting management actions on contaminated sediments. The workshop outcome is being developed into 5 full length articles: 1) and 2) provide a literature review of PSM use in sediments for organics and metals, respectively; 3) outlines the rationale for using freely dissolved concentrations derived from PSMs; 4) outlines practical guidance for lab and field deployment of PSMs and 5) discusses management application of PSMs. This talk will provide an overview of the workshop outcome and focus on paper #3 on practical guidance. The objective of this paper is to provide practical guidance that advances the use of PSMs in regulatory contexts. The paper focuses on defining methodological principles that when applied correctly will provide accurate, reliable, and consistent data across different polymer/sampling formats including the translation of polymer concentrations to C_{free} of hydrophobic organic chemicals in sediment pore water. Both laboratory-based "ex-situ" and field-based "in-situ" approaches are discussed along with key research questions and other practical considerations that influence approach selection. The paper includes an example of the application of using C_{free} data in the decision-making process for a contaminated sediment site. Finally, a brief list of future research questions are provided to further improve the collection of better quality and more scientifically robust data for making environmental management decisions.

WP093 Using sorption data to predict the effectiveness of in situ remediation treatments of contaminated sediments

J.L. Gomez-Eyles, Y. Zhou, UMBC; U. Ghosh, University of Maryland Baltimore County / Department of Chemical, Biochemical, and Environmental Engineering

Enhancing sediment sorption characteristics using *in situ* amendments (e.g., activated carbon (AC)) can reduce contaminant bioavailability by decreasing freely dissolved concentrations. The sorption characteristics of typical sediment contaminants to different sorbents can be used for the selection of the most appropriate amendment to reduce contaminant bioavailability, and also to predict amendment effectiveness on a site specific basis. Bioavailability tools (e.g., polyoxymethylene solid phase extraction (POM-SPE)) can be used to assess these sorption characteristics and also measure amendment effectiveness. ACs have a high affinity for many hydrophobic organic chemicals (HOCs), but more recently biochars have been suggested as lower cost alternatives. However, there are few comparative sorption data on which to base the choice of AC or biochars for sediment remediation. Here we characterize the sorption of PAHs, PCBs, DDTs to a wide range of ACs and biochars at environmentally relevant concentrations. This sorption data is used to model the predicted reductions that would be achieved in typical contaminated sediments after amendment. These modeled predictions are compared with actual reductions in sediment porewater concentrations measured using POM-SPE. The effectiveness of the carbons was found to be closely related to their surface area and pore size distribution. Modeled predictions suggested high surface area ACs are more effective than biochars at reducing HOC bioavailability. Modeled reductions were in close agreement with observed reductions. Sediment porewater PCB concentration reductions of 18-80% were observed after amendment with unactivated biochars, and >99% after AC amendment. However, modeled reductions generally over predicted amendment effectiveness. It is likely for this to occur as a result of dissolved organic matter within the sediment sorbing onto the carbon, blocking sorption sites and reducing the effective surface area of the carbon (fouling). New sorption isotherms using fouled ACs and biochars were performed and confirmed a decrease in sorption effectiveness with increasing dissolved organic matter concentrations. This fouling effect was greater in the ACs than the biochars. Extensive characterization of the virgin and fouled carbons confirmed this was due to a reduction in surface area of the fouled carbons. The fouled carbon sorption data is currently being used to refine model predictions of carbon effectiveness.

WP094 Utilizing thin-film solid-phase extraction to assess the effect of organic carbon amendments on the bioavailability of DDT and dieldrin to earthworms

N.A. Andrade, Civil and Environmental Engineering; T. Centofanti, University of Maryland; L.L. McConnell, US Department of Agriculture-ARS / Environmental Quality Laboratory; C.J. Hapeman, USDA-ARS; A. Torrents, University of Maryland / Civil and Environmental Eng.; A. Nguyen, USDA-ARS; N.

Beyer, US Geological Survey / Patuxent Wildlife Research Center; R. Chaney, J. Novak, USDA-ARS; M. Anderson, University of Maryland; K. Cantrell, USDA-ARS

Improved approaches are needed to rapidly and accurately assess the bioavailability of persistent, hydrophobic organic compounds in soils at contaminated sites. The performance of a thin-film solid-phase extraction (TF-SPE) assay using vials coated with ethylene vinyl acetate polymer was compared to an earthworm bioassay (*Lumbricus terrestris)*. Experiments utilized, as a control, contaminated soil from a former orchard that received routine DDT and dieldrin applications >40 years ago. The soil was amended with four different organic carbon materials at 5% by weight to assess the change in pesticide bioavailability. In both assays, bioavailability of 4,4'-DDE, 4,4'-DDD, and dieldrin was higher than 4,4'-DDT in the control soil. Addition of organic carbon amendments significantly lowered bioavailability for all compounds except for 4,4'-DDT where bioavailability was significantly higher for three out of four amendments. Equilibrium concentrations of dieldrin and 4,4'-DDT + 4,4'-DDE in the polymer coating were strongly correlated with uptake by earthworms after 48 d exposure ($R^2 = 0.97$; $p < 0.001$) indicating TF-SPE provided an accurate simulation of uptake by *L. terrestris*. In a further test of the TF-SPE method, estimated bioavailability of dieldrin and DDX residues in the orchard soil was compared with a soil that was spiked with the same compounds and aged for 90 days in the laboratory. Differences in residue bioavailability in the two soils were observed using TF-SPE. Dieldrin and DDX were only 18% and 11% less bioavailable, respectively, in the orchard soil relative to the spiked soil despite >40 years of aging. Results show that TF-SPE will be a useful tool in examining the potential risks associated with contaminated soils and to test the effectiveness of remediation efforts.

What Worked and Why?

WP095 A Streamlined Approach to Ecological Risk Assessment

A.M. Rodolakis, AMEC Earth & Environmental, Inc.; D. Rae, AMEC; L. McFarlane, H. McCleave, Public Works and Government Services Canada / Environmental Services

Public Works and Government Services Canada (PWGSC) is responsible for assessing environmental liability at hundreds of coastal lightstations across the Maritimes. One of the goals of this program was to efficiently ascertain which sites had actionable risk. In order to meet this goal, the project team developed a risk assessment template that streamlined the ecological risk assessment process. Most ecological risk assessments in Canada and the United States recommend beginning with a Screening Level Ecological Risk Assessment (SLERA) that relies on conservative assumptions, and then proceeding to a detailed quantitative assessment (or Baseline Ecological Risk Assessment). Even these detailed risk assessments often default to conservative assumptions that later get replaced with more realistic assumptions in an uncertainty assessment or risk characterization. We streamlined the process by basing the ERA on realistic exposure assumptions from the outset. This approach worked because all the sites in this program have similar histories and contaminants, and the nature and extent of release at a given site is already well delineated (i.e., Phase II/III ESA completed) by the time the ERA is begun. As a result we have been able to meet the project goals of efficiently identifying which sites require remedial action.

WP096 Risk Management: The Key To A Cost Effective Sediment Remedy In A Northeastern Lake Severely Impacted By Lead And Chromium

R. Schuck, Haley & Aldrich, Inc; S.R. Clough, Haley & Aldrich, Inc / Risk Assessment

Risk management tools that were used to develop practical remedial solutions for a 110 acre lake located in Massachusetts. Lake sediments are impacted by lead and chromium due to past disposal practices of a former paint pigment factory operating along its shoreline from 1848-1928. Maximum lead and chromium concentrations in sediment were 94,000 mg/kg and 15,000mg/kg, respectively. Hydrodynamic modeling, human health and ecological risk assessments, long term monitoring and sampling approaches were all tools used to develop a cost effective remedial solution that addressed risks without destroying the lakes ecosystem. Hydrodynamic modeling conducted as part of the sediment transport study by the lead regulatory agency indicates that the lake is depositional in nature with very little sediment output. The modeling also determined that resuspension and subsequent transport of sediments is not likely to occur under conditions modeled. Human health and environmental risk assessments were used to develop risk-based remedial approach along a very limited portion of the lake to eliminate the significant risks. The remedial action utilized precise, mechanical dredging. Sample analysis on site for lead and chromium using a field portable XRF in near real time guided the dredging. A total of 6,500 cubic yards of sediment were excavated and disposed in an upland area beneath an engineered barrier. Monitoring of the remediated shoreline began after dredging was completed. Minimal recontamination has been observed, confirming the conclusions of the hydrodynamic model. For the rest of the Lake we were able to implement a No Action with monitoring remedy even though fish growth rates were reduced and surface water did exceed the NRWQC. No Action with monitoring was accepted as the remedial solutions (e.g., dredging or capping) would cause more harm than the risks posed by the current state. However, since the remedial dredging was completed, post remediation monitoring of the warm water fishery also show marked improvements of fish condition factors. The data no longer suggest reduced fish growth rates and no further action is proposed regarding the fishery. Long term monitoring of Surface water quality also indicates that a chronic exceedance does not exist and we are proposing to discontinue monitoring. As such, the site is in the process of being closed out.

WP097 Using a Shared Vision of Wetland Resource Protection to Focus the Design and Communication of a Baseline-Level ERA

C.A. Claytor, GEI Consultants, Inc.; K.B. Bradley, GEI Consultants; S.A. Roark, GEI Consultants, Inc.; C. Bradley, M. Westray, GEI Consultants

A baseline-level ecological risk assessment (ERA) was conducted at a former manufactured gas plant (MGP) site in the southeastern United States. The ERA was designed specifically to help make risk management decisions regarding the remediation of a wetland adjacent to the former facility and surface waters further downgradient. Moreover, the regulators and property owners shared a desire to limit active remedial measures in the wetland, which had previously been shown to provide important ecosystem services worth maintaining, to the extent possible. Thus, several lines of evidence along a gradient from predictive to empirical measures were evaluated, including: hazard quotients developed by comparing exposure point concentrations to ecological screening values; metals and PAH bioavailability-based toxicity predictions; benthic invertebrate community evaluations, wetland assessments; and laboratory sediment toxicity tests. Among these lines of evidence, only the hazard quotient assessment suggested the potential for adverse effects could not be ruled out, in contrast to the bioavailability, benthic community structure, wetland function and value, and toxicity assessments. Upon comparison of these lines of evidence, as well as consideration of their predictive or empirical bases, the ERA identified no site-related conditions requiring remedial actions, especially given the environmental injuries potentially caused by such actions would likely outweigh the benefits provided by the high-quality wetland. Regulatory response to the ERA's conclusions has, so far, been favorable, which is likely a reflection of the mutual objective identified at the outset of the project. Designing the ERA to rigorously address all risk questions related to this common goal was also critical to the project's success. Part of that rigor involved utilizing state-of-the-science methods to more-realistically characterize impacts at the site; and these methods were considered trailblazing given the state-level regulatory context of this project. Final approval by the regulatory agency is still pending, but this collaboration so far has been a fine example of risk managers and responsible parties using sound science to determine the potential for realizing a shared vision of lasting environmental benefits.

WP098 Adoption of a New Risk-based Approach for Deriving Water Quality Criteria for Copper in Colorado

R.W. Gensemer, GEI Consultants / Ecological Division; J. Gondek, GEI Consultants; S.D. Baker, GEI Consultants, Inc.; S.P. Canton, GEI Consultants, Inc. / Ecological Division; T. Foster, Law Office of Tad Foster; W. Burks, Tri-Lakes Wastewater Treatment Facility; J.W. Gorsuch, Copper Development Association Inc.

There is general consensus that hardness-based aquatic life criteria for copper and most other metals are scientifically outdated compared to derivation methods that use the biotic ligand model (BLM). In most states, however, current copper criteria are based on hardness even though EPA's national recommended criteria use the BLM. In Colorado, temporary criteria have been in place on several CO rivers until EPA developed more specific guidance on how to apply BLM-based criteria given the high temporal and spatial variability in instantaneous water quality criteria (IWQC) that

typically result from applying the BLM to large datasets. While EPA has not yet formally released such guidance, they have developed a new method, called the Fixed Monitoring Benchmark (FMB). The FMB is a probabilistic statistical tool that compares IWQC derived using the BLM to measured dissolved Cu concentrations in the receiving water to derive a single "fixed" benchmark Cu concentration that, if not exceeded, ensures that aquatic life uses will be protected using a 1-in-3 year exceedence frequency. This presentation summarizes our approach for developing and successfully implementing a new set of copper criteria for upper portions of Monument Creek that use both the BLM and FMB methods. While CO regulatory agencies have long been interested in BLM-based criteria, significant barriers have existed to implementing these criteria including availability of water quality data, sufficient amounts of detectable copper concentration data, and the challenges of educating stakeholders as to the basis and levels of aquatic life protection afforded by these new methods. Our experience showed that breaking through these barriers entails a thorough scientific understanding of the new methods, the willingness of the permittee to collect sufficient data, and careful attention to preparing a clear and simple technical proposal to facilitate stakeholder review and ensure that the outcome was fully protective of aquatic life uses. The result was, to our knowledge, the first successful adoption of FMB-based aquatic life Cu criteria in the US.

WP099 Chemical, biological and ecological indicators provide an integrative framework for measuring remediation success: examples from the Clark Fork River

M. Hornberger, D.J. Cain, US Geological Survey / Water Resources Division; T.M. Short, US Geological Survey; K. Edwards, USEPA; S.N. Luoma, University of California, Davis / John Muir Institute of the Environment

Remediating a metal contaminated Superfund site like the Clark Fork River presents complex challenges: multiagency performance objectives are not always co-aligned; environmental indicators representing remediation success can be confounded by issues of scale. Here we present two examples where multiple indicators are incorporated into a weight-of-evidence approach to determine remediation success. First, environmental condition is defined by dissolved and bed sediment metal concentrations; second, bioaccumulation studies link exposure to metal bioavailability; and third bioassessment studies provide ecological context at the community level. Collectively, they provide a holistic framework for sound risk management decisions. One example is based on a 15-year dataset and evaluates the physical and chemical disturbance related to the removal of Milltown Dam located on the Clark Fork River, MT. Soon after removal of contaminated sediments stored behind the dam, Cu concentrations in water, bed sediment and the biomonitor *Hydropsyche* doubled below the dam. In recent years, Cu in these indicators declined to levels at or below pre-dam levels. Coincident with these changes were shifts in relative proportions of invertebrate feeding-trait activities, primarily filter feeding, suggesting that restoration of natural channel flows and upstream connectivity were likely additional remediation outcomes. The second example is from a site on Silver Bow Creek, where remediation began in 2003 and is near completion. Since 2006, Cu concentrations in sediment, water, and *Hydropsyche* have declined by ~50% from pre-remediation years, though remain 2-10 fold higher than performance standards. Despite the persistence of elevated Cu concentrations, the marked decline in overall concentrations demonstrates that remediation efforts are working, though ongoing monitoring is necessary to determine long-term recovery. In both examples, sediment from mine tailings is the primary source of metal and the focus of remediation efforts. But determination of remediation success requires a broader understanding of the physical, chemical, and biological properties that define disturbance-response relationships. Incorporation of multiple response measures that individually integrate disturbance effects at different levels of severity and scales of activity is fundamental to providing resource managers with a sound scientific basis for decision-making.

WP100 Ecological Risk Assessment of a Complex Superfund Site on the Hudson River

M.T. Sorensen, ENVIRON International Corp; P.A. Leitman, ENVIRON International Corp; C. Beals, Environ International; L.B. Martello, ENVIRON International Corp / Department of Health Sciences; M. Nielsen, ENVIRON International Corp

A Baseline Ecological Risk Assessment (BERA) was conducted at a Superfund Site on the Hudson River. The subject property was used for the manufacture of paving and roofing materials from approximately 1896 to 1971. In 1974, a portion of the Site was reoccupied and leased for oil recycling. Primary chemicals of interest included PAHs in sediments attributable to nonaqueous phase liquids released from the site. The assessment endpoints evaluated in the BERA included: benthic community structure and integrity, survival and reproduction of fish populations, survival and reproduction of bird populations, and survival and reproduction of mammal populations. Multiple lines of evidence were used to evaluate potential risks to these receptors including site-specific sediment toxicity testing on sediment-dwelling amphipods and fish, benthic community assessments, solid-phase microextraction of pore water for estimates of bioavailable PAHs for comparison to early life stage fish ecotoxicity benchmarks, dilution series testing to evaluate potential site-specific effects levels for benthic organisms through the evaluation of dose-response relationship for PAHs, consideration of USEPA's equilibrium partitioning of PAHs as they may relate to toxicity, bulk sediment chemistry and comparison to sediment quality benchmarks, food web modeling for mammals and birds, and consideration of potential oiling of birds. Based on the BERA, site-specific protective thresholds were determined to be 74 to 144 mg total PAH/kg, equivalent to a threshold toxicity unit (TU) of 2 where TUs greater than 2 result in adverse effects that can are likely attributed to PAH toxicity. This BERA approach greatly informs future risk management decisions.

WP101 Risk assessment of the Canol Trail – a complex, linear corridor

H. Phillips, M. Tai, T. Brown, SENES Consultants; J. Hoyt, Public Works and Government Services Canada; J. Mace, Aboriginal Affairs and Northern Development Canada; S.L. Fernandes, SENES Consultants

The Canol Project was a cooperative effort between the United States and Canada to ensure a continuous supply of oil to American forces stationed in the Pacific during World War II. The project, which included the construction and short term operation of a crude oil pipeline and associated infrastructure, covered a total distance of approximately 800 km from Norman Wells in the Northwest Territories (NWT) to Whitehorse in the Yukon. The construction of the Canol Project was a massive undertaking in a harsh and remote environment. In April 1945, less than a year after the first oil reached Whitehorse, the entire project was abandoned. Environmental site assessments were carried out at sites locations along the corridor where infrastructure was constructed to support the pipeline (e.g., pump houses, maintenance yards, etc.). In addition, there are spill sites which are found randomly along the pipeline route. The Canol Project was constructed on traditional Sahtu lands and are used by Sahtu residents for hunting, trapping and recreation. In addition to Aboriginal use, there are two active lodges along the trail with guided outfitting, hunting and naturalist excursions. The trail also attracts "extreme" enthusiasts who are looking for rugged wilderness challenge for hiking, biking and the use of all-terrain vehicles. To support the use of the site a risk assessment that was undertaken that included potential effects on wildlife and people that may use the Canol Trail. An innovative approach to characterizing the site for the risk assessment was developed using a Monte Carlo approach. The risk assessment considered petroleum hydrocarbon (PHC) and metal contaminants in the terrestrial and aquatic environments. Small mammals were evaluated on the individual Trail sites as they would potentially be the most exposed and a methodology was developed to evaluate large animals such as moose. As part of the process there was extensive consultation with stakeholders and regulators.

Environmental or Analytical Chemistry

WP102 A comprehensive model for chemical bioavailability and toxicity of organic chemicals based on first principles

J. Khim, Korea University / Division of Environmental Science and Ecological Engineering, Seoul National University / School of Earth and Environmental Sciences; S. Hong, Seoul National University / Division of Environmental Science and Ecological Engineering / School of Earth and Environmental Sciences; J. Forrest, P. Bazylewski, R. Bauer, University of Saskatchewan; C. Kim, Canadian Light Source; J.P. Giesy, University of Saskatchewan / Toxicology Centre and Department of Veterinary Biomedical Sciences; G. Chang, University of Saskatchewan

While much is known about the toxicity of polychlorinated biphenyls (PCBs), there are tons of chemicals in the environment that can activate the aryl hydrocarbon receptor (AhR) and thus cause toxicity. Since it is difficult to conduct toxicity studies of all those compounds on all species and for all possible toxicological endpoints, here we present a novel model based on a first principles approach targeting basic electronic characteristics. The

predictive model is based on ab initio density functional theory. The model suggests HOMO-LUMO energy gap as the overarching indicator of PCBs toxicity, which was shown to be the primary factor predicting toxicity, but not the only factor. The model clearly explains why chlorination of both para positions is required for maximum toxic potency. To rank toxic potency, the "dipole moment" in relation to the most chemically active Cl-sites was critical. This finding was consistent with the accepted toxic equivalency factor (TEF) model for these molecules, and was also able to improve on ranking toxic potency of PCBs with similar TEFs. This novel model also includes a 13th toxic dioxin-like PCB, PCB 74, not considered in the current TEF model. Predictions of HOMO-LUMO gap made with the model were consistent with measured values determined by synchrotron based X-ray spectroscopy for a subset of PCBs. HOMO-LUMO gap can also be used to predict bioaccumulation of PCBs. Overall, the new model provides an in silico method to screen a wide range of chemicals to predict their bioavailability to act as an AhR agonist.

WP103 A Fast and Effective Approach for Running EPA Method 539: Determination of Hormones in Drinking Water using SPE and LC/MS/MS

A. Haghani, A. Eaton, Eurofins US; J. Wei, M. Trass, Phenomenex

Hormones have been found in a wide range of water supplies throughout the world. These compounds, including ethinyl-estradiol, the active ingredient in commonly prescribed birth-control medication, can cause detrimental affects to both aquatic life and humans. Because of the significant risk to human health and aquatic life, there is a rapidly growing public interest in monitoring these compounds. EPA method 539 was developed to address this large and growing public concern. EPA method 539 is a challenging analysis because not only does it require very low detection limits (0.1 part per trillion for some compounds), it also requires mass-spectrometer analysis in both positive and negative mode. In addition, more than 1,000 utilities are running EPA 539 in the United States alone. Therefore, it is extremely important to have a fast, accurate and reproducible analytical testing method. This work follows the EPA method extraction protocol, in conjunction with an optimized LC/MS/MS method. The extraction protocol, after collection of the water sample, involves preservation and then solid phase extraction. The extract is then analyzed by LC/MS/MS under high pH mobile phase conditions. The optimized LC conditions result in a rapid analysis time of ~ 9 minutes. Aside from a fast analysis time, the analytical procedure results in excellent linearity and reproducibility (R^2 values of 0.99 for all compounds with 1/X quadratic fit). Because EPA 539 is such an important and widely used assay, the presented method is of great assistance to laboratories wanting to improve efficiency and productivity.

WP104 A Multi-Analyte Method for the Determination of Selected Pesticides, and PBDEs in Human Milk: Minimum Reporting Limits – LOQs or LCMRLs

S. Boone, USEPA / Office of Chemical Safety and Pollution Prevention; C. Byrne, J. Ferrario, USEPA/OCSPP/OPP/BEAD/ECB

A multi-analyte method to determine selected current-use and legacy pesticides and the predominant polybrominated diphenyl ethers (PBDEs) was developed in human milk to evaluate potential exposures of these chemicals to infants during breast feeding. The method uses a limited sample volume with minimal preparation time at relevant low detection limits. The compounds of interest in this method include: aldicarb, atrazine and its metabolites (DEA, DIA, DACT), bifenthrin, carbaryl, chlorpropham, chlorpyrifos, cyfluthrin, cyhalothrin, cypermethrin, deltamethrin, p,pα-DDE, diazinon, diphenylamine, endosulfan (α, β), endosulfan sulfate, fenvalerate, fipronil, hexachlorobenzene, imazalil, malathion, o-phenylphenol, PBDEs (47, 99, 100, 153, 154), pentachloronitrobenzene, permethrin (cis, trans), tau-fluvalinate, thiabendazole, and triclosan. Twelve surrogates are used in this method: the deuterated forms of atrazine, chlorpyrifos, trans-cypermethrin, and diazinon and the carbon labeled forms of carbaryl, p,pα-DDE, hexachlorobenzene, cis-permethrin, trans-permethrin, o-phenylphenol, thiabendazole, and triclosan. The extraction method is a modified QuEChERs method which includes the extraction of milk with acetonitrile, C18 SPE column clean-up, and three separate PSA SPE columns: elution with acetonitrile for LC/MS/MS, elution with acetone/toluene for GC/MSD-CI, and elution with acetone/toluene on a modified PSA SPE column with the addition of a neutral alumina top bed volume for GC/HRMS. Matrix matched calibration procedures are required for the analyses by GC/MSD-CI and LC/MS/MS. The Limit of quantitation (LOQs) for the selected compounds range from 0.01-0.5 ppb, which was based upon seven replications of selected concentrations expected to be within 3 to 5x of the minimum detection limits (MDLs). Lowest concentration minimal reporting limits (LCMRLs) and detection limits (DLs) were calculated from the matrix-matched curves from six sets of data ranging over 8 months to compare to the LOQs. The LCMRL takes advantage of multiple concentrations and eliminates the need for the analyst to choose the concentrations used to calculate LOQs and MDLs. This is an added benefit when dealing with multiple compounds with a large range of detection limits. The LCMRL is being adopted for widespread use by the USEPA Office of Water.

WP105 Addressing Analytical Challenges When Using Cotton Garments to Estimate Dermal Exposure to PBDEs, Insecticides, and Pyrethroid Transformation Products

S.S. Clifton, N.S. Tulve, USEPA / Office of Research and Development, National Exposure Research Laboratory

Measurement of dermal exposure to chemicals in the indoor environment is important to fully understand aggregate and cumulative exposures. To estimate dermal exposure, samples may be collected on cotton garments, such as union suits and socks, which are worn by study participants for a set period of time while they perform normal activities inside their homes. Chemical residues are transferred from contacted surfaces to the garment. Unfortunately, cotton garments present many analytical challenges that create difficulties for trace residue analysis. These challenges may include safe and effective pre-cleaning prior to field deployment and an optimized extraction and clean-up procedure to minimize the mass of co-extracted materials from the garment. We have developed procedures to address these challenges for trace analysis of selected PBDEs, organophosphates, pyrethroids, and pyrethroid transformation products (TPs). Garments were pre-cleaned by Soxhlet extraction prior to deployment. After samples are collected, they are stored at freezer conditions (<-20° C) until analysis. The samples are then Soxhlet extracted and cleaned-up using a combination of partitioning steps designed to remove the bulk of the co-extracted material. This is followed by a selective solid phase extraction (SPE) procedure for purification of PBDEs and insecticides and derivatization of pyrethroid TPs. Method detection limits per sample were <12 ng for most PBDEs, <15 ng for insecticides, and <34 ng for pyrethroid TPs when analyzed by GC/MS. Most mean recoveries at 50 and 500 ng/sample ranged between 70 and 130% for PBDEs and insecticides with precision (n=3) <20% RSD. Pyrethroid TPs demonstrated recoveries between 83 and 106% with precision < 25%RSD. Additional methodological approaches will be presented along with results of the method evaluation and a summary of results from a study where these procedures were applied.

WP106 Air-water partitioning of benzobicyclon and benzobicyclon hydrolysate under California rice field conditions

K. Williams, University of California – Davis / Department of Agricultural and Environmental Chemistry; R.S. Tjeerdema, University of California – Davis / Environmental Toxicology

The rise in herbicide resistance observed in California rice field weeds, such as *Scirpus juncoides* and *Lindernia attenuata*, has generated increasing interest in herbicides with alternative modes of action from those currently in use. One such herbicide is benzobicyclon, a triketone herbicide, which, upon hydrolysis within the plant to its active form, benzobicyclon hydrolysate, inhibits hydroxyphenylpyruvate dioxygenase (HPPD) activity. This subsequently halts plant photosynthesis through the destruction of chlorophyll and leads to plant bleaching and death. The mode of action of this HPPD-inhibitor herbicide has the potential to overcome sulfonylurea-resistant weed persistence, however benzobicyclon and benzobicyclon hydrolysate's behavior in a central Californian environment should be understood prior to its application to California rice fields. In this study we will attempt to create an analytical method of analysis for benzobicyclon and benzobicyclon hydrolysate using LC-MS/MS, and elucidate the partitioning of benzobicyclon and benzobicyclon hydrolysate between air and water through the determination of their Henry's Law constants, representative of volatilization potential, considering California rice field conditions. A gas-stripping apparatus with mist trap incubated at two temperatures, 25 °C and 37 °C, will be used to determine the Henry's Law constants for benzobicyclon and benzobicyclon hydrolysate. These temperatures have been chosen such that the range in temperatures observed by California rice fields will be accounted for.

WP107 Application of novel mass spectrometry technologies to the analysis of hydrocarbons of environmental concern

J. Mather, A. Tyler, G. Perkins, Perkin Elmer

The need for robust analytical procedures to assess hydrocarbons found in many types of environmental samples is well known. While gas chromatography combined with mass spectrometry (GC/MS) is long established as a staple method for this task, however, significant limitations remain. Specifically, a lack of molecular signal and extensive molecular ion fragmentation means that method-dependent retention time becomes a critical factor in assigning formulae, and challenges quantitation. Furthermore, distinction between isomers can be challenging. We have examined a series of environmentally-relevant samples for their hydrocarbon content using Perkinelmer's innovative AxION iQT GC/MS/MS technology. This product has been designed with overcoming these limitations, which are common to numerous analytical areas accessible to GC/MS. This instrument allows the formation of genuine molecular ions from hydrocarbons, facilitating more robust quantitation. In addition, the novel time-of-flight detection system allows collection of fragment ions of all masses from the selected molecular signal. This allows easier distinction of isomers, based upon their distinctive fragmentation patterns.

WP108 Application of passive sampling devices for underwater unexploded ordnance exposure assessment

J.B. Belden, Oklahoma State University / Department of Zoology; G.H. Rosen, SPAWAR Systems Center Pacific / Environmental; G.R. Lotufo, U. S. Army ERDC / Environmental Laboratory; B. Wild, SPAWAR Systems Center Pacific

Munitions constituent (MC) exposure to marine biota from potentially leaking unexploded ordnance (UXO) is likely localized and episodic in nature. Thus, passive sampling devices (PSDs) are potentially valuable tools for assessment of MC exposure in the marine environment. The use, and optimization, of commercially available PSDs for MC characterization in marine/estuarine sediments and surface waters was explored. Equilibrium passive sampling involving solid phase microextraction (SPME) fibers was evaluated for estimation of sediment porewater MC concentrations, while polar organic chemical integrative samplers (POCIS) were used for determination of time-weighted average water column concentrations. The approach involved calculating loading rates for both passive samplers, determining if POCIS samplers are integrative across a series of pulsed exposures, and validating sampling techniques in mesocosms with different turnover rates. In the mesocosms, Composition B (59.5% RDX, 39.5% TNT, 1% wax) fragments were placed in 10 gallon tanks to represent exposure to a MC source in shallow, tropical waters. Varying turnover rates, up to 5 per day, were evaluated. MCs investigated include TNT, primary TNT degradation products, and RDX. POCIS samplers had linear uptake over the first 14-28d and were integrative during pulsed exposures as loading rates were within 20% of experiments conducted using continuous exposure. Within the mesocosm study, both passive sampling techniques were able to detect and quantify MCs even when five turnovers per day were occurring and when Composition B was partially encased. Pending field validation, the successful demonstration and availability of the use of these tools should greatly enhance the ability to conduct exposure assessments of MC at sites containing UXO.

WP109 Asbestos Data Verification vs. Asbestos Data Validation: How they differ and why they should be used to improve data quality of asbestos results

E. Formanek, CDM Smith / Enviromental Management Division; L. Woodbury, CDM Smith / Environmental Management Division

Compared to standard chemical analysis data, asbestos analysis data are unique because they can yield complex, detailed information on the specific attributes of each grid opening examined and asbestos structure observed. Another distinctive feature of asbestos data is that they can be reviewed *post hoc* for inaccuracies, and data may then be corrected if errors are discovered. This process is called "data verification." Asbestos data verification entails reviewing the laboratory benchsheets to ensure that structure counting and recording rules have been followed, and that the analysis achieved the analytical stopping rules as specified in the analytical method and other applicable governing documents (e.g., target sensitivity). In addition, asbestos data can be reviewed to check that information recorded on the laboratory benchsheet is transferred accurately to the electronic data deliverable. If any errors are discovered during the data verification process, these issues can be resolved by the laboratory, thus improving the quality of the data. Asbestos data verification differs from "data validation." The purpose of the verification process is to identify and correct errors, whereas data validation includes a review of components of an analysis that have the potential to impact analytical results which cannot be corrected. The goal of data validation is to alert data users to potential data quality issues by assigning appropriate data qualifiers. Data validation includes a review of sample shipping and chain of custody information, grid preparation procedures, calibration information, scope alignment, and quality control analysis results. Data users often confuse the differences between asbestos data verification and asbestos data validation. When both verification and validation efforts are completed, data users can have higher confidence in the overall quality of their asbestos results.

WP110 Assessing Changes to Organic Contaminant Fluxes from Contaminated Sediments Following Removal of a Low Head Dam in an Urbanized River

M.G. Cantwell, Atlantic Ecology Division; M.M. Perron, EPA; D.R. Katz, USEPA / Atlantic Ecology Division / ORD / NHEERL / AED; R.M. Burgess, USEPA / Atlantic Ecology Division, National Health and Environmental Effects Research Laboratory / ORD / NHEERL

Restoration of rivers and their associated ecosystems is a growing priority for government agencies (e.g., NOAA, USEPA), as well as conservation organizations. Dam removal is a major component of many restoration projects credited with reintroducing fish species, improving water and habitat quality, and increasing recreation potential. In this study, methods and approaches were tested to assess changes in contaminant fluxes resulting from dam removal. Sediment traps and passive samplers were deployed to measure suspended particulate and dissolved polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in the water column prior to and following removal of a low head dam in the Pawtuxet River, an urbanized river located in Cranston, RI, USA. During the course of the study, concentrations of particulate and dissolved PAHs ranged from 25-103 ug/g and 49-164 ng/L. Trends of PAHs showed no increases in either following dam removal. Dissolved concentrations of PCBs were low, remaining below1.5 ng/L, while particulate PCB concentrations showed slightly greater variability, ranging from 80-469 ng/g throughout the study. There was no indication that dam removal influenced any increases in either particulate or dissolved PCBs. Variations in river flow during the study did not have an effect on the concentration of contaminants in the dissolved or particulate phases, but did influence the flux rate of contaminants exiting the river. Overall, the employment of passive sampling technology and sediment traps was highly effective in monitoring the concentrations and flux of contaminants moving through the river system. Results from this study will be used to improve methods that assess the short and long-term impacts ecological restoration activities such as dam removal have on the release of previously discharged, sediment-bound contaminants.

WP111 Assessment of Perfluorinated Compounds (PFCs) in Fish from US Rivers and the Great Lakes

L.L. Stahl, USEPA / Office of Science and Technology; B.D. Snyder, Tetra Tech, Inc. / Center for Ecological Sciences; J.B. Wathen, USEPA / Office of Science and Technology, Office of Water / Office of Science and Technology, Standards and Health Protection Division; A.R. Olsen, USEPA Office of Research and Development / Western Ecology Division, National Health and Environmental Effects Laboratory; H.B. McCarty, CSC

Since 2000, PFCs have emerged as contaminants of concern because they are broadly distributed and persistent in the environment, linked to a number of potential health effects, and associated with endocrine disruption and cancer in animal studies. Based on potential health risks to people exposed to PFCs in fish, EPA's Office of Science and Technology (OST) within the Office of Water identified the need for a comprehensive characterization of PFC contamination in US fish. As a result, OST planned and conducted a national-scale study of urban rivers and a regional-scale study of the Great Lakes to evaluate the extent of PFC contamination in freshwater fish. The purpose of the studies is to develop national and regional estimates of the median concentrations of PFCs in fish from US urban rivers and the Great Lakes, respectively. The probabilistic design of the studies also allows estimation of the percentage of river miles or nearshore lake area with fish tissue concentrations above a specified human health threshold. These assessments included analysis of PFCs in fish fillet tissue collected from 164 randomly selected urban river locations throughout the lower 48 states (in 2008 and 2009) and from 157 randomly selected nearshore locations in the five Great

Lakes (during 2010). Both studies were conducted under the framework of EPA's statistically designed National Aquatic Resource Surveys (NARS), namely the National Rivers and Streams Assessment (NRSA) and the Great Lakes component of the National Coastal Condition Assessment (NCCA). Fish tissue samples (fillet composites) were analyzed for 13 PFCs including PFOA and PFOS, since elevated concentrations of PFOA and PFOS in human blood have been linked to a number of potential health effects. Study data show that had a low frequency of occurrence (detected in less than 12% of all samples), however PFOS was detected at quantifiable levels in 73% and 100% of fish samples collected for the urban river and Great Lakes studies, respectively. Based on results from this probabilistic study, the median concentration of PFOS is 10.7 ppb in fish from U. S urban rivers and 14.6 ppb in fish from the Great Lakes.

WP112 Caracterization of humic acid obtained from agroindustrial vermicomposts by ultraviolet visible light absorption (UV–vis): soil sustainable management

L.B. Pigatin, F. Benetti, R.N. Rodrigues, M. Landgraf, IQSC-USP; A.V. Borsato, Embrapa Pantanal; M.O. Rezende, IQSC-USP

The correct managment of organic solid wastes improves soil health; however the incorrect application can favor mechanisms that negatively influence climate changes. Vermicomposting is a biotechnological process in which earthworms are employed to convert the organic solid waste into an organic fertilizer with high content of humic substances and their humification are directly related to soil quality. This research investigates the humification of humic acids (HA) obtained from manure waste (HA-M), orange peel+manure waste (HA-OP), and filter cake+manure waste (HA-FC) by UV-Vis. The extraction, split of the humic substances and HA purification were done according to the method suggested by the International Humic Substances Society. Absorbance scans were measured over a wavelength range of 800–200 nm for 200 mg L^{-1} of HA material dissolved in 0.05 mol L^{-1} $NaHCO_3$. E_4/E_6 and E_2/E_4 ratios, used as humification parameters, were obtained from UV–Vis light absorption spectra and relates the absorption intensities at 465 and 665 nm for the first one and at 270 and 407 nm for the second. HA-M showed expected E_4/E_6 ratio around 4.5 during the total vermicomposting period of 135 days indicating relatively more condensed structures and higher degree of humification. For humic acids HA-OP and HA-FC values were: HA-OP: 7.0 during all time and HA-FC: 15.5 after 90 days. High values in the E_4/E_6 ratio, compared with HA from soil, reflect low aromatic condensation, presence of high proportions of aliphatic structures, and low degree of humification. Studies with composted manure and organic sludge showed values above the reference value for HA from soils what indicates the presence of HA less evolved from non-pedogenic organic matter. Higher values are correlated with more open structure and lower complexity. The ratio E_2/E_4 has been used to distinguish different sources of organic matter. For HA-FC the values increased from 3.0 to 5.1 as a function of time, indicating porfirinics structures consumption or condensed aromatic structure not attached to oxygen function formation. For HA-OP values increased from 3.7 to 4.7 and HA-M constant values about 4.8. Low E_2/E_4 ratios are related to greater porfirinic structures participation compared to structures of lignins. These findings provide significantly informations about the aromaticity and to the degree of condensation of the chain of aromatic carbons of the HA.

WP113 Characterizing Bio Oil Composition from an Environmental Forensics Perspective

J.A. Layshock, Battelle Memorial Institute / Analytical Chemistry Services; R. Taha, Battelle Memorial Institute

Pyrolysis of plant-based feed stocks for the generation of renewable liquid fuel is a growing industry with researchers developing innovative ways to convert the fuel into a refined product that is compatible with current fossil fuel systems. Methods to rapidly characterize the chemical composition of the step products of bio oil refinement are in development and are necessary to assess the compatibility of biofuels with current infrastructure. In this study, numerous analytical methods including those routinely employed for petroleum forensics were used to characterize catalyzed and non catalyzed pine wood bio oil samples produced by an auger pyrolysis reactor. Quantitative results were obtained for nearly 100 GC-amendable compounds and tentative identifications of hundreds more were possible from TIC reports and LC-based methods. Chemical concentrations detected in the non catalyzed and catalyzed bio oil were statistically different (p-value < 0.05). Phenols were the most concentrated type of chemical with totals of 4,800 and 17,000 mg/kg before and after catalysis, respectively. All GC-amendable concentrations increased after catalysis with an 11-fold increase in volatiles, a 5-fold increase in PAHs and a 3-fold increase in phenols. LC data suggests the removal of some oxygenated species (e.g., Levoglucosan) upon catalysis. The similarity of bio oil to traditional petroleum crude in respect to PAH profiles will be presented. The identification of possible diagnostic markers for environmental forensic applications will also be introduced and explored.

WP114 Chemical clustering methodology in hazard assessment: phthalate alternatives case study

M. Kawa, J. Rhoades, SRC, Inc; C. Baier-Anderson, USEPA / Design for the Environment; K.E. Mayo-Bean, USEPA / OPPT; J.L. Tunkel, SRC, Inc. / Environmental Health Analysis

Phthalates are produced in high volume (470 million pounds per year) in the United States. They are found in many products, primarily as plasticizers; however they are used in cosmetics, personal care products and solvents as well. The USEPA published an Action Plan for eight phthalates in 2010 due to concern about the toxicity of phthalates and the evidence of pervasive human and environmental exposure. A Design for the Environment (DfE) alternatives assessment (AA) is being performed for the phthalate chemicals as discussed in the EPA's Action Plan. AA's are published by the DfE program to help industries identify safer chemicals and provide a comparison of potential human health and environmental impacts of chemical alternatives. DfE hazard criteria are used to assign hazard designations for human health toxicity, ecological toxicity and environmental fate endpoints. Over 70 substances were identified as potential alternatives to the eight action plan phthalates. Some of the 70 alternatives are well characterized for all endpoints, while experimental human health and environmental fate data for other alternatives is scarce in the publically available literature. In the absence of experimental data, DfE assessment methodology designates hazards based on a read across approach to structurally similar compounds or analogs. This poster describes a novel technique for green chemistry and hazard screening that uses the EPA's Office of Pollution Prevention and Toxics ChemACE program to automate the clustering of the alternatives based on common structures, functional groups, and molecular architectures. The ChemACE program identifies structural similarities and generates reliable and organized results. Read across (analog) data from data-rich chemicals was used to assist in assigning and justifying hazard designations for data poor chemicals within a cluster. This technique efficiently identifies and standardizes analog selection to support informed decision making in alternative assessments. This poster will present some examples of how the read across methodology for designating environmental fate and ecotoxicological hazards of phthalate alternatives was used.

WP115 Chemicals of High Concern to Children in Children's Products

A. Stone, J. Grice, K. Dunne, Washington Dept. of Ecology; S.K. van Bergen, Washington Dept. of Ecology

In 2008, the Washington State legislature passed the Children's Safe Product Act (CSPA, 70.240 RCW). The CSPA Reporting Rule was finalized in June of 2011 and implemented the reporting requirements under the CSPA. Under the rule, companies making children's products must report on 66 specific or class of chemicals if found in children's products. The list includes chemicals that have primarily either been found in children's products or have been documented by biomonitoring studies to be present in human tissues. Reporting requirements began with the largest manufacturers who make products intended for mouth or skin contact or any product that is mouthable for children 3 and under. Other manufacturers will report using a phased-in schedule included in the rule. In response to the CSPA Reporting Rule, the Washington State Department of Ecology (Ecology) analyzed children's products for approximately half of the 66 chemicals or classes of chemicals of high concern to children. Chemicals analyzed under this program included four parabens, 8 phthalates, 6 metals, formaldehyde and 14 volatile organic compounds. The objectives of this study were to 1) verify that a wide range of children's products could be analyzed for the chemicals of concern to children at the levels required, 2) evaluate compliance with the reporting requirements of the CSPA and 3) evaluate levels of these chemicals in a wide range of children's products. Approximately 800 children's products were purchased from local stores and internet retailers for testing. Special emphasis was placed on products applied to the skin or ingested. The products were separated into three components; packaging, containers and

product. For example, a container of children's lip gloss was separated into packaging, product (lip gloss itself) and container (used to store and apply the product). Depending upon its construction, the container or product could be separated into different components as identified in the CSPA rule. Packaging is not covered under the CSPA but was retained for potential analysis as four toxic metals are restricted by Washington toxics in packaging legislation. Sampling was completed in 2013. This presentation will provide the final results of the study and information on the presence of these chemicals in children's products.

WP116 Comparison of a Time- Integrative Solid Phase Extractive Sampler to Passive and Discrete Sampling Methods for TOCs in Waste Water Effluent

*B.A. Hepner, C.I. Agent Storm Water Solutions / Research & Development; J. Aderhold, C.I. Agent Storm*Water Solutions; A.L. Coes, N.V. Paretti, US Geological Survey / Arizona Water Science Center; W.T. Foreman, J.L. Iverson, US Geological Survey / National Water Quality Laboratory; D.A. Alvarez, US Geological Survey / Columbia Environmental Research Center*

Abstract The need to sample natural waters for a diverse array of trace organic compounds (TOCs) is rapidly intensifying. Recent advances in sampling technologies now can provide rapid, low level, time- integrative options for identifying TOCs in water. A newly developed continuous solid phase extractive (SPE) sampling method was compared to a passive sampling method, and a standard discrete sampling method. The continuous low-level aquatic monitoring (C.L.A.M.) sampler is a submersible, SPE, time-integrative, active extractive sampler. C.L.A.M. samplers were deployed at two stream field sites in conjunction with the deployment of polar organic chemical integrative samplers (POCIS) and the collection of standard discrete 1-L water samples. All samples were analyzed for a suite of 69 organic waste-indicator TOCs. The C.L.A.M. and POCIS samples represent time-integrated samples that accumulate the TOC present in the water over the deployment period (19-23 hours for C.L.A.M. and 29 days for POCIS); the discrete samples represent only the TOCs present in the water at the moment of sampling. Non-metric multi-dimensional scaling was used to examine patterns in both TOC detections and relative concentrations between the three sampling methods. This work was supported by the US Geological Survey Water Science Field Team, and the US Geological Survey National Water Quality Laboratory. This presentation will summarize the findings of this work and compare qualitative, and quantitative capabilities, contrast bias, recovery, and performance of each method.

WP117 Determination of antioxidant compounds in sediment and sewage sludge

Z. Zhang; E. Sverko, Environment Canada; C. Marvin, Environment Canada

Benzenamine, N-phenyl-, reaction products with styrene and 2,4,4-trimethylpentene (BNST), reaction product of N-phenyl-benzeneamine and 2-propanone (PREPOD) and 1,4-Benzenediamine, N,N'-mixed phenyl and tolyl derivatives (BENPAT) are are widely used as antioxidant additive in vehicle engine oils, commercial/industrial lubricants and rubber products. They are UVCB (Unknown or Variable Composition, Complex Reaction Products, or Biological Materials) mixture, and, as such, contain a number of components in different concentrations. These compounds are persistent and bio-accumulative, and the presence in the environment results primarily from human activity. In addition, modeled acute aquatic toxicity data indicate that PREPOD is potentially highly hazardous to aquatic organism. Releases of BNST, PREPOD and BENPAT to soil, municipal sewer, surface water and sediment are expected due to industrial wastewater discharge, leaks and spills of lubricants/oils during use and also due to the improper disposal. Despite this, there are no existing environmental measurements of these compounds in any media. In this study, we determined 17 antioxidant compounds in sediment and sewage sludge samples using gas chromatography-tandem mass spectrometry (GC-MS/MS). Recoveries of target compounds spiked into sample matrices and passed through the entire analytical procedure ranged from 70% to 130%, except for 3 BENPAT compounds (35-50%).

WP118 Determining Sources of Organic Pollution in Natural Waters by LC-MS Analysis

J.J. Harwood, S.L. Narayanan, Tennessee Technological University / Chemistry

We are developing a comprehensive, rapid method to identify the sources of organic pollution in streams and rivers. The method utilizes liquid chromatography-mass spectrometry (LC-MS) to analyze for "molecular markers", compounds which indicate specific sources of pollution. The artificial sweeteners acesulfame-K and sucralose have been found to be excellent markers of human wastewater input. These compounds are very stable, resisting both metabolism and degradation in wastewater treatment, and are present in analyzable concentration in natural waters. These markers can be analyzed in a single determination with electrospray ionization LC-MS. We have found ion pair chromatography to be necessary for quantitative analysis of acesulfame-K. Analytical sensitivity for sucralose benefits significantly by post-column addition of TRIS buffer. Preconcentration of the sweeteners can be achieved by solid phase extraction (SPE). We present comparison of pretreatment recoveries obtained with the Waters Oasis HLB and the Jordi DVB SPE cartridges. In field studies, we are investigating possible correlation of sweetener concentration with total organic carbon (TOC), and also correlation with estrogenic compounds. The profile of bile acids and fecal sterols associated with particulates can identify inputs of animal waste. Sterols do not ionize in the LC-MS electrospray source, and we have developed the common alternative atmospheric pressure ionization (APCI) for this application. We have developed methodology to separate and analyze the bile acids and fecal sterols commonly suggested as molecular markers to distinguish pollution from different animal species; both classes of compounds can be analyzed in a single LC-MS analysis. To isolate the compounds, are presently extracting sediment and particulate samples. We plan to investigate using the polar organic chemical integrative sampler (POCIS) to sample both these markers and the artificial sweeteners. We present the results of this method development, including results of field testing the methods by analyzing samples from sites on the Cumberland Plateau of Tennessee.

WP119 Developing a Method for the Analysis of Chemical Waste Markers in Groundwater to Identify Sources of Nitrate Contamination

W. DeVita, University of Wisconsin – Stevens Point / Water and Environmental Analysis Lab; A. Nitka, Water and Environmental Analysis Lab; P. McGinley, University of Wisconsin – Stevens Point / Fisheries and Water Resources

Nitrate-nitrogen is a common groundwater contaminant. While nitrate-nitrogen occurs in groundwater naturally at low levels, anthropogenic influences can impact groundwater quality to levels above drinking water standards. Nitrogen-containing fertilizers and nitrate from manure applications intended to increase crop production can leach into groundwater. Nitrate from human waste in septic systems can also enter groundwater. Identification of the nitrate source is critical to remediation of the condition through changes in land use practices. Laboratory analysis of groundwater for pesticides and waste markers may indicate the source of nitrate contamination for a particular well or group of wells. The objective of this study is to develop a method for the simultaneous analysis of a group of pharmaceuticals, personal care products, and artificial sweeteners, which can be used in tandem with traditional pesticide analysis to identify sources of nitrate contamination. The method being developed will be used for analysis of nine pharmaceuticals, personal care products, and artificial sweeteners. Selection of these compounds was based upon frequency of detection in other published reports, and spans a range of chemicals that would be useful in segregating human septic system impact from agricultural impacts, such as landspread animal waste. Consideration was also given to the stability of each compound and reported or potential mobility in groundwater. The suite of chemical waste markers for this method includes: acesulfame (artificial sweetener), acetaminophen (analgesic), caffeine (stimulant), carbamazepine (anti-convulsant), cotinine (nicotine metabolite), paraxanthine (caffeine metabolite), sucralose (artificial sweetener), sulfamethazine (bovine antibiotic), and triclosan (anti-microbial). Solid phase extraction (SPE) was used to increase sensitivity and achieve a lower method detection limit. Results from various SPE elution schemes will be presented. Following SPE, samples were analyzed by ESI-LC-MS/MS. Average recoveries for carbamazepine, cotinine, paraxanthine, and sulfamethazine ranged from 80-106% (n=13 for cotinine and paraxanthine, n=14 for carbamazepine and sulfamethazine), with limits of detection ranging from 1.0-4.2 ppt using SPE. The test concentration for carbamazepine was 3.75 ppt, and 7.5 ppt for cotinine, paraxanthine, and sulfamethazine.

WP120 Development and testing of polymeric thin-film passive samplers to determine gas-phase air concentrations of semivolatile organic compounds

L. Guo, H. Lee, National University of Singapore / Department of Chemistry; B.C. Kelly, National University of Singapore

The conventional approach for determination of aerial concentrations of semivolatile organic compounds (SVOCs) is to utilize high volume air

samplers. This approach can be time-consuming, costly and impractical, especially for studies of remote locations without readily available power sources. The objective of the present study was to develop polymeric thin-film (PTF) passive samplers as low-cost alternative for monitoring legacy POPs and emerging contaminants of concern in air. The developed PTF samplers are equilibrium type passive samplers and require chemical equilibrium be attained before analysis. The samplers consist of thin-films (1 to 3 µm) of ethylene vinyl acetate (EVA) coated on150 mm glass fiber filters. Calibration experiments (45 days) were conducted at the National University of Singapore's Atmospheric Monitoring Station to assess uptake kinetics and polymer-air partition coefficients (K_{PA}). Field-testing involved 45-day deployment of PTFs at a remote mangrove forest site in Singapore. Concentrations of SVOCs extracted from the PTFs were determined using gas chromatography triple-quadrupole tandem mass spectrometry (GC-QqQ-MS/MS). SVOCs investigated included polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), brominated flame retardants (BFRs) and synthetic musks. The studied chemicals span a wide-range of octanol-air partition coefficients (log K_{OA}), ranging between 6 and 13. Equilibrium was confirmed by simultaneous deployment and analysis of two sets of PTFs having different polymer coating thicknesses (3:1 EVA mass ratio). Calibration experiments showed that low K_{OA} chemicals reach equilibrium in the PTFs relatively quickly (2-10 days), while high K_{OA} compounds exhibited longer equilibrium times. In field experiments, equilibrium was apparent for many compounds, as indicated by a 3:1 mass sequestration ratio in the 3 µm and 1 µm PTFs. Estimated gas-phase concentrations of PCB congeners 44, 52, 66 and hexachlorobenzene in the field were 0.76 pg/m^3, 1.2 pg/m^3, 0.82 pg/m^3 and 50.73 pg/m^3, respectively. These estimates are in good agreement with concentration data using conventional high volume air samplers. High K_{OA} compounds typically did not reach equilibrium during the deployment period. Determination of gas-phase concentrations of these less volatile compounds using PTF based equilibrium samplers will likely require use of thinner films (i.e., submicron films < 1 µm) coated over a larger surface area.

WP121 Development of an HPLC-MS Method for Quantification of BPA, Triclosan, Five PFC's and Four Phenols in Biological and Environmental Matrices

A. Breaud; W. Clarke, The Johns Hopkins University

The ability to rapidly and reliably detect and quantify environmental toxicants has improved as the implementation of high performance liquid chromatography (HPLC)-mass spectrometric (MS) instrumentation has evolved. The use of these instruments allows for development of assays for myriad toxicological agents. We have developed a method using HPLC and a high resolution accurate mass (HRAM) mass spectrometer in full scan mode for detection and quantification of bisphenol A, triclosan, five perfluorinated compounds (PFC's): PFHxS, PFOS, PFOA, PFNA and PFDA; and ethyl-methyl-, butyl- and propyl- parabens in biological or environmental matrices. Though some methods exist for groups of these compounds, there is currently no method which can detect all analytes in this panel from a single sample and with a single analysis. Deuterated internal standards for each analyte are added to the sample during sample preparation, which consists of a simple protein precipitation step with methanol and a 1:1 dilution of the sample buffer with water to effect retention of all analytes on the analytical column. The ionization of BPA and triclosan require different source conditions which were achieved by programming a gas pressure and voltage switching step within the mass spectrometer instrument method. The chromatography method is 7-minutes long and includes a simple gradient from 0-100% acetonitrile containing 0.01% acetic acid. We report a limit of quantification of < 1 ng/mL (< 1 ppb). The use of HRAM mass spectrometery in full scan mode has an added benefit in environmental analysis as data may be retrospectively reviewed for the presence of compounds not initially queried by application of mass filters with mass accuracy to four decimal places.

WP122 Effect of cassava (Manihot esculenta) effluent on Oreochromis niloticus (Nile Tilapia) and Clarias gariepinus (African mud catfish)

O.S. Olatunji, Cape Peninsula University of Technology / Department of Chemistry; O.S. Fatoki, Cape Peninsula University of Technology, Cape Town, South Africa. / Department of Chemistry; B.O. Opeolu, Cape Peninsula University of Technology / Faculty of Applied Sciences; B. Ximba, Cape Peninsula University of Technology, / Chemistry

Acute bio-assays were conducted on *Oreochromis niloticus* (Nile tilapia) and *Clarias gariepinus* (African mud catfish) species in order to evaluate the effect of cassava effluent on fish growth and haematology under laboratory conditions. Cassava effluent was collected from fermentation drain of a Cassava processing plant, and characterized for pH, cyanide (CN^-), ammonia (as NH_4^+), cadmium (Cd), chromium (Cr) and lead (Pb) using standard methods. Haematological parameters (packed cell volume (PCV), haemoglobin (Hb), red blood count (RBC), white blood count (WBC), total protein (TP), albumin (Alb) and globulin (Glb)) were determined using standard laboratory methods at 96, 192 and 288 chronic exposure hours, and the LC_{50} was estimated using probit method. The result of the characterization of cassava effluent showed: pH, 3.95±0.01; CN^-, 190.69 mg/l; NH_4^+, 4.26±0.04 mg/l; Pb 0.05±0.01 mg/l and Cr 0.16±0.01 mg/l, while Cd was not detected. The LC_{50} at 96 hours chronic exposure to effluent concentration was 0.25% for *O. niloticus* and 0.45% for *C. gariepinus*. There was significant (p< 0.05) body weight loss in both species (2.58 – 8.91% and 3.19 – 6.70% for *O. Niloticus* and *C. Gariepinus* respectively) with increasing cassava effluent concentration. The toxicity induced by cassava effluent on the haematological parameters of *O. niloticus* varied: PCV, 4.82 – 51.85%; RBC, 5.33 – 64.03%; WBC, 1.38 – 66.43%; Hb, 4.57 – 45.45%; TP, 28.42 – 63.82%; Alb, 14.97 – 58.19%; and Glb, 46.00 – 83.87%, while those for *C. Gariepinus* catfish varied; PCV, 5.27 – 52.86%; RBC, 4.48 – 46.85%; WBC, 4.35 – 15.65%; Hb, 3.62 – 45.93%; TP, 11.59 – 71.90%; Alb, 10.77 – 71.18% and Glb, 14.71 – 75.00%. The LC_{50}-96 hours showed that *C. Gariepinus* was nearly twice resistant to the cassava effluent compared to *O. niloticus*. The reduction in the body weight and haematological parameters of both fish species showed that exposure to cassava effluent could lead to serious health disorders in fishes. Therefore, effluents with quality such those of cassava peels should be adequately treated before release into water bodies.

WP123 Effects of Sediment Organic Matter and Water Depth on the Environmental Fate of a POEA Surfactant

J. Rodriguez Gil, University of Guelph / School of Environmental Sciences; M.L. Hanson, University of Manitoba / Department of Environment and Geography; A. Ross, Fisheries and Oceans Canada / Ocean Sciences Division, Institute of Ocean Sciences; K.R. Solomon, University of Guelph / School of Environmental Sciences

The environmental fate of the POEA surfactant MON 0818 was evaluated with a 28 day outdoor mesocosm study under varying conditions of sediment organic matter (SOM) and water depth. A total of nine microcosms were prepared with a synthetic sediment consisting of 64-76% sand, 18-22% clay, and 1.5, 5.0 and 10.0% SOM from peat (w/w) with water depths of 15, 30 and 90 cm (i.e., 1884, 3768, and 11304 L, respectively) in a randomized 3 x 3 factorial design. The mesocosms were sprayed uniformly with 1.4 g of MON 0818, which is equivalent to the maximum application rate stated on the label of the Visionmax® formulation for use in forestry. The difference in water depth among treatments yielded nominal water concentrations at time zero of 760, 380 and 127 µg/L at 15, 30 and 90 cm, respectively. Concentrations of POEA in the water column were measured by LC-MS/MS up to 336 h after treatment with intensive sampling intervals during the first 48 h. First order half-lives (DT50s) in the water column ranged from 9.4 h (at the 15 cm and 10 % SOM treatment) to 16.2 h (at the 30 cm and 10% SOM treatment). Proportion of SOM in sediment did not significantly affect DT50s. No significant differences in DT50s were found between the 90 and 30 cm water depths; however, those measured in the 15-cm microcosms were significantly shorter. These results are consistent with observations of rapid adsorption of POEA to sediments and suggest that risks from direct overspray of shallow surface waters are mitigated by more rapid adsorption to sediment and reduced biological availability.

WP124 EPA's Assessment of Contaminants of Emerging Concern and Legacy Contaminants in Fish from US Rivers and Streams

L.L. Stahl, USEPA / Office of Science and Technology; B.D. Snyder, Tetra Tech, Inc. / Center for Ecological Sciences; J.B. Wathen, USEPA / Office of Science and Technology, Office of Water / Office of Science and Technology, Standards and Health Protection Division; A.R. Olsen, USEPA Office of Research and Development / Western Ecology Division, National Health and Environmental Effects Laboratory; H.B. McCarty, CSC

EPA's Office of Water and Office of Research and Development are collaborating to conduct a national study of fish tissue contamination in US rivers and streams. This study adds a human health component to the ecological assessments EPA is conducting under the statistically designed National Rivers and Streams Assessment (NRSA). Results from the 2008-2009 NRSA fish tissue indicator generated a national baseline for fish contamination data

(i.e., PFCs, PBDEs, and legacy contaminants including mercury, selenium, PCBs, and pesticides) in rivers and streams. EPA's inclusion of fish tissue analysis in the 2013-2014 NRSA will provide the first probability-based national fish contamination trends data for US rivers. Sampling in 2013-2014 will involve the collection of fish tissue at a statistically representative subset of over 400 river locations (5th order or greater) assessed during the 2008-2009 NRSA. This subset provides sufficient sample size to develop national estimates of toxic chemical concentrations in fish with acceptable confidence intervals. Assessment of contaminants in rivers fish for human health applications involves collecting one fish composite sample from each of the river sites consisting of five similarly sized adult fish of the same species that are commonly consumed by humans. Fillet tissue from each 2013-2014 composite sample will be analyzed for mercury and 13 perfluorinated compounds (PFCs) including perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS). Samples will also be archived for potential future analysis of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs). Sample collection is proceeding in 2013 and 2014, fish tissue analysis and data quality review will be completed in 2015, and EPA anticipates having results available to report in 2016.

WP125 Fast and Accurate Analysis of PBDEs in a Single Run, Including PBDE 209

K. Kelly, M. Trass, Phenomenex

Polybrominated Diphenyl Ethers (PBDEs) are aromatic and non-polar compounds that were used as flame retardants. After extensive usage, it was determined that these compounds are toxic and have been restricted or banned in many areas, including under the Stockholm Convention. PBDEs result in reproductive and other health effects, are toxic at low levels, and are subject to bioaccumulation. It is therefore important to measure these compounds at very low levels from environmental, food, and biological sources. PBDEs consist of 209 individual conformations called congeners, which vary in toxicity. It's therefore important to measure and quantitate the individual congeners separately. To achieve the lowest levels of detection and highest degrees of confidence, high resolution gas chromatography with high resolution mass spectrometry (HRGC/HRMS) is used. Even using this advanced instrumentation, accurate separation of all congeners is difficult and requires long run times to provide enough resolution. In addition, not all congeners are stable and may degrade if activity exists in the system. One example is the most substituted congener 209, which often requires a separate analysis using a shorter column to reduce activity and provide sufficient results. This work utilizes a new technology that allows for fast quantitation of toxic congeners with short run times, and includes the quantitation of congener 209 in the same analytical run. This eliminates the need for an extra instrument using an alternative column dimension to quantitate the necessary congeners. Comparison of existing methods and the proposed method are included highlighting improved sensitivity and shorter run times.

WP126 GC/MS Software and Database for Screening Analysis: Mechanism and Evaluation

K. Arizono, Prefectural University of Kumamoto / Faculty of Env. & Symbiotic Science; S. Nakashima, T. Yamagami, Nishikawa Keisoku Co. Ltd.; Y. Ishibashi, Pref. Univ. Kumamoto

Simultaneous analysis of trace organic compounds using GC/MS is coming popular and important. However, for such analysis, particularly in the case of numerous target compounds, the problem of complicity and difficulty becomes obvious for not only analysis but also the management of standard substance and solution. GC/MS trace analysis requires the two kinds of target compound's information, one is retention time for qualification and another is response factor for quantification. These normally vary among instruments used and analysis periods. To obtain these, thus, standard should be analyzed at the same time as sample analysis. And several analytical parameters such as calibration curve are revised based upon acquired values. This is the one of reasons why the simultaneous numerous compounds analysis is so complicate and difficult. For the purpose of improving the problem, we developed dedicated software and database for GC/MS screening analysis. Three kinds of database: retention time, relative response factor with internal standard (RRF), and mass spectrum were constructed for approximately 600 chemicals. The software operates by associating with the databases which were built aiming to "fix" retention time and RRF as specific values for each compound. Using the databases as calibration curve, the software enables GC/MS to determine numerous chemicals without standards except for a few internal standards.

WP127 Herbicides in Stream Water Following a Silvicultural Application According to Modern Best Management Practices

J. Louch, G. Allen, NCASI; T. Garland, Oregon State University; C. Hale, Nutter and Associates; G. Ice, NCASI

Dissolved glyphosate, aminomethylphosphonic acid (AMPA), imazapyr, sulfometuron methyl, and metsulfuron methyl were measured in a small stream in the Oregon Coast Range both during and after application of herbicide(s) following a commercial timber harvest. The stream drained a small watershed, the upper portions of which were the harvest unit. Application rates were 681 g/ac glyphosate (a.e.), 85 g/ac imazapyr (a.e.), 64 g/ac sulfometuron methyl (a.i.), and 17 g/ac metsulfuron methyl (a.i.), and all herbicides were applied by helicopter in a single tank mix. Baseflow and storm event samples were collected at three sites along the hydraulic gradient: NBH (at the fish/no fish interface in the middle of the harvest unit), NBU (at the bottom of the harvest unit), and NBL (downstream of the harvest unit). AMPA, imazapyr, sulfometuron methyl and metsulfuron methyl were not detected in any sample at 15 ng/L, 600 ng/L, 500 ng/L and 1000 ng/L, respectively. However, a clear pulse of dissolved glyphosate manifested at the highest elevation site (NBH) during the application (baseflow conditions). This pulse maximized at ≈50 ng/L and persisted for two to three hours. An associated pulse was not detected (<20 ng/L) at downstream sites. Subsequent baseflow samples collected three days after treatment (DAT) showed ≈25 ng/L dissolved glyphosate at all three sites, and all sites were <20 ng/L dissolved glyphosate by 19 DAT. Samples collected during the first storm event (8 DAT) showed a clear pulse of dissolved glyphosate at NBU, but not at NBH or NBL. The maximum glyphosate concentration observed during this pulse at NBU was 115 ng/L, and the pulse persisted for about six hours. During the next storm event (10 DAT) a clear pulse of dissolved glyphosate manifested at NBH, but not at NBU or NBL. The maximum concentration observed was 42 ng/L, and this pulse persisted for about ten hours. Results from all subsequent storm events showed < 20 ng/L dissolved glyphosate at all three sites. A limited number of analyses on suspended sediment (SS) showed that SS held *de minimis* masses of glyphosate and AMPA following application. These results show that exposure of aquatic organisms to herbicides from silvicultural applications conforming to modern best management practices are short lived and of low concentration (<1 μg/L).

WP128 Hurricane Released Pollutants in Surface Waters: A Gulf Coast Analysis

G. Okome, Texas Southern University – Department of Environmental Science and Technology

Hurricanes have the potential to cause widespread environmental pollution from flooding and runoffs flowing into streams and rivers. This research identifies hurricane induced release of pollutants from point and non-point sources into surface waters in the Gulf coast area and the transient response of pollutant flow after the storm. Pre and post Hurricane Ike event surface water quality monitoring data for rivers and lakes in and around Galveston, Texas city and Port Arthur were analyzed to identify the chemicals released and their concentration in these bodies of water.

WP129 In Situ Field Extraction Providing Quantitative Ultra-low Time Integrative Extracts for PBDE's and PCB Congeners in the Water Column

*B.A. Hepner, C.I. Agent Storm Water Solutions / Research & Development, ; J. Aderhold, C.I.Agent Storm*Water Solutions; B.J. Era-Miller, Washington State Department of Ecology / EA Program – Toxics Studies Unit*

PBDE's and PCB's in the water column are often extremely low. In the past, estimation of concentrations in the water column were obtained from indicator organism tissue residue studies, or passive samplers using equilibrium partitioning calculations. These studies were time consuming and supplied qualitative data at best of the dissolved water fraction. A time integrative solid phase extraction device was developed to provide both quantitative, ultralow total whole water or dissolved values. The device is in the form of a small submersible extractive sampler. The device actively draws water in a submersed state through a SPE disk that has special lofted pre-filtration filters, Its small foot print allows transportation to remote areas sequestering hundreds of liters of water, using only four AA batteries. A small field hardened SPE disk designed for transport, durability and storage, is all that

is sent to the lab for elution and analysis. The Washington State Dept. of Ecology obtained funding to evaluate this technology in Spokane river dam reservoirs for PBDEs and PCBs. The study compares the field extracted disk values to grab water samples using HRMS methods, and non-HRMS methods. The study evaluates and compares QA/QC issues, reporting levels, transportation, laboratory analysis issues, and running multiple methods by splitting the solvent extract of the eluted disk.

WP130 Influence of the Humic Substances on the Degradation of Fipronil Pesticide by the bacterium Burkholderia sp.

L.T. Cappelini, Universidade de São Paulo / Chemistry; J.V. Alberice, E. Pozzi, J.P. Lima, L.G. Diniz, R.T. Verbinnen, E. Carrilho, E.M. Vieira, Universidade de Sao Paulo

Due to great interest in knowing the soil microbiology that is utilized on agricultural crops front to use of humic substances. The present study aims to determine soil bacteria able to use fipronil as the sole carbon source and check the action of humic substances in the process of degradation of fipronil. Four non-comercial techniques were used: Viestel, Selbach,Griffiths, Direito to DNA extraction and a comercial kit with the same function. The efficiency of two primers, 968FGC 1392R and 27F/1100R, was assessed through these methods. None of the extraction methods was efficient in the direct extraction of soil DNA, therefore an indirect extraction method was choosen. The DNA of selected bacteria was extracted through indirect technique. The PCR was run using the primer 27F/1100R and its product was cloned and sequenced. The identified bacteria were: Clostridiumsp, Bdellovibriosp, Flavisolibactersp, Burkholderiaspe, Herbaspirillumsp. The selected gender to perform the research was Burkholderiasp, using the pure strain of Burkholderiathailondensis due its potencial of degradation cited in the literature. A method QuEChERS GC-MS was validated for the determination of fipronil and its methabolites, sulfide fipronil and sulfone fipronil. The following results were found: detection and quantification limits of 0,06 mgL^{-1} and 0,25 mgL^{-1}, respectively, linearity of 0,99 and precision from 10,9% to 7,3%, at low level, and from 1,84% to 1,24%, at high level. The recovery was between 78% and 98%, and accuracy around 99%. The method is robust with 95% of confidence and has values of repeatability no higher than 11%.Subsequently, eight treatments were set up in order to measure fipronil degradation in view of the utilization of SH. *Burkholderiathailondensis* deteriorated about 0,75 mg L^{-1} of fipronil, with or without the presence of SH into the studied soil. The quantification of byproducts was not possible, because the concentration of both methabolites were below LOQ.

WP131 Influence of the presence of dispersants in commercial deltamethrin on acute toxicity in Eisenia foetida earthworms

F. Benetti, L.B. Pigatin, IQSC-USP; M.M. Kanashiro, R.N. Rodrigues, University of São Paulo; M. Landgraf, M.O. Rezende, IQSC-USP

Deltamethrin is widely used in Brazilian crops to combat cockroaches and other invasive insects, and although the pesticide is not present commercially in its isolated form, it is presented with a number of propellants and surfactants that improve its solubility in water. Thus, it is interesting to know whether besides the deltamethrin, these principles also cause deleterious effects on earthworms and in which proportion. Acute toxicity tests were performed according to ISO 11268-1 and ISO 11268-2. Every tests were performed with five repetitions, each result being the arithmetic mean of these replicates, and were performed with soil collected in rural area. The ratio used was 2x4x5, in which two treatments (with and without deltamethrin solution – the suspension without the active principle was provided by the manufacturer, for academic purposes), with four different concentrations (5 mg/kg – recommended concentration by the manufacturer – 100, 250 and 500 mg/kg of 25% commercial deltamethrin) in five replicates over 5 control vases. In each vase, the humidity was maintained at 60% and 10 adult worms were placed. To monitor toxicity, acute earthworms were weighed on days 0, 7 and 14. Compared to control, in experiments with and without deltamethrin there were no sharp differences in their development at a concentration of 5 mg/kg (with biomass gain in the first 7 days and loss from the 14th day). In the experiments of 100 mg/kg it can be already noticed differences in the development of earthworms, where the presence of deltamethrin begins to negatively influence their development (with loss of biomass right on the 7th day (-6.83%) while in the experiment without deltamethrin, biomass loss was not noticed. From 250 mg/kg, on it can be already observed a decrease in biomass gain right on the 7th day, but the vases with deltamethrin the decrease is much more acute than in vases where the pyrethroid is not present. What can be noticed with these results is that deltamethrin presents relevant acute toxicity to earthworms and, despite the presence of propellants and surfactants be necessary for better solubility of deltamethrin in aqueous medium, they also present health danger to earthworms, even in the absence of pyrethroid. Thus, it is interesting to state that the use of commercial deltamethrin outside the manufacturer's recommendations should not be practiced by farmers, since it can cause harmful effects to living organisms that lives the soil.

WP132 Inorganic analysis of challenging environmental samples

E. Pruszkowski, C. Bosnak, PerkinElmer, Inc.

The objective of the poster is to show the capability of the current ICP-MS technology for analysis of difficult environmental samples. It would be shown that the ICP-MS is a perfect technique not only for analysis of drinking water, soils and sediments but also for such challenging matrices as Flue Gas Desulfurization wastewaters (FGDWW). FGD wastewaters are arguably some of the most complex and challenging samples to analyze by ICP-MS. These FGD samples are extremely acidic and saturated with high concentrations of gypsum, heavy metals, alkali earth metals, chlorides and dissolved organic compounds. The USEPA has recently taken on the task of revising the rules affecting these wastewaters with the final rule expected by April 2014. ICP-MS instrumentation is needed to achieve the sensitivity required for elemental determinations but also must be capable of handling the challenging nature of the matrix and must be able to correct for the very significant interferences posed by a myriad of polyatomic specifies formed in the plasma as combinations of ambient and plasma gases such as Ar, O2, and N2 with the high concentrations of elements in the samples such as Ca, S, Cl, and Na. This poster will show that modern ICP-MS instrumentation equipped with interference removal cell technologies (DRC/KED) and robust sample introduction hardware can effectively address all the concerns. Using modifications to existing EPA Methods 200.8 and 1638, the matrix and interferences can be addressed and controlled while meeting all the required EPA quality control criteria. In addition, long term stability is maintained for many hours of continuous analysis of this difficult matrix. With the ability to handle difficult samples like FGD wastewaters, current ICP-MS instrumentation can also easily handle drinking water samples using EPA Method 200.8 and solid wastes or soils using the EPA Method 6020. Data will be shown to illustrate this capability.

WP133 Measurement of Fipronil and a metabolite in dosed rodents and human biological samples

R. McMahen, USEPA; M.J. Strynar, USEPA / National Exposure Research Laboratory; D. Herr, G. Moser, S. Dagnino, USEPA; S. Garantziotis, NIEHS; E. Andersen, L. McMillan, USEPA; A.B. Lindstrom, USEPA / National Exposure Research Laboratory

Fipronil is a phenyl pyrazole insecticide used to control termites, fleas, roaches, ants, and other pests in residential and agricultural areas. It has widespread use, fipronil has been found in dust (indoor and outdoor) and surface water runoff, suggesting that there may be many pathways for human exposure. Fipronil causes thyroid follicular cell tumors in both male and female rats, and the USEPA has classified it as a possible human carcinogen. Technical grade fipronil is also considered moderately toxic by ingestion with an oral LD50 of 97 mg/kg in rats. To understand more about fipronil, it is important to evaluate its toxicity in test animals and to develop biomarkers for use in human exposure studies. In an attempt to identify biomarkers of exposure, adult male Long Evans rats were dosed with fipronil acutely (0, 5, 10, 25, 50 mg/kg) and chronically (0, 5, 10 mg/kg/day) for 2 weeks. Resulting serum samples were evaluated via LC/MS/MS for fipronil and its primary metabolites, fipronil sulfone, fipronil sulfide, and fipronil amide. Fipronil was found at relatively low concentrations at all doses, but the metabolite fipronil sulfone varied from 500 to 3500 ng/mL in both a dose and time dependant manner. The method was then applied in an evaluation of human serum samples obtained from NIEHS from individuals with no known pesticide exposures. Similar to the rats, only trace levels (< 0.1 ng/mL) of fipronil were observed in these human serum samples while fipronil sulfone was present in approximately 50% of the individuals at concentrations ranging from 0.1 – 4 ng/mL. Together these results suggest that fipronil sulfone is a primary metabolite in mammalian systems that could be useful as a biomarker in human studies. These results will be presented suggesting that human exposure to fipronil may be widespread and in need

of more extensive characterization. Pharmacokinetic studies in animals and humans are also needed to fully evaluate the potential utility of fipronil sulfone as a biomarker of exposure to fipronil.

WP134 Mercury concentrations in Pacific walrus (Odobenus rosmarus divergens) muscle tissue

L. Aguilar Lazaro, Baylor University / Environmental Science; S. Trumble, S. Usenko, Baylor University

Mercury is emitted from natural and anthropogenic sources and is capable of undergoing long-range atmospheric transport to remote regions, such as the Arctic. Mercury is known to accumulate in colder environments, enter the food web, and bioaccumulate into higher trophic organisms. As a result, many Arctic mercury monitoring studies have focused on marine mammals, such as walrus. The study of mercury concentrations in walrus tissue is also important for human health, due to its role in the Inuit diet. In addition, previous studies have highlighted that environmental mercury concentrations are influenced by changes in emission sources, climate, food availability, and ice coverage. Due to the logistical and financial challenges of Arctic monitoring there are very limited studies that examine mercury in walrus tissue. Current walrus mercury datasets from Alaska, Canada, and Greenland have intermittent data from 1975 to 2004. The purpose of this study is to measure mercury concentrations in Pacific walrus tissue collected in 2009 (n=15) and 2010 (n=15) using cold vapor atomic fluorescence spectroscopy according to EPA method 1613. The expanded dataset will be used to examine walrus mercury concentrations over time and will evaluate the relationship between mercury concentrations and physical environmental variables, such as changes in sea surface temperatures.

WP135 Non-targeted evaluation of pharmaceutical degradation and byproduct formation in wastewater treated with an innovative advanced oxidation reactor

E. Parry, University of California, Davis / Agricultural and Environmental Chemistry Graduate Group; T.M. Young, University of California-Davis / Dept. of Civil & Environ. Engineering

Wastewater discharge is a source of pharmaceuticals and personal care products (PCPPs) to surface water. Treatment with advanced oxidation has been shown to degrade PCPPs, but research has been targeted to known contaminates and byproducts. Use of high resolution mass spectrometry (MS) in combination with MS/MS techniques makes a non-targeted examination of a reactor's effectiveness possible. This approach will provide a more comprehensive view of the reactions and byproducts that result. A pilot scale (50 GPM) innovative UV reactor system with the addition of two different hydrogen peroxide concentrations, 15 mg/L and 200 mg/L, was operated at the UC Davis wastewater treatment plant. Samples were collected before and after treatment, extracted and analyzed by high resolution liquid chromatography MS. Acquired data was blank subtracted and filtered by presence/absence in two or more replicates. Fold change comparison between influent and effluent replicates show that the number of statistically significant transformed features was much higher in the 200 mg/L treatment. Higher hydroxy radical concentrations present with a higher peroxide addition support this result. Tentative molecular formula identification was done using an in-house database of wastewater contaminants and a purchased database. A score of 70 out of a 100 was required for a match to be made. This methodology utilizing high resolution MS to assess reactor performance and to optimize operational parameters could be used in combination with bioassays to better focus water treatment strategies for compounds (both parent compounds and transformation products) of the greatest ecological importance.

WP136 Optical properties of chromophoric dissolved organic matter (CDOM) in salt marsh pore water

J. Bowen, C. Clark, Chapman University

Optical properties of chromophoric dissolved organic matter (CDOM) were measured in salt marsh as a function of vertical depth and distance from oil drilling activity in coastal Southern California. Absorption coefficients and fluorescence intensities increased with depth, while fluorescence:absorbance ratios (flu/abs) were higher in pore waters at -25.5 cm for regions closest to the rig and -7.5 cm for regions furthest from the rig. Flu/abs and spectral slopes showed a decrease as emission peak maxima were red-shifted. Terrestrial humic peaks A and C indicated increasing fluorescence intensity with depth for all distances from drilling activity. Greater amounts of humic substance in lower anoxic regions form as a result of the biodegradation of organic matter. The optical properties showed distinct bands for tryptophan-type and tyrosine-type protein peaks T and B at regions near the upper anoxic region. T and B peaks correspond to higher sulfate depletion in samples closest to the rig as a result of anaerobic microbial activity. These anoxic regions were similarly characterized by greater amounts of the reduced Fe(II) species. Fluorescence intensities for these samples were depicted using 3D excitation-emission matrix (EEM) fluorescence spectroscopy. CDOM optical properties and distribution suggest greater anaerobic microbial activity in salt marsh regions closest to oil drilling.

WP137 Organotin and imposex contamination in South American coastal areas: an updated review

I. Castro, Universidade Federal do Rio Grande / Institute of Oceanography; G. Fillmann, Universidade Federal do Rio Grande / Institute of Oceanography

Organotin compounds (OTs) were used in antifouling paints for more than four decades. However, due to their widespread intensive use and high toxicity, undesirable effects in non-target marine organisms have been detected since the early 1980s. Consequently, the International Maritime Organization banned new maritime applications of these products on January 1, 2003 and their presence on ship hulls from January 1, 2008. Although extensively studied in Europe, North America, Oceania, and Asia, and despite the recent efforts, environmental levels and effects of organotin contamination are still partially known for South America. Thus, an update on the current status of this problem for South America, including new data for coastal areas of Colombia, Venezuela, Chile and other areas of Brazil, is presented and compared with the review published in 2012. An overview of the OTs concentrations in sediment and biota and their effects, mainly imposex in marine gastropods, are presented. On the Atlantic side of South America, organotin contamination has been reasonable studied along the coast of Argentina, Uruguay and Brazil, but remains unstudied for Suriname, Guyana and French Guyana. Despite some "hot spots" of OTs contamination, recent data have indicated trends of reduction in the contamination for some areas, while others are still showing fresh inputs of TBT. On the other hand, for the Pacific coast of South America, organotin contamination remains unknown for northern Chile and southwestern Colombia. Extremely high and increasing TBT and imposex levels were registered in Peru. These data confirmed the widespread TBT contamination along the South American coastal areas and, despite clear decreasing trends in the contamination levels for some areas (i.e., southern part of Brazil), levels are still environmentally relevant and increasing in other areas (i.e., Callao, Peru). Therefore the establishment of baselines in areas with no previous studies and temporal trend studies in the main South American coastal areas is crucial to verify the effectiveness of local regulations and OTs global ban, and to map the most sensitive areas related to present and future antifouling impacts.

WP138 Perchlorate and thiocyanate in human serum from North Vietnam and their association with thyroid hormone and iodide levels

A. Eguchi, National Institute for Environmental Studies / Center for Environmental Health Sciences; T. Kunisue, Tottori University / Faculty of Agriculture; Q. Wu, School of Public Health, State University of New York at Albany / Environmental Health Sciences, Wadsworth Center, New York State Department of Health; P.T. Trang, P.H. Viet, Hanoi University of Science / Centre for Environmental Technology and Sustainable Development; K. Kannan, New York State Department of Health / Wadsworth Center; S. Tanabe, Ehime University / Center for Marine Environmental Studies (CMES)

Perchlorate (ClO_4^-) and thiocyanate (SCN^-) interfere with iodine (I) uptake by the sodium-iodide symporter (NIS) and thereby these anions affect thyroid hormone (TH) production in the thyroid gland. Studies have reported human exposure to perchlorate and thiocyanate in the United States (US) and Europe, but very few investigations are available in Asian developing countries. In this study, we determined the concentrations of perchlorate, thiocyanate, and iodide in 131 serum samples from two locations in North Vietnam, Bui Dau (BD: e-waste recycling site) and Duong Quang (DQ: rural site), and examined the association of serum levels of these anions with thyroid hormones. The median concentrations of perchlorate, thiocyanate, and iodide detected in Vietnamese sera were 0.104 ng/ml, 2020 ng/ml, and 3.11 ng/ml, respectively. Perchlorate levels in the BD population (median: 0.116 ng/ml) were significantly higher than those (median: 0.086 ng/ml) in the DQ population, indicating the presence of perchlorate sources in e-wastes and specific exposure for the BD residents. The median daily

exposure doses of perchlorate estimated from the serum levels in DQ and BD populations were 0.033 and 0.048μg/kg bw/day, respectively: the values were one order of magnitude lower than the USEPA's reference dose (RfD), 0.7 μg/kg bw/day. However, the highest daily dose (0.81μg/kg bw/day) of perchlorate, estimated from the serum levels in an e-waste recycling worker, exceeded the USEPA's RfD. Significantly higher concentrations of thiocyanate were observed in sera of the smoking males, compared to those in the non-smoking males and in the females, indicating that smoking activity is a major exposure source of thiocyanate for humans, as reported previously in other countries. In order to assess the association between serum levels of the anions and thyroid hormones (THs), we implemented a stepwise multiple linear model. No associations were found between the TH concentrations and the levels of perchlorate or thiocyanate in the Vietnamese population.

WP139 Presence of Perfluorinated Compounds in Source and Treated Drinking Waters from 25 Drinking Water Treatment Plants in the United States

S. Boone, USEPA / Office of Chemical Safety and Pollution Prevention; C. Byrne, J. Ferrario, USEPA/OCSPP/OPP/BEAD/ECB

The USEPA and USGS conducted a national study sampling source and treated water from 25 drinking water treatment plants (DWTPs) in the US for the detection of over 200 contaminants of emerging concern (CECs). The source waters from these facilities included aquifers, reservoirs, and rivers. The facilities tested used various treatment processes with production rates ranging from 0.036 to 500 million gallons per day (mgd) at the time of sampling. Seventeen perfluorinated compounds (PFCs) were analyzed in both source and treated water by the current method developed by the USEPA Environmental Chemistry Laboratory using LC/MS/MS detection. At least one PFC was detected at all sampling locations. Based upon the mean concentration in both source and treated water from the 25 sites, the most abundant PFCs in descending order were: PFPeA > PFOA > PFBA > PFHpA > PFHxA > PFOS > PFHxS > PFNA = PFDA = PFBS > PFUnDA > PFDoDA; and upon detection frequency, the most prevalent PFCs in descending order were: PFHxA > PFBS > PFBA = PFPeA = PFHpA > PFNA > PFHxS > PFOS > PFOA > PFDA > PFUnDA > PFDoDA. Five PFCs were never detected: PFTrDA, PFTeDA, PFHxDA, PFOcDA, and PFDS. The highest individual level of detected PFCs (ng/L: ppt) were: PFPeA (510), PFHpA (180), PFOA (110), PFBA (100), PFHxA (60), PFOS (50), PFHxS (50), PFNA (40), and PFDA (30). The USEPA provisional health advisory for PFOA is 400 ppt and 200 ppt for PFOS. The concentrations of individual PFCs in treated waters were similar to their source waters at all sites tested but one. At that site, powdered and granular activated carbon (PAC and GAC) was used, and the PFCs detected were measurably reduced: PFHpA, PFOA, PFNA, PFDA, PFUnDA, PFHxS, and PFOS (>96%), PFHxA (75%), PFPeA (40%), and PFBA (20%). This DWTP had the highest amount of GAC, with the longest reportable empty bed contact time (EBCT), and the shortest activated carbon replacement time. Six other DWTPs used PAC, and eleven other DWTPs used GAC. In most cases, the levels of PFCs were not affected by the more commonly used drinking water treatment processes. While this study was designed to provide important baseline information on CECs in source and treated drinking waters, it was not designed to be representative of all such sources and drinking waters throughout the United States.

WP140 Rapid Evaluation of Sub-Lethal Toxic Effects of Nanoparticles in Escherichia coli

C. Kaweeteerawat, University of California, Los Angeles / Molecular Toxicology; A. Ivask, National Institute of Chemical Physics and Biophysics / Environmental Toxicoloy; R. Liu, University of California, Los Angeles; P.A. Holden, University of California; H. Godwin, UCLA Fielding School of Public Health

Synthesis and use of nanoparticles has skyrocketed during the past decade. To ensure that nanotechnology is safely and sustainably developed, toxicity of nanoparticles is needed to be determined. Here, we report the application of a suite of sub-lethal assay as well as a growth inhibition assay to a series of silver and metal oxide nanoparticles in bacteria (*Escherichia coli*). Fluorescent assays such as PI/SYTO, XTT, DiBAC, and H2DCFDA were used to measure viability, respiration rate, membrane potential, and ROS production, respectively. These studies revealed that toxicity of the silver particles studied correlates with size and surface charge, with smaller particles and particles with a more positive surface charge being more toxic. By contrast, the toxicity of the metal oxide particles studied correlates most with the energy of the conduction band, although dissolution of some of the particles also plays an important role.

WP141 Reconstructing the historical nutrient status of Lake Diefenbaker, Saskatchewan, Canada, using sediment algal pigment composition

T.J. Tse, Toxicology Centre / Toxicology Graduate Program; Z. Quinones-Rivera, P.R. Leavitt, University of Regina / Department of Biology; L.E. Doig, University of Saskatchewan / Toxicology Centre; B. Lucas, University of Saskatchewan / Toxicology Graduate Program; L. Karsten, J. Giesy, University of Saskatchewan / Toxicology Centre; H. Wheater, University of Saskatchewan / Global Institute for Water Security; P.D. Jones, University of Saskatchewan / Toxicology Centre

Lake Diefenbaker is the largest multi-purpose reservoir (225-km long) in southern Saskatchewan, Canada. Anecdotal evidence has suggested an increase in frequency and density of algal blooms occurring in recent years. However, there is limited information available regarding the historic nutrient status of Lake Diefenbaker. Sedimentary pigments were used in a paleolimnological investigation of photophysiology and structure of past and present algal communities and to provide an estimate of the historical trophic status of the lake. Cores of sediments were collected from Swift Current Creek, near the Wild West Steelhead fish farm, a cattle location, and the Qu'Appelle and Gardiner arms, in Lake Diefenbaker. Sediments exhibited a composition of algal pigments representative of: cyanobacteria, diatoms, *cryptophytes*, and *chlorophytes* The Qu'Appelle and Gardiner arms demonstrated clear trends of increasing concentrations of some of these algal pigments per gram total organic carbon (TOC), including the cyanobacterial pigments: canthaxanthin (262 nmol/g TOC in the Gardiner arm and 109 nmol/g TOC in the Qu'Appelle arm); echinenone (64 nmol/g TOC in the Gardiner arm and 27 nmol/g TOC in the Qu'Appelle arm). An increasing trend in myxoxanthophyll (filamentous/colonial cyanobacteria pigment) was clearly seen only in the Gardiner arm sediment core with concentrations ranging between 13 nmol/g TOC to 123 nmol/g TOC. Increasing phototrophic and cyanobacterial activity might be becoming more frequent at some locations within Lake Diefenbaker, primarily towards the Qu'Appelle and Gardiner dams.

WP142 Seasonal survey of selected pharmaceuticals in drinking water across Canada

L. Bragg, University of Waterloo / Department of Biology; H. Li, Trent University; C.D. Metcalfe, Trent University / Environmental & Resource Studies; A. Tugulea, Health Canada / Exposure and Biomonitoring Division, HECSB; M.R. Servos, University of Waterloo / Department of Biology

An area of growing interest for water research is the occurrence of many emerging contaminants in drinking water, particularly pharmaceuticals. These chemicals are not regulated or monitored in drinking water systems. This national survey is a part of a larger drinking water surveillance program, looking at various disinfection byproducts, inorganics and emerging contaminants across Canadian provinces and territories. This presentation will focus on the selected pharmaceuticals studied from sites across Canada and at two sites where a wider range of contaminants were examined. Sixty drinking water plants, with various water treatment processes, water sources and populations, were visited during summer and winter sampling campaigns of 2009 and 2010. At each site, duplicate samples were taken from the raw water prior to treatment, and from the final treated drinking water. Samples spiked with surrogates were extracted using solid phase cartridges and quantified using LC-MS/MS with electrospray ionization (both positive and negative). Overall, concentrations of all pharmaceuticals and emerging contaminants were found to be very low or below the detection limits in both sampling periods in the treated water. The analytes with highest percent occurrence in the source water were caffeine, carbamazepine, diclofenac, ibuprofen, naproxen and venlafaxine. Some treatment plants were able to successfully remove or reduce these contaminants at even lower levels through their treatment process, whereas others were less efficient in this respect. For the majority of analytes, occurrence was increased in the summer compared to the winter sampling period. A variety of additional pharmaceuticals and personal care products were detected at the two sites examined in more detail. Many source waters in Canada have very low but detectable levels of pharmaceuticals that may not always be removed by treatment. However the current weight of evidence does not indicate any risk for human health at the levels that have been detected.

WP143 Sediment Bioavailability Initiative: Development of Standard Methods for the Use of Passive Samplers in Management of Contaminated Sediment

S.B. Kane Driscoll, Exponent, Inc.; C.A. Menzie, Exponent; U. Ghosh, University of Maryland Baltimore County / Department of Chemical, Biochemical, and Environmental Engineering; D.D. Reible, The University of Texas-Austin / Environmental and Water Resources / Dept. of Civil & Environmental Eng.; G. Burton, University of Michigan / School of Natural Resources & Environment and Cooperative Institute for Limnology & Ecosystem Research; P. Gschwend, Massachusetts Institute of Technology / Department of Civil and Environmental Engineering

This presentation will introduce a new program that focuses on the development of standard operating procedures and approaches for the use of passive sampling methods (PSMs) in sediment management. Over the last 20 years, a substantial amount of research has been conducted on the use of PSMs to characterize the fraction of total contaminant in bulk sediment that is available to be taken up by organisms. PSMs are defined as techniques that rely on the partitioning of contaminants to a sampling media that is placed in contact with the sediment. Unfortunately, despite considerable advances in the development of PSMs to measure bioavailability, significant barriers to the use and regulatory acceptance of these methods persist. These barriers have been attributed to uncertainty regarding advantages and limitations of the methods, lack of standard methods that have been critically reviewed and validated, and lack of consensus on technical guidance for use in regulatory decision-making. This presentation will focus on four PSMs that have been studied expensively: solid phase microextraction (SPME) fibers that are coated with a layer of polydimethysiloxane (PDMS), polyoxymethylene (POM), and polyethylene (PE) for organic contaminants, and diffusive gradient in thin films (DGT) for metals. This presentation will present current state-of-the-practice for PSMs, including standard operating procedures for field and laboratory applications. Measures of bioavailability are meaningful only if they inform the assessment and management of exposure. The presentation will discuss issues related to the successful use of passive samples, including: 1) need for documentation of the reliability of PSMs; 2) information on the strengths and limitations of PSMs for specific uses; and 3) necessity of linking PSM measures to an assessment and management framework so that the information generated make sense to the user community.

WP144 Selective Pressurized Liquid Extraction of Marine Mammal Blubber (a lipophilic matrix)

E.M. Robinson, Baylor University / The Institute of Ecological, Earth, and Environmental Sciences; S. Usenko, Baylor University

Analytical methods for unique and rare samples, such as marine mammal blubber, should be improved to eliminate possibilities for sample loss and contamination. Historically, blubber analytical methodologies for organic contaminant analysis required an extraction step followed by multiple cleanup steps (e.g., traditional column chromatography and size-exclusion chromatography), which increase the chance for sample loss and contamination. Selective pressurized liquid extraction (SPLE; an analytical technique that combines PLE with adsorbent cleanup) has reduced or eliminated the number of steps associated with sample extraction and cleanup. SPLE utilizing acidic and neutral silica at a 1:30 sample to total adsorbent ratio eliminated the need for size-exclusion chromatography and required no additional cleanup for the analysis of organochlorine pesticides, polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) in rare bowhead whale blubber. Analysis was performed using gas chromatography-mass spectrometry. Pesticide, PCB, and PBDE triplicate recoveries averaged 75, 83, and 84%, respectively.

WP145 Sensitive screening for polybrominated environmental contaminants in complex matrices using GCxGC-ICP-MS

N. Rosenfelder, W.C. Davis, National Institute of Standards and Technology / Chemical Sciences Division/ Hollings Marine Laboratory; J.R. Kucklick, National Institute of Standards & Technology / Chemical Sciences Division/ Hollings Marine Laboratory

Polybrominated compounds (PBCs) have been used in high quantities worldwide with many of these chemicals known to be widespread and persistent in the environment. In addition to man-made compounds, some regularly detected compounds are known to be produced naturally. The determination of PBCs is usually performed using gas chromatography (GC)/ electron impact (EI)-mass spectrometry (MS) or GC/electron capture negative ion (ECNI)-MS. However, recent studies predict that there are many compounds in the environment that have never been measured and some of these compounds are only present at very low concentrations. For this reason, dedicated and sensitive screening methods are needed to detect the presence of other relevant known and more important unknown pollutants at trace levels. Multidimensional separation technologies such as two dimensional GC (GCxGC) are powerful tools to enhance analytical selectivity and increase resolving power allowing for the identification of a larger number of chemicals. Targeted as well as non-targeted screenings using GCxGC coupled to electron capture detectors (ECDs) or time of flight (TOF) MS have been described but only a few studies deal with the simultaneous screening of different classes of PBCs. Inductively coupled plasma MS (ICP-MS) is a promising alternative detector for analyzing PBCs in complex samples at trace level concentrations. Here we explore the feasibility of ICP-MS coupled to GCxGC for the selective determination of PBCs. Maximized separation and selective detection allows for the simultaneous analysis of both within and among different classes of PBCs as well as separation from co-extracted matrix compounds. Resulting structured chromatograms will be used as an additional feature to identify analytes and classes of compounds. Furthermore, the linear bromine response in ICP-MS allows the quantification of unknown or rarely described PBCs for which reference standards are not available. The potential of this method for the sensitive screening of PBCs will be illustrated by analyzing different complex matrices, e.g. house dust, marine mammal blubber, sponge tissues, including simplified small-scale sample preparations as an alternative to targeted pre-separation of specific classes of compounds.

WP146 Soil humic-like organic compounds in fire emissions

M. Chalbot, J. Brown, University of Arkansas for Medical Sciences; G. Gamboa da Costa, National Center for Toxicological Research; I. Kavouras, University of Arkansas for Medical Sciences

Dust particles, when mixed with fresh combustion emissions, substantially enhance the atmospheric oxidative capacity, particle formation and microphysical properties of clouds, influencing the climatic and toxicological responses of particles. Owing to the large emissions of combustion aerosol during fires, an analysis of the release of dust particles from soil surfaces that are subjected to intense heating and shear stress has, so far, been lacking. Here we present the chemical characterization of the water-soluble organic carbon fraction of atmospheric aerosol collected during a prescribed fire burn in relation to soil organic matter and biomass combustion. Using nuclear magnetic resonance spectroscopy, we observed that humic-like substances in fire emissions have been associated with soil organic matter rather than biomass. Using a chemical mass balance model, we estimated that soil organic matter may contribute up to 41% of organic hydrogen and up to 27% of water-soluble organic carbon in fire emissions.

WP147 Soil remediation: Simultaneous adsorption of copper, chromium and lead by vermicompost using glass column

L.A. Mendes; L.P. Bucater, L.B. Pigatin, M. Landgraf, M. Rezende, IQSC-USP

Human activities have altered the global cycle of toxic elements. This is evidenced by the concentrations of these elements not only found in rivers, soils and sediments, but also by the testimonies that provide altered areas, with increasing concentrations coinciding with the beginning of the industrial activities. The apparent concentration increase of these elements in the environment has caused harm to human health, flora and fauna. Thus, the demand for the development of technological solutions has increased to meet environmental legislation. One of these is the vermicomposting, a process that worms are used to degrade recent organic matter, transforming it into a more stable material, capable of adsorbing and/or complexing with toxic elements, making them unavailable to the environment. The central idea of this work was to evaluate the competition between the metal species Cr^{+3}, Cu^{+2} and Pb^{+2} in vermicompost in order to assess the dynamics with which these elements are retained in the matrix. Before the competition study it was done the characterization of the adsorbent material. To study metal species competition, in the same mass of humus (5.0 g) was percolated through the column 30 aliquots of 25 ml of the mixed solution at a concentration of 500 mg L^{-1} for each metal ion. In this study, it was used a single mass of vermicompost (5.0 g), in order to verify to what extent the vermicompost may be used without the already adsorbed metals are leached. The results show that the toxic element Pb^{+2} pass without interacting with the column after the addition of the 12th aliquot (300 mL). That is, as the concentrations of Cr^{+3} and Cu^{+2} increases, the amount of adsorbed Pb^{+2} decreases. Possibly one of the

metals or both metals, Cr^{+3} and Cu^{+2}, are moving Pb^{+2} from the adsorption sites of the vermicompost and then taking them. One possible explanation for this phenomenon would be that the chromium is trivalent in relation to copper and lead and the latter still has a greater ionic radius, getting harder to retain the active sites. Thus, it was found that competition of species by metal active sites showed that the lead begins to be displaced by the chromium and copper as the concentration of these increases, this is due to its larger ionic radius that makes its displacement easier.

WP148 Soil-Water Partitioning of Clothianidin Under Simulated California Rice Field Conditions

R. Mulligan, University of California, Davis; R.S. Tjeerdema, University of California – Davis / Environmental Toxicology

Clothianidin, (E)-1-(2-chloro-1,3-thiazol-5-ylmethyl)-3-methyl-2-nitroguanidine, was initially registered by the U. S. Environmental Protection Agency (USEPA) in 2003 and was recently registered for use as a commercial seed treatment for protection from the rice water weevil and grape colaspis and is currently being approved for use in California. However, little is known about its environmental fate and no peer-reviewed studies concerning its abiotic dissipation in a flooded rice field area available. Sorption-desorption behavior of clothianidin in four soils collected from rice fields in the Sacramento Valley was investigated using the batch equilibrium technique at rice field relevant temperatures. Isotherm data were fitted to the Freundlich equation. All curves were L-shaped, indicating that sorption affinity of clothianidin decreased as aqueous concentrations increased for all temperature treatments. Desorption hysteresis was observed for all soils and temperature treatments. Clothianidin, with an aqueous solubility of 327 μg mL^{-1}, sorbs poorly to rice soils typical of California. Organic-carbon normalized partitioning coefficient K_{oc} values were low, ranging from 55 to 680 L kg^{-1} and 68 to 497 L kg^{-1} for 22 °C and 37 °C temperature treatments, respectively. A significant decrease in K_{oc} values was observed for 37 °C treatments ($p < 0.05$). The low sorption coefficients suggest that clothianidin is weakly sorbed by rice field soils and that sorption is inhibited at elevated temperatures.

WP149 Spatial Distribution and Time Trend of Selected Chlorinated and Brominated Compounds in the Sediments of Lake Michigan

J. Guo, University of Illinois – Chicago / School of Public Health; A. Li, University of Illinois at Chicago / School of Public Health; M. Corcoran, University of Illinois at Chicago; K.J. Rockne, University of Illinois at Chicago / Department of Civil and Materials Engineering; J.P. Giesy, University of Saskatchewan / Toxicology Centre and Department of Veterinary Biomedical Sciences; N. Sturchio, University of Illinois at Chicago / Department of Earth and Environmental Sciences

This work aims at revealing the spatial distribution and the temporal trend of selected legacy and emerging organic pollutants in the sediment of Lake Michigan. Chemicals from seven analyte groups were measured in 29 surface sediment grab samples plus segments of a number of cores collected in 2010 and 2011 were analyzed using gas chromatography coupled with either electron impact ionization triple quadruple or electron capture chemical ionization single quadruple mass spectrometry. Target chemicals include seven groups: polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs), polychlorinated biphenyls (PCBs), polychlorinated dipenyl ethers (PCDEs), polychlorinated naphthalenes (PCNs), organochlorine pesticides (OCPs), halogenated flame retardants (XFRs) and musk fragrances (MFs). For OCPs, except for p,p'-DDE, p,p'-DDD and p,p'-DDT, hexachlorobenzene (HCB), α-chlordane, γ-chlordane and trans-nonachlor were relatively high. Dieldrin, aldrin, endrin and dacthal were also found in certain core samples. PCN-28, 52 and 66 were relatively abundant. For XFRs, except for PBDEs, syn-DP, anti-DP, BTBPE and DBDPE, HBB, BB153, Dec602 and Dec603 were detected in most samples. In surface grab samples, the high concentrations of most target analytes were found at the sites near southeast of the lake, east of the midlake and the sites near sleeping bear dune. In core sediments, PCNs and OCPs peaked around 1950s to 1970s while XFRs are relatively high in recent years.

WP150 Spatiotemporal evaluation of TBT and Imposex occurrence in Todos os Santos bay, Northeast Brazil

V.A. Pereira, I.B. Castro, Universidade Federal do Rio Grande / Institute of Oceanography; G. Fillmann, Universidade Federal do Rio Grande / Institute of Oceanography

A spatial and temporal evolution of tributyltin (TBT) contamination was performed in Todos os Santos bay using surface sediments, bivalve tissues and imposex occurrence. The spatial approach used chemical analyses of surface sediments (10 sites) and bivalve samples (9 sites) (*Mytella guyanensis* and *Anomalocardia brasiliana*) collected in 2010. For temporal analyses, 7 sediment samples were recollected in 2012 in the same places sampled in 2010. In addition, the imposex occurrence (RPLI, FPLI, VDSI and % imposex) was verified in *Stramonita rustica* (11 sites) in 2004 and 2012. All sampled sites were chosen based in the presence of ship and/or boat traffic, which are very intense inside Todos os Santos bay. The spatial evaluation shown that higher TBT levels (262 ng Sn g^{-1} for surface sediments and 421 ng Sn g^{-1} for bivalve tissues) were found at the innermost part of bay, where is less hydrodynamic and surface sediment is finer and richer in organic carbon. On the other hand, comparatively lower levels of TBT were found in surface sediments (< 2 to 12 ng Sn g^{-1}) and bivalve tissues (12 to 43 ng Sn g^{-1}) collected around the entrance of the bay. These findings suggested that TBT spatial contamination in Todos os Santos bay is not only affected by direct sources but also by the hydrodynamic and sediment characteristics. Considering the temporal evaluation, no difference (p>0.05) was seen in TBT concentrations between sediments collected in 2010 and 2012. However, the butyltin degradation index, which assesses the historic TBT contributions based on proportional concentrations of its degradation products (dibutyltin and monobutyltin), indicated more metabolites in sediments sampled during 2012. Additionally, a significant reduction in the imposex incidence was detected in *S. rustica* from 2004 to 2012, indicating a possible reduction of TBT contamination around the entrance of the bay during the last decade. This improvement is related to the global TBT ban issued by the International Maritime Organization associated to local restrictions on TBT-based antifouling paints. Nevertheless, TBT contamination levels are still relevant especially in the inner part of Todos os Santos bay.

WP151 Specific Characteristics of Kentucky Lake Waters that Prevent the Reproduction of Zebra Mussels

S. Dasari, Murray State University / Department of Chemistry and Watershed Studies Institute; B.G. Loganathan, Murray State University / Department of Chemistry & Watershed Studies Institute; K.S. Sajwan, Savannah State University / Department of Natural Sciences; D. White, Murray State University / Department of Biological Sciences and Watershed Studies Institute

Every plant and animal species require ideal environmental conditions (physico-chemical characteristics) to survive, reproduce and establish their population. Zebra Mussel (*Dreissena polymorpha*; Bivalvia, Dreissenidae) is an exotic and invasive mollusk that spread extensively over various rivers and lakes in the United States. However, these mussels could not reproduce and establish population in the Kentucky Lake. The objective of this study was to explore various physical (DO, pH, Light penetration) and chemical parameters (trace elements) that may be crucial for the reproduction and development of zebra mussels in Kentucky Lake. In this study, water samples were collected from selected locations in Kentucky Lake, Ohio River, Hudson River, Clarks River and Murray Waste Water Treatment Plant to delineate differences in physical and chemical parameters that support zebra mussels to reproduce and develop in the waters. Standard analytical procedures were followed including field sampling, sample preparation and analysis using Atomic Absorption Spectrophotometer. In general, detectable concentrations of various inorganic elements were found in almost all samples analyzed. Elemental concentrations ranged from μg L^{-1} to mg L^{-1} in depending on analyte type and sampling locations. Among the various elements analyzed calcium contained highest concentrations (Range: 7-18 mg/L) in almost all samples from the Kentucky Lake waters. Copper was barely detected/below the detection limit (< 0.1 μg/L) in all samples tested. The concentration pattern followed the order of Ca > Na > Mg > K > Mn > Zn > Cu. Considering the inorganic elemental concentrations in other rivers and natural lakes, the calcium levels in Kentucky Lake waters were relatively low. Based on calcium-based invasion risk assessment report, calcium level is considered low range to enable zebra mussels to establish population in the Kentucky Lake waters.

WP152 Stability of Silver Nanoparticles in Aqueous Media Determined by Single Particle ICP-MS

L. Telgmann, H. Hintelmann, Trent University / Chemistry; C.D. Metcalfe, Trent University / Environmental & Resource Studies

Silver nanoparticles (AgNP) are integrated into a wide range of products for antibacterial purposes, including socks, underwear, shoe liners, food containers, deodorants, washing machines and refrigerators. The increased use of these nanomaterials in consumer products may lead to the release of

nanoparticles into the environment, especially surface waters. Currently, knowledge about the ways in which these particles impact on aquatic ecosystems is limited. The stability of the AgNP in water is of major importance and needs to be evaluated. Previous studies in lake mesocosms showed that the mean particle sizes of AgNPs declined over time. In this study, we developed a method using single particle inductively coupled plasma mass spectrometry (spICP-MS) and applied this technique to bench scale evaluations of the effect of various water quality parameters on AgNP particle aging. ICP-MS parameters such as dwell time, sample uptake rate and particle concentration were optimized in order to achieve low run times and low particle coincidence rates for spICP-MS. Also, the influence of additional dissolved silver (dAg) on AgNP detection was investigated. A particle size detection threshold of 35 nm was achieved. Particle sizes and concentrations of citrate and PVP-capped AgNPs were monitored over time in aqueous solutions with varying pH, temperature, particle size, particle concentration, chloride and dissolved organic carbon (DOC) concentrations. In general, a decline in particle size and number, indicating dissolution of the AgNPs was observed in almost all solutions. In milliQ water stored at 21°C, the AgNPs showed a silver mass decrease of 25% in the first two days and a decrease of 60% after two weeks. However, storage of milliQ samples at 4°C prevented such dissolution. Acidification of the solutions accelerated the dissolution process, with the greatest stability occurring at neutral pH. Separate treatments with additions of chloride and DOC led to an almost complete dissolution of the particles within a few days. At the same time, an increase in the silver background signal indicated an increase in dAg. The results of this study show the strong impact of water quality parameters on AgNP stability. These investigations will aid in the interpretation of data on the fate of AgNPs in natural water samples. Additionally, these results demonstrate the utility of spICP-MS as powerful tool for research on the fate of AgNPs in aqueous matrices.

WP153 The effect of nitrogen and phosphorus concentrations on painted turtle (Chrysemys picta) density

C. Ragsdale, Environmental Studies; K. Cecala, Sewanee: University of the South / Department of Biology; C. Hauser, Davidson College / Department of Chemistry

Small changes in nitrogen and phosphorous concentrations can have large impacts on freshwater systems. Several anthropogenic sources can contribute to nitrogen and phosphorous content in a system including storm water runoff and the use of cleaning and landscaping chemicals. Although freshwater productivity is often limited by nitrogen and phosphorus, eutrophication can yield increased primary productivity. Research has demonstrated that trophic cascades may translate increased productivity to differences in growth patterns and density for species in higher trophic levels. Heavy use of fertilizers on golf courses relative to rural and urban land-uses may explain observations of higher densities of turtles in golf course ponds, rather than similar ponds in urban and rural landscapes. Our objective was to determine whether golf course ponds that harbor high densities of painted turtles also have high concentrations of nitrogen and phosphorus. We compared water samples from ponds located on golf courses with those in other rural and urban locations. Phosphorous concentrations were determined using titrimetric nesslerization and nitrogen was quantified using a colorimetric testing kit and measuring for absorbance. We also examined whether changes in nutrient concentration altered pond food webs by estimating macrophyte cover and algal biomass. Ponds with high concentrations of nitrogen and phosphorus that also have concomitant increases in primary production are more likely to have high densities of painted turtles.

WP154 The spatial distribution of selected antiandrogens and pharmaceuticals during low flow conditions in the Grand River watershed, Ontario, Canada

M. Arlos, University of Waterloo / Department of Civil and Environmental Engineering; Department of Biology; G. Tetreault, Environment Canada / Aquatic Ecosystem Protection Research Division; L. Bragg, University of Waterloo / Department of Biology; W. Parker, University of Waterloo / Department of Civil and Environmental Engineering; M.R. Servos, University of Waterloo / Department of Biology

Endocrine disruption and high occurrence of intersex have been observed in wild fish associated with wastewater treatment plant (WWTP) effluents in the urbanized reaches of the Grand River watershed located in southern Ontario, Canada. WWTP effluent is a complex matrix with diverse aquatic environmental contaminants and stressors. The objective of this study is to characterize the spatial distribution and fate of two antiandrogenic personal care products (PCPs) (chlorophene and triclosan), along with selected pharmaceuticals (carbamazepine, ibuprofen, naproxen, venlafaxine) and the herbicide, atrazine. Water sampling of 29 sites which cover six municipal WWTPs and 100 km of river length was completed during summer low flows (July 2012). Many of the target pharmaceuticals and triclosan were detected in WWTP effluents in the Grand River watershed, especially those that did not nitrify (minimal treatment with high ammonia). Chlorophene was not detected or was at trace levels in the effluents. Under low flow conditions, triclosan and several other pharmaceuticals exhibit a spatial pattern where concentrations increased directly downstream of the WWTPs then decreased with distance downstream (dilution and degradation). Chlorophene, in contrast, was not found downstream of most of the WWTP outfalls but was first detected at a site 5 km upstream of a WWTP and then continued with relatively constant concentrations for approximately 29 km downstream. Atrazine was consistently found in all sampling locations which reflects the agricultural non-point source nature of this compound. The results of this study are being integrated into a fate and transport model to predict the spatial and temporal concentrations of pharmaceuticals and other compounds in the watershed.

WP155 Three stage BCR sequential extraction procedure for the fraction of heavy metals in contaminated soil samples of an electronic waste disposal site

A.O. Bankole, Basel Convention Coordinating Center for Training for West Africa Sub Region / Department of Chemistry University of Ibadan, Nigeria; O. Osibanjo, Department of Chemistry University of Ibadan / Department of Chemistry University of Ibadan; D.V. Perez, Embrapa Soils, Rio de aneiro Brasil; J.M. Godoy, Puc – Rio / Department of Chemistry

Determinations of total metal content are poor indicator of metal bioavailability; mobility and toxicity. This study investigates the mobility and availability of heavy metals from a contaminated electronic waste disposal site. Three stage BCR sequential extraction scheme developed by the Community Bureau of Reference protocol was applied to analyse the concentrations of ten surface soil samples for cadmium (Cd), Chromium, (Cr), Copper (Cu), Nickel, (Ni), Lead (Pb) and Zinc (Zn) from the polluted disposal site.The metal fractions obtained were analysed using the ICP-OES and FAAS. The BCR 141r and 701 certified reference materials were used to evaluate the analytical performance, accuracy and precision of the method. Good recoveries were obtained for all the heavy metals studied except for Pb. The behaviour of the soil samples when they come in contact with water was studied by the leaching test (DIN 38414-S4) and the result showed that the levels of extractable heavy metals were low compared to those values obtained by the acid-soluble fraction of the BCR protocol. In comparison, the results showed that the BCR fractions generally agreed well with the pseudototal digestion results with aqua regia.Total analysis of the soil samples were performed in order to calculate the Enrichment factors based upon abundances of the elements in the earth crust for soils. Sequential leaching tests revealed that Cu, Pb, Cr, Zn, Cd and Ni in the four fractions varied significantly among the soil samples and were predominantly associated with the oxidizable and reducible fractions.

WP156 Trophic magnification and isomer fractionation of perfluoroalkyl substances in Taihu Lake food web, China

S. Fang, X. Chen, Y. Zhang, Nankai University / College of Environmental Science and Engineering; W. Jiang, L. Zhu, Nankai University

Biomagnification of perfluoroalkyl substances (PFASs) are well studied in marine food webs, but related information in fresh water ecosystem and knowledge on fractionation of their isomers along the food chain are also limited. The distribution, bioaccumulation, magnification and isomer fractionation of PFASs were investigated in a food web of Taihu Lake, China. Perfluorooctanesulfonate (PFOS) and perfluorocarboxylates (PFCAs) with longer carbon chain length, such as perfluorodecanoate (PFDA) and perfluoroundecanoate (PFUnA), were predominant in bioorganisms, while PFHxA and perfluorooctanoate (∑PFOA) contributed more in water phase. Consistent profile signature of PFOA isomers in water phase with ECF products by 3M suggests that ECF production of PFOA still exists in China. Liner proportions of PFOA, PFOS and perfluorooctane sulfonamide (PFOSA) were in the range of 91.9-100%, 78.6-95.5% and 72.2-95.5%, respectively in the biota, indicating discrimination in isomer bioaccumulation. Trophic magnification was found for PFDA (2.43), perfluorododecanoate (PFDoA) (2.68) and PFOS (3.46) when all biota were included. Moreover, the trophic magnification factor (TMF) of *n*-PFOS (3.86), followed by 3+5*m*-PFOS (3.35), 4*m*-PFOS (3.32), 1*m*-PFOS (2.92) and m_2-PFOS

(2.67), *iso*-PFOS (2.59), is roughly identical to the eluted order on a FluoroSep-RP Octyl column, suggesting that hydrophobicity may be an important contributor for the isomers discrimination in biotas.

WP157 Two Stage Physiologically Based Extraction Test for Aluminum, Cobalt, Manganese and Vanadium in Soil

M. Jones; C. Schreier, Prima Environmental Inc.; R. Sahu, L. Farris, Basic Remediation Company

Typically, for environmental risk assessments, the relative oral bioavailability of all chemicals of potential concern are conservatively assumed to be 100 percent, an assumption necessitated by the fact that data to support alternatives are often not available. *In vitro* physiologically based extraction tests (PBETs) have been designed to simulate the human gastrointestinal tract in order to assess the mobilization of compounds from soil during the digestion process, to provide a reasonable approximation of bioaccessibility. The objective of the present study was to provide site-specific information to evaluate the relative oral bioavailability of selected metals from site soils. Much of the research effort around bioavailability and bioaccessibility testing has centered on arsenic and lead. This study was implemented specifically for the *in vitro* bioaccessibility testing of four metals: aluminum, cobalt, manganese, and vanadium. These metals are generally the greatest contributors to the risk estimates for the subject site. In addition, arsenic was also included in the study to evaluate the consistency with other similar studies. While it is recognized that there may be a paucity of *in vivo* validation data, there are several studies to indicate the applicability of PBET techniques for these four metals. Oral bioavailability varies with oxidation state, chemical form, and minerology. The four metals in the study are all cationic, with oxidation states typically present in soils similar to either arsenic or lead. The results of the PBET study indicate that the study data were of good quality, with consistent bioaccessibility values between samples for each of the four metals. Results of the PBET test were incorporated into the risk assessment conducted for the site. An evaluation of the effects of the bioaccessiblity values on the risk estimates was included in the risk assessment, as well as a discussion on the uncertainties associated with their inclusion in the risk estimates. Potential next steps include inter-laboratory comparisons, *in vivo* studies, and evaluation of background vs. site soil bioaccessibility.

WP158 Using an Extended Ready Biodegradability Method to Establish Ultimate Biodegradation of Metformin

S.P. McLaughlin, Smithers Viscient / Department of Environmental Fate; A. Griffith

The OECD 301B Guideline 'CO_2 Evolution Test' also known as the 'Modified Sturm Test' is the standard for demonstrating if a test substance is 'readily biodegradable'. If a test substance exposed to a dilute inoculum generally comprised of activated sludge achieves 60% biodegradation equivalent to 60% of its organic carbon being converted to CO_2 within a 10-day window (once reaching 10% CO_2 evolution) during the 28-day test, the test substance can be labeled 'ready biodegradable'. Based on the method in its current form, human medicines are rarely classified as ready biodegradable. A 301B study design was modified in this experiment by augmenting the activated sludge inoculum with soil microorganisms and by extending the 28-day exposure to nearly 3 months. Metformin, a small molecule pharmaceutical product was used to evaluate the performance of the modified 301B system. In previous publications, it has been demonstrated that Metformin undergoes primary biodegradation, but in this modified 301B exposure, Metformin was shown to undergo nearly complete conversion to CO_2. A preliminary test showed that Metformin was converted to approximately 30% CO_2 using two different inoculum conditions after 28 days. During the definitive test, Metformin showed no conversion to CO_2 until the test was extended beyond 28 days. The conversion of CO_2 reached 20%, 60%, 80% and 90% after approximately 45, 55, 66 and 98 days. The results demonstrate that under certain conditions, Metformin can be completely mineralized to CO_2 with a dilute inoculum.

WP159 Volatile Organic Compounds (VOCs) in groundwater from the karstic aquifer of Yucatan, Mexico

E. Norena-Barroso, G. Puch-Hau, M. Chan-Cocom, S. Capella-Vizcaino, Universidad Nacional Autonoma de Mexico / Unidad de Quimica-Sisal, Facultad de Química; J. Pacheco-Avila, A. Cabrera-Sansores, Universidad Autonoma de Yucatan / Facultad de Ingenieria

In Yucatan, groundwater is the only source of drinking water and, because of the karstic nature of the aquifer, it is susceptible of being polluted by antropogenic activities. Merida, the capital city of Yucatan, has shown a constant population growth during the last decades and pressure upon the aquifer has increased. As a part of larger study to assess the quality of the aquifer in order to preserve it, levels of volatile organic compounds (BTEX and trihalomethanes) in water were determined. Samples were taken during the rainy and dry seasons (July 2012 to March 2013) from a network of wells that provide drinking water to the Metropolitan Zone of Yucatan (conformed by the cities of Merida, Uman, Kanasin, Conkal and Ucu). VOCs were extracted by head-space SPME using a PDMS/DVB fiber and individual compounds were quantified by GC-MS. A total of 260 water samples were analyzed. Chloroform was present in most of the sites, but the other THMs were seldom detected. Regarding BTEX, toluene, ethylbenzene, m-xylene, p-xylene and o-xylene were also identified in most of the sampling sites. Highest levels of chloroform were found close to Progreso, a coastal city with an increasing population and touristic activities; on the other hand, maximum levels of BTEX were detected in Kanasin, a city that belongs to Merida's conurbation. Concentrations of VOCs found in the samples were lower than the maximum levels allowed by Mexican law (NOM-127-SSA1-1994) for drinking water.

WP160 Developing an economical analytical technique for identifying manure contamination

J. Hall, University of Wisconsin – Stevens Point; P. McGinley, University of Wisconsin – Stevens Point / Fisheries and Water Resources

Human and animal waste poses a serious threat to the quality of groundwater, surface water and sources of drinking water. This is especially of concern for drinking water in areas of Wisconsin where agricultural land spreading of livestock waste occurs on thin soils overlaying fractured bedrock. Current microbial source tracking methods for reliable source identification requires the use of expensive and time consuming microbiological testing using PCR techniques. Due to cost the use of these tests, it is often not an option for homeowners or small municipalities with limited resources. The Water and Environmental Analysis Laboratory (WEAL) seeks to develop a method to determine concentrations of fecal sterols in ground and surface waters using solid phase extraction techniques combined with atmospheric pressure chemical ionization in tandem with a triple quad mass spectrometer (APCI-LC/MS/MS). The combined techniques will allow the detection of fecal sterols in the sub part per billion levels. The presence and ratios of fecal sterols can indicate fecal contamination from point sources such as sewage treatment plants, septic leachate or large scale confined animal feeding operations. Sterols of interest include; stigmastanol, stigmasterol, sitosterol, 24-ethylcoprostanol and coprostanol. These compounds have been identified as reliable candidates to identify the source of fecal contamination. Fecal samples will be analyzed from known point sources (bovine, swine, chicken) to establish a baseline sterol profile for each species of interest. Well samples where suspected contamination is present will be analyzed for fecal sterols and human waste indicators. These results will then be compared to microbiological results from the Wisconsin State Lab of Hygiene.

WP161 Improving the effectiveness of parking lot stormwater filters at Mammoth Cave, Kentucky

A.C. West, Tennessee State University / Civil & Environmental Engineering; H. Ho, Tennessee State University / Civil Engineering; D. Solomon, Tennessee State University / Civil Engr; R. Toomey, MACA NPS; T. Byl, US Geological Survey

MammothCavewas designated an international Biosphere Reserve because of the unique organisms that live in the cave and the surface. The National Park Service installed parking lot storm filters to remove oil and metals in 2002. The filters included an oil-grit separator, followed by an organic carbon filtration system. The organic carbon cartridges were later replaced with zeolite-perlite-granular activated carbon (ZPG). The objective of this project was to determine what chemicals were coming from the parking lots and evaluate the efficacy of the filters to remove those chemicals. The first evaluation took place eight years after the filters had been installed with the carbon filter cartridges in place. Grab samples were collected at the inlet and outlet of the filter systems, and analyzed for oil and grease, sediments, turbidity, gasoline compounds, nitrate, ammonia, fecal bacteria, dissolved iron, and chemical oxygen demand. The contaminant concentrations at the outlet were similar to those at the inlet, with the exception of removing 20-70 percent of the oil and grease. The second phase of sampling took place after the filters had been serviced and the carbon cartridges were replaced with ZPG. The modified filters were effective at removing petroleum aromatic ring compounds, but were in-effective at removing zinc and copper. Regression

analysis established a correlation between decreasing filter efficiency for copper with increasing parking lot size. Also, there was a positive correlation between increasing parking lot size and increasing copper concentration in the runoff. Quaternary ammonia compounds (QACs) are a new concern because of their use in recreational vehicle sanitation tanks. The filters that received the highest QAC concentrations during storm runoff were effective at reducing 40-90% of the QAC concentrations. Replacing the filter cartridges with ZPG reduced the efficiency of the filters to absorb QAC. Lab sorption isotherm studies indicate that the filters could be improved by increasing the ratio of granular activated carbon to 33% of the ZPG volume.

WP162 Mutual Impacts of Wheat (Triticum aestivum L.) and Earthworm (Eisenia fetida) on the Bioavailability of Perfluoroalkyl substances (PFASs) in Soil

S. Zhao, Nankai University

Wheat and earthworm were exposed to perfluoroalkyl substances (PFASs) contaminated soil individually and together. Wheat took up PFASs from soil mainly through root while foliar uptake was negligible. The root concentration factors (RCFs, 0.016-1.225) and bioconcentration factors of PFASs in wheat (BCFs, 0.026-2.119) decreased while the biota-to-soil accumulation factors (BSAFs) in earthworm increased with the perfluorinated carbon chain length. The translocation factors (TF, 0.033-1.91) of PFASs in wheat peaked at perfluorohexanoic acid, and decreased significantly with increasing or decreasing the carbon chain length. The presence of wheat stimulated the bioaccumulation of PFASs in earthworms ($p< 0.05$) especially for those with short chain length, while earthworm enhanced the BCFs in wheat for perfluorinated carboxylates (PFCAs) with perfluorinated carbon chain length C£7, but depressed the BCFs for PFCAs with C>7 and perfluoroalkane sulfonates ($p< 0.05$). In general, the co-presence of wheat and earthworm enhanced the bioavailability of PFASs in soil.

WP163 Quantitative Determination of Levonorgestrel in Fish Plasma using UPLC-MS/MS

S. Jayaraman, M. Cantwell, L. Mills, D. Borsay Horwitz, USEPA

In this study, a sensitive high-performance liquid chromatography electrospray tandem mass spectrometric method was developed for the determination of levonorgestrel in fish plasma using levonorgestrel-d_6 as an internal standard (IS). In the laboratory, the fish cunner, (*Tautogolabrus adspersus*) was dosed by oral gavage with 7.5 mg/kg levonorgestrel in a methylcellulose carrier. Blood was taken from the caudal vein of three individual fish at each sampling time after gavage (15 minutes, 1, 3, 6, 18, 24, 48, 72 and 96 hours), plasma was separated, and samples were stored at -80°C until chemical analysis. To quantify levonorgestrel, 150 µL plasma was extracted by an ether extraction procedure, followed by solid phase purification. Waters Xevo tandem quadrupole mass spectrometer (TQD) with positive electrospray ionization was operated in the multiple reaction monitoring (MRM) for the analysis of levonorgestrel. Extract (10 uL) was injected on a Waters BEH C18, 50 mm x 2.1 mm, 1.7 um using water and acetonitrile as the mobile phase. The method was validated over a concentration range of 0.93 and 238 ng mL^{-1}. The transitions of precursor ions were *m/z* 313→109 for levonorgestrel and *m/z* for 319→251 for IS. The mean recovery of levonorgestrel from plasma (n =3) was 83.5% and 88.2% for IS. Matrix Effect (ME) was measured using the post-extraction spiked method and was calculated to be 70%. Results show the levonorgestrel concentration in plasma peaked at 0.8% of the gavage amount at three hours after treatment, and then gradually declined.

Nanotechnology

WP164 Biodegradability of 14C-SWNT by pure and mixed microbial cultures

A.N. Parks, USEPA; X. Liu, Duke University / Department of Chemistry; L. Ferguson, Pratt School of Engineering / Department of Civil and Environmental Engineering, Duke University

Single walled carbon nanotubes (SWNT) are highly hydrophobic nanomaterials that may enter the aquatic environment by release through wastewater treatment or manufacturing processes. Once released, SWNT may aggregate and associate with other organic matter under environmental conditions, including sediments. Because of their strong association with aquatic sediments, detailed knowledge of the ultimate fate and persistence of SWNT requires investigation of possible biotransformations (i.e., biodegradation) in environmental media. Significant biotransformation/degradation could lead to formation of more hydrophilic degradation products that are more readily transported and have greater toxicological effects. In this work, ^{14}C-SWNT were utilized in order to track the biodegradability of this carbon nanomaterial over a six month time period by pure and mixed microbial cultures. Three microbial consortia were chosen to test their SWNT biodegradation potential. The first was a pure liquid culture of *Trametes versicolor*, a white-rot fungus previously shown to degrade fullerols. The remaining two media were chosen as more environmentally-relevant media where SWNT will likely accumulate and encounter natural microbial communities; isolates from field-collected sediment from New Bedford Harbor and from an aerated wastewater treatment plant sludge. For each treatment, ^{14}C activity was assessed in the solid phase, aqueous phase, and $^{14}CO_2$ phase to determine the amount of intact SWNT, partial soluble SWNT degradation products, and mineralized SWNT generated, respectively, during the course of the experiment. Mass balance based on radiocarbon activity was determined to be approximately 100% over the course of the six month experiment. Over six months, no significant degradation of SWNT was observed in any of the three cultures tested. Approximately 99% of the ^{14}C-activity remained in the solid phase, 0.8% in the aqueous phase, and less than 0.1% was mineralized to $^{14}CO_2$, regardless of culture type. These results suggest that SWNT are not readily biodegraded by pure fungal culture or environmental microbial communities. Therefore, it is likely that, once in the environment, SWNT will be relatively persistent nanoparticulate contaminants associated with sediments.

WP165 Characterization of nanoparticles using Centrifugal Field-Flow Fractionation coupled to ICP-MS and Dynamic light scattering

S. Tadjiki, Postnova Analytics Inc.; S. Assemi, T. Tepci, University of Utah, Department of Metallurgical Engineering; E. Moldenhauer, T. Pfaffe, Postnova Analytics GmbH

To fully understand the environmental effects of engineered nanoparticles (ENPs), powerful and sensitive analytical techniques are needed to detect and characterize ENPs in complex environmental matrices at environmentally relevant concentrations (sub mg to ng per litter). Centrifugal Field-Flow Fractionation (CFFF) is a high resolution mass-based separation device used to separate and characterize natural and engineered nanoparticles dispersed in natural and biological media over the size range of 6 nm to 30 µm. The separation takes place in an open channel where the nanoparticles are subject to an external centrifugal field. The sample constituents are swept out of the channel at different speeds resulting in elution of small (or light) components first and large (or heavy) components second.In these studies, The CF2000 system (CFFF) was interfaced directly with ICP-MS system, Dynamic Light Scatrering detector and UV/Vis spectrometer to characterize a variety of Au and Ag nanoparticle (NP) standards (spheres and rods, 10-60 nm in size). The results of four different applications will be presented: 1) The CF2000-ICPMS system was used to separate a mixture of Ag and Au NPs with respect to their densities. The elemental intensities exposed a small amount of Ag content in the Au NPs. 2) The CFFF-DLS system was used to study the absorption of BSA on the Ag NPs. The absorption isotherm was obtained and the thickness of the BSA coating was measured using the CFFF theory and DLS measurement. 3) A mixture of two Au nano-rod standards (both with the length of 30 nm) was separated based on their axial dimensions (10 nm and 25 nm). A third structure (not reported by the manufacture) was also detected which eluted between the two nano-rod distributions. 4) Mixtures of Au NPs (NIST) and Ag NPs (nanoComposix) were analyzed for reproducibility and total sample recovery. The CF2000 system exhibited a RSD value of ≤ 0.1% in retention time and a recovery of ≥ 94% for both Ag and Au NP mixtures.

WP166 Chronic Toxicity of Silver Nanoparticles in Daphnia magna – The Influence of Feeding Level

A. Mackevica, Technical University of Denmark / Department of Environmental Engineering; L. Skjolding, DTU / Department of Environmental Engineering; A. Gergs, Roskilde University / Department of Environmental, Social and Spatial Change; A. Palmqvist, Roskilde University / Dept.of Environmental, Social & Spatial Change; A. Baun, Technical University of Denmark / Department of Environmental Engineering

The number of available studies on the acute effects of silver nanoparticles (AgNP) on aquatic organisms has increased dramatically in recent years, however very limited information is available on chronic effects. In this

study, a series of *Daphnia magna* 21-days reproduction test (performed as a slightly modified OECD 211) were performed using 30 nm citric acid stabilized AgNP. The aim of the study was to investigate the influence of the abundance of food on the reproductive toxicity of AgNP in *D. magna*. The exposure concentrations applied ranged from 2 to 50 µgAg/L, and the test animals were fed with green algae *Pseudokirchneriella subcapitata* of approximately 0.12 mgC/*Daphnia*/day for standard treatment or 0.36 mgC/*Daphnia*/day for high food availability treatment. Parameters of interest were survival, growth of mother animals and number of neonates produced. Data obtained from the chronic tests were analyzed by means of a Dynamic Energy Budget (DEB) model. In test medium both concentration of silver and size of AgNP were quantified by ICP-OES and DLS, respectively. The high food availability treatment increased animal survival, growth and reproduction. Detrimental effects on survival, growth and reproduction were observed in concentrations higher than 20 µgAg/L, whereas in standard treatment the threshold was above 10 µgAg/L. The DEB model was fitted using scaled internal concentration and assuming somatic and maturity maintenance to be the physiological mode of action. The resulting parameter fits indicated that with higher amounts of food available, the assimilation of food increases, but also toxicant elimination rate and no effect concentration is higher. In conclusion, this study shows that food availability highly influences the toxic effects of AgNP on growth and reproduction in *D. magna* and clearly demonstrates the importance of gaining ecological understanding by performing thoroughly designed long-term studies.

WP167 Comparison of silver uptake in a freshwater snail after waterborne exposure to ionic and nanoparticulate Ag at different water hardnesses

T.L. Stoiber, University of California-Davis / John Muir Institute of the Environment; M.N. Croteau, US Geological Survey; S.N. Luoma, University of California, Davis / John Muir Institute of the Environment

We investigated the bioavailability of ionic silver (Ag) and Ag nanoparticles coated with polyvinylpyrrolidone (PVP-AgNP) and thiolated polyethylene glycol (PEG-SH AgNP) in the freshwater snail, *Lymnaea stagnalis.* Specifically, uptake rates of Ag in snails were measured after short-term waterborne exposures to a range of Ag concentrations at water hardness that varied up to 100-fold. Water hardness did not influence the uptake of ionic silver *in L. stagnalis*, as there was no significant difference among the rate constants of Ag uptake (k_{uw})measured from experiments conducted in soft, moderately hard, and hard water (k_{uw} ~ 3 l g^{-1} d^{-1}). In contrast, water hardness did affect the uptake of nano-sized silver by *L. stagnalis*. The k_{uw}s for PVP-NPs into snail tissue decreased 1.7-fold (from 2.2 ± 0.02 to 1.3 ± 0.01 l g^{-1} d^{-1}) when experiments were conducted in deionized (D.I.) and very hard water, respectively. The k_{uw} for PEG-NP decreased 16-fold (from 3.1 ± 0.7 l g^{-1} d^{-1} to 0.2± 0.01 l g^{-1} d^{-1}) when experiments were conducted in D.I. and very hard water, respectively. Surface coating and water hardness affected the dissolution of the nano-sized silver. As estimated using centrifugal ultrafiltration, dissolution was observed to be less for PEG-NP compared to the PVP coated particles. We incorporated estimates of dissolved silver into the biotic ligand model and the biodynamic model to predict Ag delivery to the snail from Ag-NPs. The k_{uw} and silver bound to the biotic ligand (BL-Ag) are well-correlated, linking the parameters and predictive power of these two models. The AgNP coating also affected the rate constant of loss (k_{ew} d^{-1}) as the k_{ew} for PEG-NPs was statistically not significant to zero and that for PVP-NP was 0.035 ±0.01 (S.E). Uptake and elimination of Ag in biological tissues from AgNP's are unique compared to ionic silver, as water chemistry and the type of particle coating must be considered to predict bioavailability.

WP168 Determination of carbon nanotube uptake, translocation and bioaccumulation in corn (Zea mays L.) in soil using a microwave-induced heating method

A.M. Parra, Environmental Toxicology; B. Shrestha, Texas Tech University / Environmental Toxicology; F. Irin, M.J. Green, Texas Tech University / Department of Chemical Engineering; J.E. Cañas-Carrell, Texas Tech University / Department of Environmental Toxicology

There has been a substantial growth in carbon nanotube (CNT) applications and production. With uses in agriculture, environmental remediation, and wastewater, there is a likelihood of CNTs entering the terrestrial environment. Once in the environment, there is potential for plant uptake and dietary exposure to humans. This study addresses the uptake and bioaccumulation of various CNTs in corn using a microwave-induced heating technique which can quantitatively determine CNT concentrations in biological samples. All CNTs were characterized using thermogravimetric analysis and scanning electron microscopy (SEM). Corn was grown in 0, 10, 100 and 1000 mg/kg of multiwalled nanotubes (MWNTs) in soil for a period of 40 d. Uptake of MWNT concentrations in roots were < 10 µg/g in exposed plants. MWNTs were also translocated to stem and leaf portions but at lower concentrations (≤ 6 µg/g). Studies with various types of single walled nanotubes (SWNTs) are ongoing. Corn was grown in 0, 10 and 100 mg/kg of unmodified, surfactant stabilized and covalently modified SWNTs in soil for 40 d. After harvest, roots, stems and leaves were dried, grounded and analyzed using the microwave-induced heating technique to determine CNT concentrations. Results from these plant uptake, translocation, and bioaccumulation studies will provide data needed for future CNT risk assessments for ecological and human exposure.

WP169 Effects of Hydroxylated Fullerenes and Silver Nanoparticles on Early Life Stages of the fathead minnow, Pimephales promelas

C. Tseng, University of Georgia / Environmental Health Science

Fullerenes and silver nanoparticles (AgNPs) have emerged as two important classes of nanomaterials, with a wide range of industrial and commercial applications. Hydroxylated fullerenes have been developed to increase their industrial use. The extensive use of nanomaterials has raised concerns that they could contaminate aquatic systems and have effects on aquatic organisms. The objective of this study was to investigate the toxicity of hydroxylated fullerenes and AgNPs on early life stages of the fathead minnow (*Pimephales promelas*) and to assess metabolomic changes in exposed embryos. Fathead minnow embryos (≤24 h post fertilization) were collected and exposed to nanomaterials for seven days. Exposure concentrations were 1–4000 mg/L for hydroxylated fullerenes and 10–6000 mg/L for AgNPs. Mortality and teratogenic endpoints were evaluated daily by microscopy. Embryos were collected at different stages of embryogenesis (12, 48, 72, and 120 h post exposure) and metabolite levels were detected using ^{1}H-NMR and GC-MS. For embryos exposed to hydroxylated fullerenes, early hatching was observed at the highest concentration (4000 mg/L). No significant mortality or other teratogenic endpoints were observed. In embryos treated with AgNPs mortality was first observed at 750 mg/L and 100 percent mortality was attained at 3000 mg/L. The 120 h LC50 for AgNP-treated embryos was 1450 mg/L. Spinal curvature, edema, and morbidity were significantly increased at concentrations higher than 750 mg/L of AgNPs. Multivariate analysis was applied to identify metabolomic changes in exposed embryos. Changes in metabolomic profiles after nanoparticles exposure can help determine the toxic mechanisms and serve as more sensitive biomarkers of exposure to nanomaterials.

WP170 Effects of nanosilver and silver nitrate exposure on mucus production, gene expression and Na+/K+-ATPase activity in fathead minnows

A.D. Hawkins, Department of Pharmacology, Environmental Toxicology Research Program; C.E. Thornton, University of Mississippi / Department of Pharmacology and Environmental Toxicology Research Program; A. Harmon, US Army Engineer Research and Development Center / Environmental Laboratory; A.J. Kennedy, US Army Engineer Research and Development Center, CEERD-EPR; J. Steevens, US Army Engineer Research and Development Center; N. Garcia-Reyero, Mississippi State University / Institute for Genomics Biocomputing and Biotechnology; A.J. Bednar, US Army Engineer Research and Development Center / Environmental Laboratory; K. Bu, J. Cizdziel, University of Mississippi / Department of Chemistry; K.L. Willett, University of Mississippi / Environmental Toxicology Research Program

Mucus has both increased and decreased the toxicity of different xenobiotics by either concentrating contaminants on the gills and body or encapsulating toxicants to prevent exposure. Ionic regulation in fish gills can be perturbed by silver nitrate ($AgNO_3$) exposure primarily due to the inhibition of Na+/K+-ATPase. One proposed toxicity mechanism of silver nanoparticles (Ag-NPs) is thought to be derived from the release of silver ions; however, Ag-NPs can accumulate and have unique toxic effects on different biological targets. In order to understand the relationship between mucus, toxicity and silver exposure, fathead minnows (FHM) were exposed for 96 hr to nominally 20 nm PVP- or citrate-coated silver nanoparticles (PVP-AgNPs; citrate-AgNPs) or $AgNO_3$ at two nominal concentrations (20 and 200 µg/L) or (2 and 6 µg/L), respectively. After 4 hr, FHM produced significantly more dose-dependent mucus secretion in every silver treatment compared to the control fish as measured by a phenol-sulfuric acid method. The distribution

of silver was quantitated in skin, liver, brain, gill and GI tract by acid digestion and ICP-MS analysis. No detectable accumulation occurred in the brain. In all other tissues, the highest bioaccumulation was observed in the 6 µg/L $AgNO_3$ with the exception of the GI tract in the 200 µg/L citrate-AgNPs exposure. Ag-NP size was characterized in GI tract and gill tissue after exposure using field-flow fractionation interfaced to ICP-MS. AgNPs detected in GI tissue displayed heteroaggregation with particle sizes ranging from 20-70 nm, while AgNPs in gill tissue were less aggregated with particle sizes from 20-40 nm. Na+/K+-ATPase activity was measured in fish gills and GI tract in order to further understand the effects of the Ag-NPs on ionic exchange. Preliminary microarray analyses revealed that gill gene expression hierarchically grouped by control, and PVP treatments, but some of the citrate and $AgNO_3$ samples clustered together. AgNPs display many of the same toxic effects as $AgNO_3$ due to the release of silver ions, but accumulation and microarray data suggests that unique particle effects do occur. This research is supported by US Army ERDC grant: W912HZ-09-C-0033.

WP171 Effects of single walled carbon nanotubes on the expression of genes responsible for nutrient processing and uptake during fish feeding studies

J.H. Bisesi, Clemson University / Institute of Environmental Toxicology (CU-ENTOX), University of Florida / Department of Environmental and Global Health; S. Ponnavolu, University of Florida / Department of Environmental and Global Health; K. Liu, Duke University / Department of Chemistry; N.B. Saleh, University of South Carolina / Department of Civil and Environmental Engineering; L. Ferguson, Pratt School of Engineering / Department of Civil and Environmental Engineering, Duke University; T. Sabo-Attwood, University of Florida / Department of Environmental & Global Health, Center for Environmental and Human Toxicology

Single walled carbon nanotube (SWNT) use in manufacturing and biomedical applications is steadily increasing, yet little is known about the environmental fate and effects of these potential contaminants. The hydrophobic nature of SWNTs suggests that in the aquatic environment, these materials will associate with organic matter and sediments. Therefore, foodborne exposure represents a likely route for these contaminants to impact aquatic organisms. Previous research in our lab has shown that direct exposure of SWNTs to the GI tract via gavage did not cause overt toxicity. Near infrared fluorescence (NIRF) imaging was also used to track SWNT distribution and revealed that SWNTs are not likely absorbed. While these results imply that SWNT are not absorbed in a gavage scenario their highly sorptive nature may limit nutrient availability in the presence of food as they move through the digestive system. To begin to determine whether SWNTs can impact nutrient availability and uptake in fish, we cloned a suite of genes relevant to nutrient transport and processing; peptide transporters 1 and 2 (PEPT1, PEPT2), cholecystokinin (CCK) and lipoprotein lipase (LPL). In preliminary studies, the expression levels of each gene were measured by qRT-PCR in the liver as well as proximal, middle and distal section of fathead minnow intestines. Each gene revealed a specific expression profile where PEPT1 was most highly expressed in the proximal intestine whereas LPL showed the greatest expression in the liver. To determine if SWNTs can impact the expression of these genes we are currently exposing fathead minnows to a diet consisting of ground commercial fish feed and SWNTs for 7 days. Endpoints from these fish will be compared to fish fed the same diet without SWNTs. We will also have a group of starved fish that serve as a positive control for gene expression during nutritional depletion. We will measure the expression levels of CCK, LPL, PEPT1 and PEPT2 and track and quantify SWNT by NIRF. We hypothesize that while SWNTs will not be absorbed in the intestinal tract, sorption of nutrients by the SWNTs will cause changes in gene expression profiles that mirror those of starved fish, representing a nutritional deficiency during exposure. Results from these studies will reveal whether SWNT, while not overtly toxic, may illicit indirect toxicity which can have implications for extrapolating to long term dietary exposure and overall risk assessment of SWNTs.

WP172 Exposure Effects of Silver Nanocolloids to Medaka Population through Embryo Toxicity

C. Kataoka, C. Mori, S. Furui, Toyo University; Y. Nakagame, Toyo University / Department of Life Sciences; H. Tatsuta, University of the Ryukyus; S. Kashiwada, Toyo University / Department of Life Sciences

Silver nanomaterials have been become one of the major components of healthcare products. Their environmental fate and ecological risks are not well understood yet. In this study, embryonic toxicities of silver nanocolloids (SNCs) to Japanese medaka and effects on their reproduction after hatching were investigated. Triplicate 15 medaka embryos (stage 21) were exposed to 0.064 mg/L (nominal) of SNCs in ultrapure water at pH7 and 25 ºC in dark until hatching. In SNCs exposure, ischemia, tubular heart, blood clots, vascular defects, and short spinal cords were induced significantly (ANOVA, $P< 0.05$). Also, decreasing eye diameters and body lengths, heart rates, and hatchability were observed. Time to hatch was prolonged from 8.6 to 9.6 days, and lethality for 14 days was increased from 6.7% to 37.8%. After hatching, silver concentrations in larvae were measured with ICP-MS. Detected silver concentration in post-hatch larvae was 7.49 pg/mg (7.01 pg/larvae). To evaluate embryonic toxicity of SNCs on reproduction, quintuple15 medaka embryos (stage 21, F0) were exposed to 0.01 mg/L of SNCs, and then incubated at pH7 and 25 ºC in dark condition until hatching. After hatching, larvae (F0) were moved to clean condition and cultured until sexual maturity. Five pairs of adult medaka (F0) were chosen randomly; and then, were subjected to a reproduction test for three weeks. Numbers of spawned, fertilized and hatching eggs (F1) were counted. Lengths of all post-hatch larvae (F1) were measured. After the test, the five pairs of medaka (F0) were subjected to pathological diagnoses. After measurements of body weights and lengths of other F0 medaka, they were dissected and then measured weights of liver, ovary and testis. In addition, sex of F1 (total 2646 larvae) were diagnosed using sex determination gene (DMY/DMRT1) by PCR. There was no significant difference in body weights and lengths, weights of liver, ovary and testis, and furthermore no difference in pathological diagnoses in F0 generation. In F1 generation, there is no difference in numbers of fertilized eggs and body lengths of larvae. Nevertheless spawned numbers were increased, hatching numbers were significantly ($P< 0.001$) decreased. Sex ratio of F1 was reduced to 1: 0.73 (male: female) in 200 larvae. We are going to present toxic effects of SNCs on medaka population growth.

WP173 Fate and transformation of silver nanoparticles in lake mesocosms

L. Furtado, Trent University / Environmental and Life Sciences / Environmental & Resource Studies; E. Hoque, Environmental & Resource Studies; B. Cheever, Trent University / Department of Biology; D.M. Mitrano, EMPA Technology & Society Lab / Chemistry and Geochemistry; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; H. Hintelmann, Trent University / Chemistry; C.D. Metcalfe, Trent University / Environmental & Resource Studies

Silver nanoparticles (AgNPs) are an emerging contaminant that could put aquatic ecosystems at risk. Once released into natural waters, AgNPs can remain in nanoform, heter- or homo-agglomerate, or dissociate into dissolved silver (dAg). These transformations will dictate the fate and ecological impacts associated with the release of AgNPs. Thus, it is imperative to understand AgNP transformations in natural water matrices at environmentally relevant concentrations using suitable analytical methods. In this study, sequential filtration, including ultra-filtration, Cloud Point Extraction (CPE), Single-Particle-Inductively-Coupled Plasma Mass Spectroscopy (SP-ICP-MS), and Asymmetric Flow Field Flow Fractionation with on-line ICP-MS (AF4-ICP-MS) techniques were used to characterize transformations of AgNPs added to mesocosms in the Experimental Lakes Area (ELA) of Ontario, Canada. Mesocosms (2 m diameter x 2 m depth) were deployed in Lake 239 at ELA to evaluate the fate of 50 nm capped AgNPs. In two mesocosms spiked with a single dose of polyvinylpyrrolidone (PVP) capped AgNPs at a nominal concentration of 80 ug/L, total Ag levels declined relatively slowly, with a $t_{1/2}$ of ~20 days. SP-ICP-MS indicated that the mean size of AgNPs declined over time from 50 to 30 nm over 21 days, indicating either particle dissolution or precipitation of larger particles. AF4-ICP-MS results are consistent with particle dissolution as there was an increase over time of particles in the 1-10 nm size range. However, very little dAg was detected using ultrafiltration, likely due to binding of Ag^+ to inorganic ligands or accumulation by planktonic biota. CPE results were difficult to interpret, but AgNP "weathering" may have contributed to an observed decrease in extractable AgNP. These results indicate that the AgNPs were relatively stable in the mesocosms. This stability may have been due to the low ionic strength, moderately high NOM and circum-neutral pH of the lake water. The various analytical techniques will be discussed with respect to their utility as robust and sensitive methods for studying AgNP fate at environmentally relevant concentrations.

WP174 Formation of a complex organic corona on carbon nanotubes reduces toxicity by suppressing reactive oxygen species production

J.R. Lawrence, Environment Canada / Water Science and Technology Directorate; G. Swerhone, Water Science and Technology Directorate, Environment Canada; J.J. Dynes, Canadian Light Source Inc; A.P. Hitchcock, McMaster University / Department of Chemistry; D.R. Korber, University of Saskatchewan

Nanocarbon-based materials, largely composites, are increasingly being produced at the industrial scale. These include multiwalled carbon nanotubes (MWCNT) and single wall carbon nanotubes (SWCNT). However, little is known about their fate upon entering the aquatic environment. To investigate these events, CNTs were exposed to natural river water in microcosms and during microbial biofilm development in bioreactors. After exposure periods of 5 minutes, 24 hours, 3 days, 5 days and 1 week CNTs and biofilms were recovered and transferred to silicon nitride windows for observation using the SM beamline at the Canadian Light Source (Saskatoon, SK, Canada). Scanning transmission X-ray microscopy (STXM) at the C 1s edge was used to study the fate of a variety of CNTs in river water and during the development of complex natural river biofilms. It was apparent that the nanomaterials all underwent rapid aggregation, forming bundles and flocs. CNTs were also integrated into the developing biofilm and either became associated with the biology or acted as a scaffold for growth. STXM examination revealed the rapid development of an extensive coating consisting of lipids, proteins, polysaccharides and carbonates. MWCNT had 2X the protein, 1/2 the lipid, 1/10th the polysaccharide and 1.3X as much $CaCO_3$ as did the SWCNT indicating that MWCNT possessed a higher affinity for protein and $CaCO_3$ compared to SWCNT which exhibited a greater affinity for lipid and polysaccharide. The existence of these molecular coatings indicates the development of a highly-modified surface chemistry that may influence the toxicity of these nanomaterials in the environment. Reactive oxygen species (ROS) are known to be a major source of toxicity for CNTs; therefore, we compared ROS production in bacteria exposed to naked and coated/aged CNTs. Fluorescence confocal laser microscopy of the CNT's using Carboxy-H2DCFDA (495/529 nm, Image-iT™ ROS Detection Kit) demonstrated a significant reduction in ROS production in exposed bacteria by coated CNTs. This suggests that this source of toxicity will be attenuated when these materials enter aquatic environments. These observations contribute to our understanding of the fate and potential effects of these materials in natural systems, as well as confirming the need to further evaluate the impact of these coatings on environmental effects of CNTs.

WP175 From Wastewater Treatment to Exposure: Mimicking Nanoparticle Transformations to Assess Changes in Accumulation in a Simple Aquatic Food Chain

A.T. Wray, Clemson University; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX)

Engineered nanomaterials that are marketed for consumer use are often subjected to the rigors of wastewater treatment prior to release in an aquatic ecosystem. Previous studies have monitored nanomaterials during treatment, however the subsequent impact to the local biota hasn't been pursued. To fill in this gap, we examined how contact with wastewater affects the characteristics of positively charged spherical gold nanoparticles (NP) and how these transformations change accumulation patterns in *Daphnia magna* and *Pimephales promelas*. Uptake and elimination rates were empirically derived for waterborne exposures of either pristine gold NPs or NPs mixed with synthetic wastewater (SW). Nanoparticle size and surface charge did not change significantly in the presence of SWW; yet we did observe a reduction in surface charge compared to stock solutions. Influx exhibited a sigmoidal relationship to concentration indicating adsorption was not independent. Biodynamic parameters did not change significantly between pristine (ku = 133 nmol/g/d, ke = 3.06 d-1) and SW (ku = 110 nmol/g/d, ke= 2.97 d-1) treatments; rather the influence of SW manifested in the membrane-particle interactions. After pre-treatment, binding capacity (Bmax) increased from 134 to 479 nmol/g/d while binding affinity (log K) decreased from 9.13 to 8.50. *P. promelas* did not take up NPs (0.24 nmol/g/d) to the extent observed in *D. magna* though differences between pretreated and pristine particles were analogous. The subtle change in nanoparticle properties after pre-treatment is likely a result of SW components adsorbing to particle surfaces. We did not find evidence to conclude that nanoparticles were localized outside of the gut tract or gills in our two organisms; therefore we suspect that the increased stability of the particles conferred from the protein adsorption reduced internal aggregation, hence the greater binding capacity and lower binding affinity. Based on our findings, we recommend that models incorporate pre-exposure processes into characterization and behavior so as to be better equipped to predict accumulation in contaminated environments.

WP176 Functional Gene Analyses of Medaka Embryos Exposed to Silver Nanocolloids

T. Niwa, T. Ariyoshi, Toyo University; H. Nakamura, Y. Kagami, Ecogenomics, Inc.; M. Fujita, S. Kashiwada, Toyo University

Silver nanomaterials are considered to be one of the major pollutants from nano-industries. Our group has been investigated silver nanocolloids (SNCs; colloidal diameter 43.8-66.8 nm in ultra-pure water) toxicology using medaka embryos and elucidated that SNC causes severe morphological deformities of embryogenesis such as blood clots, pericardiovascular edema, tubular hearts, small eyes, shortened body, and spinal deformities at 0.5 mg/L and 1.0 mg/L. This study explores the toxic mechanisms of SNCs in gene expression levels using medaka embryo model. *In first*, we investigated SNCs effects on gene expression using DNA microarray analyses. 118 genes were up-regulated (>2.0 fold) and 117 genes were down-regulated (< 0.5 fold). Since severe morphological defects and damage of central nervous system were observed in SNC-exposed medaka embryos, *in second*, we chose five genes that were relevant to vertebrate embryogenesis and morphogenesis, and well-studied in mammalian systems for Q-RT-PCR analyses. We validated that cathepsin L (*ctsl*), tropomyosin 1 alpha (*tpm1*), and retinol-binding protein (*rbp*) were significantly down-regulated in SNC exposed embryos, while sarcoplasmic/endoplasmic reticulum calcium ATPase 1 (SERCA1, *atp2a1*) and homeobox B6b (*hoxb6b*) were both up-regulated. *In third*, RNAi-gene knockdown techniques were performed to investigate detailed toxic cascades of SNCs based on the data of microarray and Q-RT-PCR analyses. So far, medaka embryos injected with siRNA of *hoxb6b* have induced deformities in head and eyes during embryogenesis like as embryos exposed to SNCs. We are going to present other phenotypic data in embryos suppressed or over-expressed RNA.

WP177 Impacts of differing nanomaterial surface chemistry on toxicity to Daphnia magna

J. Bozich, UWM-SFS / School of Freshwater Sciences; S. Lohse, University of Illinois at Urbana-Champaign / Chemistry; M. Torelli, UW-Madison / Chemistry; C. Murphy, University of Illinois at Urbana-Champaign; B. Hamers, UW-Madison / Chemistry; R. Klaper, University of Wisconsin-Milwaukee / School of Freshwater Sciences

Nanoparticles are an emerging technology used for a variety of industrial, biomedical, and environmental applications. As nanoparticle (NP) production increases, so to should the concern about their potentially harmful effects when present in the environment, however the mechanisms that govern the toxicity of nanomaterials is still in question. The surface chemistry of nanoparticles in production varies depending on the application. Differences in surface chemistry may influence toxicity by changing the NP properties and (or) directly contributing to toxicity. Understanding which surface functionalizations and properties make NP's toxic is imperative for assessing their impact on the environment and for creating sustainable nanotechnology. In this study the impacts of five different functionalized gold nanoparticles; citrate (CIT), cetyltrimethylammonium bromide (CTAB), polyallylamine hydrochloride (PAH), mercaptopropionic Acid (MPA), and polyethylene glycol (PEG) and four different functionalized diamond nanoparticles; carboxylated (COOH), oxidized (OX), PAH and PEG on mortality, reproduction, and growth in *Daphnia magna* were compared over 48-hour acute and 21-day exposures. Acute assays show that citrate and MPA functionalized gold nanoparticles are orders of magnitude less toxic than the PAH and CTAB functionalized gold NP's. In addition, positively charged surface chemistries are more toxic than negative or neutral charged surface chemistries. Determining which ligands and NP properties have the potential to cause the most harmful effects on an organism is the first step to informing design regarding sustainable NP production.

WP178 Maternal Transfer of Quantum Dots in Ceriodaphnia dubia

H.L. Freyaldenhoven, Hallie Freyaldenhoven / Ecotoxicology Research Facility; J.L. Bouldin, Arkansas State University / Department of Biological Sciences, Environmental Sciences Graduate Program / Biological Sciences

Nanoparticles are used in various applications ranging from biomedical diagnostics, consumer products to industrial applications because of their highly specialized capabilities. The use and manufacturing of nanoparticles is

exponentially increasing with no end in sight. Current research regarding the environmental fate of nanoparticles is insufficient in determining potential outcomes. One possible fate of nanoparticles is studied using *Ceriodaphnia dubia* as a model organism and quantum dots (QDs) with a cadmium selenium core as a model. The potential maternal transfer of QDs from adult female *C. dubia* to offspring will result in cadmium body burden in neonatal cohorts possibly affecting future generations. Maternal transfer of environmental contaminants is of particular concern as it can reduce organism fitness, possibly decimating environmental populations. Cadmium from the core of the QD is used as a tracer to determine the QD concentration. The concentration of cadmium is detected utilizing a graphite furnace atomic absorption spectrometer (240Z AA, Agilent Technologies).

WP179 Metal oxide nanoparticle characterization by collision cell single particle-ICP-MS

M.D. Montano, Colorado School of Mines / Department of Chemistry and Geochemistry; J.F. Ranville, Colorado School of Mines / Chemistry and Geochemistry; B. Majestic, Univeristy of Denver / Department of Chemistry

Metal oxides are an important class of nanomaterials used in a variety of products ranging from -bactericides, solar cells and fuel additives. In addition, there are several metal oxide nanoparticles that form naturally in the environment such as ferrihydrite and alumina. As a consequence of the growing nanotechnology market, engineered nanoparticles are expected to be emerging environmental contaminants. Emissions may come from a variety of sources, at concentrations below the detection limit of many current environmental analytical techniques. Single particle ICP-MS (spICPMS) is a novel technique that has been used to characterize nanoparticles (size, composition, number concentration) at concentrations that closely resemble those expected in the environment (ng L-1). The analysis of common metal oxides (e.g., SiO¬2, Fe2O3) is hindered by ubiquitous molecular interferences that overwhelm the signal of the desired analyte. A collision or reaction cell is required to remove the interferences and accurately characterize the sample. The characterization results for both silica and hematite nanoparticles using collision cell SP-ICP-MS are presented, and demonstrate that spICPMS is able to size these nanoparticles. Sizing results were supported by electron microscopy. Initial results of applying this technique to environmental samples, such as acid mine drainage, are presented and demonstrate the utility of this technique to characterize naturally occurring colloidal material. As metal oxide nanoparticles comprise a significant portion of the nanotechnology market, the ability to detect and characterize these materials in the environment will be essential in order to develop accurate risk assessment models.

WP180 Nanomaterial screening tools: data visualization techniques for determining potential risks despite unknowns

A.J. Kennedy, US Army Engineer Research and Development Center, CEERD-EPR; S.A. Diamond, NanoSafe Inc. / MED; A. Bednar, D. Boehm, US Army Engineer Research and Development Center; M.A. Chappell, US Army Engineer Research & Development Center; F. Hill, US Army Engineer Research and Development Center; J.K. Stanley, US Army Engineer Research and Development Center / Environmental Laboratory; J.G. Coleman, US Army Engineer Reseach and Development Center / Environmental Laboratory, US Army Engineer Research and Development Center; M. Hull, NanoSafe; J.A. Steevens, US Army Engineer Research & Development Center / Environmental Laboratory

While nanotoxicology research is increasingly robust and nuanced, the data generated is complex and unknowns persist. This confounds a logical transition of currently available information to simple, easy to understand screening and data visualization techniques tailored to researchers, regulators and green technology developers. In attempt to gain traction, a web-based tool suite was developed to provide simple particle fate and toxicology estimations. Tools in the suite include a particle dissolution estimator, a DLVO calculator, a particle number density calculator, a crystalline face surface area calculator, a toxicity visualization and screening function, bioaccumulation factor estimations, an environmental modifying factor function, and a novel dose metric converter. Each tool is connected to a database populated with empirical data from the benchtop or literature and allows user-defined flexibility. As the database continues to expand, the fidelity of each tool is enhanced. The particle stability tools (DLVO, dissolution, coating stability, crystalline dependent surface area) offer basic information regarding particle fate in media of user-defined characteristics and are important for determining likely exposure scenarios. The toxicity screening and bioaccumulation tools allow the user flexibility to filter toxicity reference values (e.g., survival, LC50, BCF/BAF) by various nanoparticles characteristics (e.g., particle size, capping agent) and test organism type (e.g., fish, cladoceran, alga) or visualize all data without discrimination (when few points are available). Tools include data modeling and QA-QC, including estimation of material- and size-specific low and high probable effects and bioaccumulation factors. Users can enter and visualize their own data using novel dose metrics, including particle number density, total surface area and the dissolved fraction (for metals or metal impurities). Finally, a tool to specifically consider relevant environmental modifying factors (e.g., organic carbon, UV light) is available to convey that toxicology data generated in pristine laboratory conditions may not represent the risk of potential releases into complex environmental media. This presentation will provide a demonstration and seeks general discussion on function and additional data for inclusion into the database.

WP181 Phototoxicity of TiO2 nanoparticles to a freshwater benthic amphipod: are benthic systems at risk?

S. Li, EPA / Environmental Toxicology; L.K. Wallis, EPA; H. Ma, Mid-Continent Ecology Division, USEPA / Mid-Continent Ecology Division; S.A. Diamond, NanoSafe Inc. / MED

With strong ultraviolet (UV) radiation absorption and photoactive properties, manufactured nano-scale titanium dioxide (nano-TiO_2) has been increasingly used in manifold applications. Due to rapid commercialization, there is an increased risk of nano-TiO_2 being released into the environment, with an estimated concentration of 0.6 mg/kg in sediment in 2012. Unfortunately, the exceptional photoreactivity of nano-TiO_2 that has led to numerous applications is also a cause for potential toxicity to organisms and the environment. This study echoes the need to investigate potential phototoxicity of TiO_2 that arises from its strong photoactivity. Specifically, this study investigated phototoxicity of nano-TiO_2 to a freshwater benthic amphipod (*Hyalella azteca*) using 48-h and 96-h bioassays. Thorough monitoring of particle interactions with exposure media (Lake Superior water, LSW) and the surface of organisms were performed using dynamic light scattering, UV/VIS spectrometry and Scanning Electron Microscope. Agglomerate size was concentration-dependent with lower nano-TiO2 concentrations showing greater agglomerate sizes. For example, 10 mg/L nano-TiO2 had an agglomerate size of 905 ± 24 nm while 750 mg/L nano-TiO2 had an agglomerate size of 158 ± 18 nm. Large agglomeration and sedimentation (>77%) in LSW was observed after 0.5 h. Specifically, after 0.5 h exposure, sedimentations of nano-TiO2 with initial masses of 0.3, 1.5, 3, 15, 30 mg in the aqueous phase were 77%, 78%, 83%, 98% and 99%, respectively. A UV-A -favored surface attachment of nanoparticles was observed, indicating an enhanced phototoxicity with increased surface attachment. A 96-h median lethal concentration (LC50) of 29.9 mg/L for *H.azteca* was calculated, with a daily 4 h UV-A exposure of 2.2 W/m^2. Phototoxicity of nano-TiO_2 under SSR had a 21 fold increase as compared to that under ambient laboratory light. Also, this phototoxicity followed the Bunsen-Roscoe photochemical law of reciprocity, with calculated LC50s around 22.9 (95% CI, 20.5 – 23.3) Wh/m^2 when exposed to 20 mg/L nano-TiO_2. Increased hiding behavior and decreased mortality were observed when aluminium foil shelters were available. This phototoxicity and its complex interactions with various environmental factors guarantee further investigations for future risk assessment of photoactive nanomaterials to benthic organisms.

WP182 Potential artifacts in nanoecotoxicology testing

E.J. Petersen, National Institute of Standards & Technology

Engineered nanoparticles are a novel technology that are expected to be incorporated into increasing numbers of consumer products given the unique properties observed with particles that possess nano-scale (1 nm to 100 nm) dimensions. However, one potential barrier to the widespread commercialization of products containing nanoparticles is uncertainty regarding the potential toxic effects that these particles could pose. Assessing such risks is hindered by uncertainty regarding the adequacy of current standard methods for assessing the ecotoxicological risks of these particles given substantial differences in behaviors between nanoparticles and traditional environmental pollutants. Public sensitivity to the potential effects of nanoparticles and the risk that miscommunication of these risks could bias the public against nanomaterials make it critical that robust nanoecotoxicology tests are developed and their results are reported accurately. One of the most important factors that can influence the accuracy of nanoecotoxicology measurements is the potential for nanoparticles to cause artifacts during testing. Artifacts

due to nanoparticles have been identified during all stages of the test setup including preparation of the nanoparticle dispersion and quantification of changes in biomarkers after nanoparticle exposure. However, these artifacts have not yet been systematically reviewed. Some of the artifacts that will be discussed during this presentation include interferences with reagents during toxicity assays, toxic byproducts produced during nanoparticle synthesis or dispersion techniques such as sonication, misinterpretation of nanoparticle distributions in tissues during electron microscopy analysis, lack of a mechanism for nanoparticle transit to organs where toxic effects are reported, and endotoxin contamination in nanoparticles tested.

WP183 Silver nanoparticle toxicity in Gulf killifish (Fundulus grandis): embryotoxicity across a salinity gradient

E. Bigorgne, Baylor University / Department Environmental Science; C.W. Matson, Baylor University / Environmental Science

To study the influence of salinity on silver nanoparticle (Ag NP) toxicity, Gulf killifish (*Fundulus grandis*) embryos were exposed for 48 hours to citrate-capped Ag NP (25 mg/L) at various salinities. After 24 hours of exposure, a V-shaped Ag NP toxicity pattern was observed with a high mortality (91.7%) in 0 ‰ artificial seawater (ASW) followed by a strong decrease in mortality at 0.1 and 0.5 ‰ ASW with 34.7 and 2.1%, respectively. At higher salinities (10 – 20 ‰ ASW), toxicity reaches between 60-80% mortality. After 48 hours, toxicity increases strongly only at 0.1‰ (EPA water) with almost 99% mortality. Cysteine, which is a natural ligand, is known to chelate Ag^+ and decrease silver toxicity. Counter to the pattern observed for $AgNO_3$, results with cysteine (4 µM) revealed no decrease in Ag NP toxicity at low salinities (0 and 0.1 ‰ ASW) after 24 or 48 hours. A significant decrease in toxicity with cysteine treatment was observed at 5, 10 and 20 ‰ ASW. Nanoparticle dissolution combined with subsequent silver speciation seems to account for 24 h toxicity, but not additional toxicity at 48 h in low salinities. A lack of cysteine rescue at low salinities and the differences between 24 and 48 h toxicity support the hypothesis that citrate-capped Ag NP demonstrate at least partially nano-specific toxicity.

WP185 Single Particle ICP-MS (SP-ICP-MS) a New Analytical Technique for Counting and Sizing ENP

C. Stephan

Engineered Nanomaterials (ENMs) are synthesized by a manufacturing process that produces and controls ENMs to have at least one dimension in the range of 1 to 100nm in size. ENMs often possess different properties than bulk materials of the same composition, making them of great interest to a broad spectrum of industrial and commercial applications. The widespread use and application of ENMs will inevitably lead to their release into the environment, which raises concern about their potential adverse effects on the ecosystems and, subsequently, human health. To better understand ENMs in the environment, the following ENM characteristics will aid in this assessment: concentration, composition, particle size, shape, and other surface characteristics. This work describes the theory and application of Single Particle- ICP-MS in analyzing Metal Based Nanoparticles. Single Particle (SP-ICP-MS) allow the differentiation between ionic and particulate signals, quantitate both the ionic and particulate fraction, measures particle concentration (part/mL), particle sizes (if shape is known), and explores agglomeration and size distribution. SP-ICP-MS is a key analytical instrument in assessing the fate, behavior and distribution of (ENMs) in several types of matrices (environment, food, etc.), evaluating ENMs bioavailability and bioaccumulation in the biota, and improving bio-labeling capabilities and advancements in the medical field.

WP186 Sorption of hydrophobic organic contaminants to single-walled carbon nanotubes: Influence of nanotube electronic structure

K. Liu, Duke University / Department of Chemistry; A.N. Parks, USEPA; N.B. Saleh, University of South Carolina / Department of Civil and Environmental Engineering; L. Ferguson, Pratt School of Engineering / Department of Civil and Environmental Engineering, Duke University

Single walled carbon nanotubes (SWNT) are unique, anthropogenic allotropes of nanoparticulate black carbon that have been applied to a range of technological applications. Because of their accelerating use, potential to enter the environment, and highly hydrophobic surface properties, concerns have emerged over the potential for SWNT to act as vectors or reservoirs for sorbed hydrophobic organic contaminants (HOC) such as PCBs and PAHs. One unique property of SWNT is the quantized nature of their electronic structure, which is dependent on the chiral wrapping angle of the sp^2 hybridized graphene sheet that comprises the wall of each SWNT species. Electronic structure of SWNT has a profound effect on the conductivity and optical spectra of these materials; however, to date no systematic investigations have been conducted to determine how SWNT chirality may affect surface-adsorption of HOCs. We have conducted experiments using probe HOCs – one planar PAH (^{14}C-naphthalene) and one halogenated aromatic (^{14}C-hexachlorobenzene) and purified conductive and semiconductive SWNT species aimed at assessing the role of SWNT chirality on HOC sorption. These experiments were conducted using a novel common-headspace partitioning method, which avoids artifacts associated with separating colloidal sorbents from sorbate probes. Results indicate that sorption isotherms are highly nonlinear for all SWNT species and that affinity of planar aromatic hydrocarbon probes (i.e., naphthalene) is higher for SWNT than for other forms of non-engineered black carbon (e.g., diesel soot). Our results will be discussed in context of how electronic structure of SWNT may control association of HOCs with nanotubes and the implications of this behavior for transport, fate, and exposure of HOCs in the aquatic environment.

WP187 Species-specific phototoxicity of TiO2 nanoparticles to three benthic invertebrates

S. Li, EPA / Environmental Toxicology; L.K. Wallis, EPA; H. Ma, Mid-Continent Ecology Division, USEPA; D.J. Hoff, Ecotoxicology Analysis Research / Mid-Continent Ecology Division; S.A. Diamond, NanoSafe Inc. / MED

The majority of ecotoxicological studies of nanomaterials have been done on pelagic species, which are the first concern in the risk assessment strategy for conventional contaminants. However, limited studies have addressed the impact of nanomaterials on benthic organisms, whose habitat is considered the ultimate repository for nanomaterials. The tendency of nanomaterials to aggregate and precipitate leads to the need to include a benthic test within the first tier of the ecotoxicity strategy for nanomaterials. Here, we investigate phototoxicity of nano-TiO_2 to three benthic organisms, *Hyalella azteca, Lumbriculus variegatus and Chironomus tentans*, aiming to fill the data gap in the nanotoxicological profile of benthic organisms. These benthic organisms were recommended by the US Environmental Protection Agency (EPA) for measuring the toxicity of sediment associated contaminants. They were selected for this study to reflect the impact of differences in physiology and life history on phototoxicity resulting from the differential exposure to both nano-TiO_2 and UV-A radiation as well as to confirm if they are proper organisms for addressing risk of nanomaterials associated with sediments. Different sample preparation techniques were also employed to explore if those techniques would lead to differences in the ultimate phototoxicity. Third, to investigate the phototaxis of different benthic organisms, matrices with different layers were applied. A series of 48-h acute assays was conducted in a constructed test chamber for phototoxicity testing of nano-TiO_2 to benthic organisms. The preliminary study discovered that: 1) nano-TiO_2 caused acute phototoxicity to benthic organisms under low UV exposure, 2) species-specific phototoxic responses existed, 3) sample preparation technique had minimal impacts on toxicity of nano-TiO_2, 4) phototaxis existed for benthic organisms dwelling in a nano-TiO_2 contaminated matrix.

WP188 The Dissolution Kinetics of Copper Oxide and Zinc Oxide Nanoparticles in Aqueous Solution: The Role of Aqueous Chemistry

P. Mwaanga, Copperbelt University / Environmental Engineering; E.R. Carraway, Clemson University / Environmental Engineering & Earth Sciences, Clemson Institute of Environmental Toxicology; M.A. Schlautman, Clemson University / Environmental Engineering and Earth Sciences

Many toxicological studies done on metal oxide nanoparticles are conducted without factoring in the impact of dissolution. In particular, both copper oxide (CuO) and zinc oxide (ZnO) nanoparticles (NPs) are toxic; however, it is not clear whether this toxicity can be wholly attributable to NPs or to the release of metal ions or to some combination of both. The aim of this study was to investigate the relative dissolution (rates) of both CuO and ZnO NPs in some commonly used media (milli-Q water, FETAX culture solution, solutions of varying dissolved NOM content and moderately hard water) for fate or toxicity studies. Moderately hard water was used for complementary short term (48 h) dissolution studies at NPs concentration range usually used for acute toxicity tests. The dissolved ions were separated from NPs by both 15 nm and 50 nm pore sized polycarbonate membrane filters and were then determined by AAS, ICP-MS or ICP-OES (there was no significant

difference between the results of the two sized filter membranes), depending on the concentration levels. In each studied medium, the dissolution of each metal oxide NPs displayed two dissolution rates, the initial higher rate over short period and a second lower rate over long period. The overall dissolution of both CuO and ZnO NPs were observed to be lowest in FETAX culture solution (ranging from 0.015 mg/L to 0.05 mg/L and from 0.85 mg/L to 1.2 mg/L for dissolved Cu and Zn respectively). The solution with the highest dissolved NOM content was observed to have the highest dissolved metal oxide NPs (ranging from 8 mg/L to 12 mg/L and from 9 mg /L to 28 mg/L for dissolved Cu and Zn respectively). The short term dissolution studies showed that the proportion of dissolved NPs is higher (25 % and 80 % for dissolved Cu and Zn respectively) at low particle concentration and lower (11 % and 40 % for dissolved Cu and Zn respectively) at higher particle concentration. Interestingly, the equilibrium concentrations for the dissolved ions of both CuO and ZnO NPs were not reached in all the tested media, even after 144 h of dissolution. The equilibrium concentrations were therefore predicted by both Visual minteq software and the empirical double exponent model. The agreement between the two models was exceptionally good. Overall, these results suggest that solution chemistry exerts a strong influence on the metal oxide NPs dissolution and that the release of metal ions can influence toxicity.

WP189 The Role of Surface Chemistry in the Toxicity of CeO2 Nanoparticles to Caenorhabditis elegans

E.K. Oostveen, University of Kentucky / Department of Plant and Soil Sciences; B. Collin, University of Kentucky / Plant and Soil Sciences; O.V. Tsyusko, University of Kentucky / Department of Plant & Soil Sciences; J.M. Unrine, University of Kentucky

Manufactured ceria (CeO2) nanoparticles (NP) are valued for their ability to transition easily between +3 and +4 oxidation states, rendering them a useful catalyst in various applications. To promote stability and decrease aggregation, surface coatings are usually applied to NPs. The charge of surface coatings can potentially affect bioaccumulation of CeO2 NPs and toxicity to soil dwelling organisms. We synthesized 4 nm CeO2 coated with a neutral 10 kDa dextran (DEX) (ζ potential =-0.731 mV in DI H2O, pH 6.88), positively charged diethylaminoethyl-dextran (DEAE) (+26.9 mV in DI H2O, pH 7.41) or negatively charged carboxymethyl-dextran (CM) (-29.3 mV in DI H2O, pH 6.19). The particles were characterized by transmission electron microscopy, dynamic light scattering, and phase analysis light scattering (to determine ζ potential). We exposed the model soil organism Caenorhabditis elegans to DEX-, DEAE-, and CM- coated CeO2 in reconstituted moderately hard water for 48 hours. DEAE-CeO2 was more lethal than both DEX- and CM-CeO2, likely due to positively charged particles interacting to a greater extent with cell membranes. We observed significant increases in mortality at DEAE-CeO2 concentrations as low as 50 mg/L while no significant mortality was observed for the Dex-CeO2 treatment at concentrations of up to 500 mg/L. We observed no significant mortality for CM-CeO2 particles up to 750 mg/L. To observe sublethal effects, reproduction rates were examined. We observed the most dramatic decrease with DEAE-CeO2 at doses as low as 2.5 mg/L. DEX- and CM-CeO2 also induced a lower reproduction rate, but required doses as high as 500 mg/L and 750 mg/L respectively. We concluded that positively charged surface coatings dramatically increase the toxicity of CeO2 NPs, while negatively charged or neutral coatings decrease particle toxicity. We are also using proteomics to determine how the molecular mechanisms of toxicity differ between three different coatings.

WP190 Toxicities of Silver Nanocolloids to Post-hatch Embryos of Japanese Medaka and Population Growth

A. Tadashi, T. Niwa, C. Kataoka, Toyo University; H. Tatsuta, University of the Ryukyus; S. Kashiwada, Toyo University / Department of Life Sciences

Post-hatch embryo stage and following early-life stage of fish are more susceptible to xenobiotics than egg embryos. It has been considered that xenobiotics exposure in early-life stage may effect on population growth of fish. In this study, we investigate biological effects of silver nanocolloids (SNCs, colloidal diameter 43.8-66.8 nm in ultra-pure water) on growth of post-hatch medaka and on population growth rate. To elucidate toxicity of SNCs, post-hatch embryo medaka was exposed to SNCs in ERM (pH7) for 7 days. Biological effects are observed at 5.0 mg/L and 10 mg/L of SNCs in day 7. In all exposures, phenotypic deformity, small body size, decrease of heart beating, and increase of lethality were observed significantly ($P<$ 0.05). To evaluate oxidative stress, we measured of CAT (catalase), MDA (malondialdehyde), GSH (glutathione) levels, and SOD (superoxide dismutase) activity in post-hatch embryos exposed to 5 mg/L SNCs at 25 °C in the dark for 6 days. Although there is no difference in CAT and MDA levels, all GSH levels was significantly ($P<$ 0.05) reduced, and SOD activity was induced by SNCs exposure. ATP activity was also measured and was significantly ($P<$ 0.05) decreased to 7.1 times lower than that of control in day 6. Due to biological effects and ATP reduction, apoptosis and relative enzyme caspase-3 were investigated. From confocal images, cell numbers stained with PI was obviously decreased in post-hatch embryos exposed to 5 mg/L SNCs for 6 days. Lower caspase-3 activities were measured ($P<$ 0.05) in SNCs exposure. To study SNCs effects on medaka population, The SNCs exposed post-hatch embryos were moved to clean condition and cultured until sexual maturity. Five mating pairs were randomly selected. Numbers of produced and fertilized eggs, hatchability, and sex ratio of produced eggs were measured. To evaluate the impact of SNCs exposures on medaka populations, we estimated *r*, a summary index that represents the ability of each population to proliferate. The index *r* was estimated by fitting the life table data for each exposure treatment to the Euler-Lotka equation. We will discuss on the impact of SNCs exposures on medaka populations.

WP191 Toxicity of platinum nanoparticles to freshwater algae and crustaceans

C. Delgado Giron, Technical University of Denmark / Dept of Environmental Engineering; S.N. Sorensen, DTU Environment / Environmental Engineering; C. Engelbrekt, Technical University of Denmark / Dept of Chemical Engineering; A. Baun, Technical University of Denmark; / Dept of Environmental Engineering

Although the aquatic fate and toxicity of various nanoparticle types has been studied intensively in recent years, there are however only very few studies investigating the effects of platinum nanoparticles (PtNPs) to aquatic organisms. From an environmental exposure perspective, PtNPs are highly relevant due to the extensive use of platinum in automobile catalytic converters and the recent findings of platinum (Pt) particles alongside roadways. The possible emissions of PtNPs to natural waters, e.g. by transport with storm-water runoff, raises the question of the potential environmental effects associated herewith. The aim of this study was to investigate the toxicity of PtNPs and platinum ions in freshwater algae and crustaceans as well as their uptake and depuration behavior in daphnia. Toxicity towards Pseudokirchneriella subcapitata was tested in both a standard algal growth inhibition test (ISO 8692:2004) for 48h and a newly developed short-term (2h) algal test, using ^{14}C-incorporation during photosynthesis as toxic endpoint. As the 2h method relies on scintillation counting to measure biomass, this may resolve some of the issues with shading, that are found to disturb the spectrophotometric chlorophyll analysis in the standard test. Toxicity to Daphnia magna was tested in a standard immobilization test according to OECD guideline 202, 2004. Uptake and depuration studies were carried out by exposing d. magna to PtNPs for 24h, followed by transfer to clean media allowing for 24h depuration. Characterization of PtNPs included ICP-OES, DLS and TEM. Ionic platinum was found to be more toxic than PtNPs in algal tests with EC_{50} values of 2.25 and 35 mg/l, respectively, suggesting that ionic release from the PtNPs may be involved in the toxicity of PtNPs. The poster will focus on the difference in toxicity between PtNPs and the ionic Pt as well as comparing the bioaccumulation of PtNPs and AuNPs in daphnia.

WP192 Understanding Uptake and Toxicity of Au Nanoparticles Using Toxicogenomic Approach and Synchrotron X-ray Based Imaging Technique

O.V. Tsyusko, University of Kentucky / Department of Plant & Soil Sciences; J.M. Unrine, University of Kentucky; B. Collin, University of Kentucky / Plant and Soil Sciences

Au nanoparticles (Au-NPs) with citrate coating were used as a model for studying particle specific effects of manufactured nanomaterials by examining toxicogenomic responses in a model organism, *Caenorhabditis elegans*. Seven hundred ninety seven significantly differentially expressed genes identified from whole genome microarray analyses were associated with seven biological pathways. One of the identified pathways was clathrin-dependent endocytosis. Endocytosis mutants for receptor mediated endocytosis (*rme-1, 2, 4, 6 and 8*) genes were compared to the wild type animals for uptake of Au-NPs of two sizes, 4nm and 40 nm. The distribution of Au in the Au-NP exposed nematodes was examined using synchrotron-based X-ray microfluorescence spectroscopy. Mutant *C. elegans* for the two *rme* genes (*rme-4* and *rme-6*)

demonstrated decreased in uptake of 4nm Au-NPs which also correlated with decreases in toxicity when compared to the wild type nematodes. The other three *rme* mutant strains (*rme-1, rme-2* and *rme-8*) did not differ from the wild type in the uptake of Au-NPs. However, *rme-2* and *rme-8* mutants exposed to 4nm Au-NP showed much lower or no mortality than the wild type animals. Also, the distribution of Au differed between wild type nematodes and *rme-2* mutants. The toxicity and uptake results were different for 40 nm of Au-NP when compared to 4nm Au-NPs. While 40nm Au-NP did not cause mortality in wild type and any *rme* mutants analyzed, there was significant increase in the uptake of Au-NPs by *rme-2*, *rme-4* and *rme-6* mutants versus the wild type. These results provide evidence for the involvement of the genes from the receptor mediated endocytosis pathway in Au-NP uptake and their subsequent toxicity. In addition, our results demonstrate size-dependent differences of Au-NPs in their uptake and toxicity, where the 40nm Au-NPs are being taken up but not causing any mortality.

WP193 Environmental fate, transport, and bioavailability of CeO2 nanoparticles in stream mesocosms

L.F. Baker, Baylor University / Department of Environmental Sciences; R.S. King, Baylor University / Biology; J.M. Unrine, University of Kentucky; C.W. Matson, Baylor University / Environmental Science

Emissions from wastewater treatment facilities ("press"-type exposures) and accidental spills ("pulse"-type exposures) are two possible entry routes of nanomaterials into aquatic environments. Significant uncertainty exists regarding the processes governing transport and environmental fate of these novel materials. Outdoor, 1870L recirculating stream mesocosms were treated with either 1) a one-time addition (pulse) of acetate-coated cerium dioxide nanoparticles (CeO2 NPs) to achieve aqueous concentration of 10 mg/L or 2) a 25-day continuous exposure (press) of the same amount of CeO2 NPs as 1), with a third stream serving as a control. Mesocosms were lined with unglazed ceramic tiles and stocked with fish, invertebrate, plant and microbial species. CeO2 NPs are known to be quite stable, elemental cerium concentrations were measured by ICP-MS. Results suggest rapid precipitation of CeO2 NPs in the pulsed dose; aqueous concentrations between 0.24-0.37 mg/L, 12-76 hours after NP addition, declining to 0.02 mg/L by cessation of the experiment on day 30. Aqueous Ce concentrations in the press-dosed stream averaged 1.2 mg/L on day 15 (12% of target) and 1.1 mg/L (11% of target dose) on day 25. Five days after completion of NP dosing in press mesocosms (day 30 of experiment), aqueous concentrations of Ce had declined to an average of 0.46 mg/L, which was still higher than any Ce concentration measured in the pulse-dosed stream. Consequently, the concentration of Ce in periphyton was lower in the press-dosed stream on day 30 (average of 9.08 µg/g of dry sample) than in the pulse-dosed stream (average of 13.11 mg/g). The press dose resulted in longer-range transport of CeO2 NPs, such that concentrations of Ce in the lower reaches of the press-dosed stream were nearly double those observed for the pulse-dosed stream (6.4 µg/g vs. 3.3 µg/g, respectively), likely because of a reduced particle-particle interaction and subsequent aggregation relative to the pulse addition. These results suggest that exposure scenarios may play a significant role in determining the environmental fate, transport, and bioavailability of stable metal oxide nanomaterials.

Agriculture & Pesticides

WP194 Acute toxicity of salominds after concurrent exposure to hypersalinity and chlorpyrifos

L.A. Maryoung, Environmental Science, University of California Riverside / Department of Environmental Sciences; R. Lavado, D. Schlenk, University of California-Riverside / Department of Environmental Sciences

Previous studies have shown that acclimation to hypersaline conditions can enhance the acute toxicity of certain thioether organophosphate pesticides to different euryhaline fish species. The present study focuses on acute toxicity of the organophosphate chlorpyrifos after hypersaline acclimation. Rainbow trout were exposed concurrently to two salinity treatments (< 0.5 ppth and 16ppth) and five chlorpyrifos treatments (20, 40, 80, 100, and 150 µg/µl). The median lethal time was significantly different between the salinity treatments at the two highest chlorpyrifos concentrations, with time to death by chlorpyrifos being more rapid in freshwater than hypersalinity. Different factors which may influence toxicity have been explored and include acetylcholinesterase inhibition, metabolism, bioavailability, and Na^+ K^+ ATPase activity. Differences in inhibition of acetylcholinesterase by the oxon metabolite between freshwater and hypersailine acclimated fish were not found. The IC_{50} was 84.5 + 26.3 and 72.01 + 19.2 nM for freshwater and hypersaline acclimated fish respectively. Hypersaline conditions enhanced the expression of cytochrome P450 3A in previous studies. To determine whether CYP3A may detoxify chlorpyrifos through cleavage of the phosphoester bond, rather than desulfuration to oxon metabolites, the ratio of the detoxified metabolite to the activated metabolite was measured in freshwater (1.47 + .40) and hypersaline (1.43 + .68) acclimated fish. Regression analysis with CYP3A expression and the cleavage detoxified product failed to show a significant relationship. Bioavailability was assessed through time course precipitation studies, however no difference was found between freshwater and hypersalinity. Previous studies have shown that acclimation to hypersalinity can upregulate overall Na^+ K^+ ATPase activity in gill. However, effects in the central nervous system have not been well characterized. After acclimation to saltwater, no significant differences were observed in Na^+ K^+ ATPase transcript levels in either brain or gill. When fish were exposed to the Na^+ K^+ ATPase inhibitor ouabain significant differences in toxicity to chlorpyrifos were not observed. Thus, other mechanisms need to be explored to characterize the mechanisms for the protective effects of saltwater acclimation on the acute toxicity of chlorpyrifos. Potential mechanisms to be studied include impacts on signal transduction pathways (T32 ES018827 and NIEHS P30ES07033).

WP195 Acute toxicity of ten chemicals to fairy shrimp relative to other crustaceans and mollusks

J.M. Besser, USGS-Biological Resources Division / Columbia Environmental Research Center; C.D. Ivey, USGS / Columbia Environmental Research Center / Toxicology; N. Wang, USGS / Columbia Environmental Research Center; C.G. Ingersoll, USGS / Columbia Environmental Research Center; C. Rogers, Kansas Biological Survey; S. Raimondo, B. Shephard, USEPA

Four species of fairy shrimp in the genus *Branchinecta* (Crustacea: Branchiopoda) are protected under the federal Endangered Species Act due to loss of seasonal wetland habitats in Oregon and California, making protection of the remaining habitat of these species imperative. However, because there is limited published toxicity data available for fairy shrimp (and almost no data for *Branchinecta* spp.), it is unknown whether current national ambient water quality criteria (WQC) adequately protect listed fairy shrimp. To address this data gap, we conducted a series of acute toxicity tests in diluted well water (100 mg/L hardness) with 10 chemicals of potential concern in fairy shrimp habitats and with varying modes of toxic action, including ammonia, herbicides, hexavalent chromium, cationic metals, and salts. Newly-hatched fairy shrimp from eggs purchased commercially (*Thamnocephalus platyurus*) or cultured in the laboratory from field-collected sediments (*Branchinecta* spp.) were used in 24-hr toxicity tests. Fairy shrimp were tested concurrently with other crustaceans (branchiopods, *Daphnia magna* and *Ceriodaphnia dubia*, and amphipod, *Hyalella azteca*; 48-hr tests) and freshwater mollusks (snails, *Physa gyrina* and *Lymnaea stagnalis*, and mussel, *Lampsilis siliquoidea*; 96-hr tests). Based on overall results of the first round of tests (with nickel, copper, zinc, sodium chloride, and potassium chloride), EC50s for fairy shrimp had a lower average rank than EC50s for other crustaceans or mollusks, indicating that fairy shrimp had a relatively high sensitivity to the chemicals tested. Fairy shrimp were the most sensitive group in tests with zinc, and EC50s for both *T. platyurus* and *B. lindahli* were slightly less than the hardness-based WQC. However, neither fairy shrimp was the most sensitive single species to any of the chemicals tested. *Ceriodaphnia dubia* was the most sensitive species to zinc, nickel, and sodium chloride, and the mussel, *L. siliquoiodea*, was the species most sensitive to copper and potassium chloride. Tests are planned with additional chemicals and with an additional species of fairy shrimp, the federally-threatened vernal pool fairy shrimp (*B. lynchi*). Results of all tests will be added to USEPA's Interspecies Correlation and Extrapolation (ICE) model database to characterize the overall sensitivity of fairy shrimp to toxicants relative to widely-tested surrogate species.

WP196 Comparing two years of Water Quality Monitoring in the Cache River Watershed, Arkansas

C.A. Rosado-Berrios, Arkansas State University / Environmental Sciences Graduate Program; J.L. Bouldin, Arkansas State University / Department of Biological Sciences, Environmental Sciences Graduate Program / Biological Sciences

The Bayou De View – Cache River Watershed is part of the White River Basin in Eastern Arkansas. The uppermost section of the watershed is located in the southeastern part of Missouri and flows through the northeast part of Arkansas through the Delta Ecoregion. The Cache River lower section is

used for fish and wildlife propagation, water source for primary and secondary recreation as well as for domestic, agricultural and industrial supply. This watershed is included in the Mississippi River Basin Initiative (MRBI) to reduce sediment and nutrient loading to the Gulf of Mexico and is cited on the ADEQ's 303d list due to the presence of toxic metals, elevated chlorides levels and total dissolved solids. The watershed is monitored for a three-year period to evaluate the basin water quality before, during, and after the initiation of conservation practices. Seven sampling sites are located in the lower subwatersheds to monitor pH, turbidity, dissolved oxygen (DO), conductivity, temperature, total suspend solids (TSS), and nutrient concentrations. This work presents the data obtained during the first two years of the monitoring project. Among all sites observed results included mean detected levels from below detection limits (BDL) to 0.458mg/L, 0.026mg/L and 0.119mg/L for dissolved nitrate, nitrite and phosphate, respectively. The monthly average for TSS ranged from 2.52mg/L to 107.60mg/L, monthly average turbidity values as high as 243.90NTU and the lowest monthly average DO level was 1.08mg/L. Long-term water quality monitoring in this watershed will provide evidence of conservation practices within the watershed.

WP197 Comparing water quality in channelized and natural streams: An Experiential Learning Fellowship (ELF) project

N. Poe, Arkansas State University; M.K. Kilmer, Arkansas State University Environmental Sciences Graduate Program; R. Word-Burkman, M. Bell, Arkansas State University; J.L. Bouldin, Arkansas State University / Department of Biological Sciences, Environmental Sciences Graduate Program / Biological Sciences; T. Marsico, Arkansas State University / Department of Biology

The continued growth of the hypoxic zone in the Gulf of Mexico has resulted in an increased awareness of potential sources of contamination in the Mississippi River Basin, particularly at the watershed level. One watershed of focus is the Cache River, located in agriculturally dominated northeastern Arkansas. This river has been consistently listed as a 303(d) impaired river by the state of Arkansas for both total dissolved solids (TDS) and lead (Pb) contamination, with the impairment suspected to be due to agriculture. In this study, we compare channelized and natural streams in a headwater sub-watershed of the Cache River, comprised of 136 km^2 used primarily as cropland (70%) with secondary uses in forest (20%) and pasture (3.5%). Channelized sites were surrounded by cropland, as channelization increases drainage from the production fields. Natural sites were located in areas considered less suitable for row-cropping and were thus surrounded by a mix of pasture, cropland and forest. Physical and chemical characteristics were measured at each site, including flow rate, canopy cover, water temperature, dissolved nutrients (nitrites, nitrates, orthophosphates), turbidity and total suspended solids (TSS). Acute toxicity tests (48-h) with *Ceriodaphnia dubia* and *Pimephales promelas* were performed with water collected from each site, using synthetic, moderately-hard water as a control. Channelized sites had higher values for flow rate, turbidity and TSS, and lower values for canopy cover than natural sites. Acute toxicity tests indicated that no significant difference existed between collected water and control water for *P. promelas*. However, water collected from channelized sites exhibited measured toxicity to *C. dubia* as compared to controls. Our results indicate that channelized streams in agricultural areas are likely to have a greater potential for impaired water quality than natural streams, especially when examining turbidity and TSS.

WP198 Construction of a Species Sensitivity Distribution (SSD) for a Fungicide Combining Fish and Aquatic Invertebrate Endpoints

A. Samel, DuPont Crop Protection / Ecotoxicology

In environmental risk assessments for crop protection products, species sensitivity distributions (SSD) are based on endpoints from toxicity tests conducted in the laboratory. The SSD is based on a distribution of similar endpoints (i.e., mortality, immobility, growth, etc.) from similar taxa (i.e., fish, algae, aquatic invertebrates). Separate SSDs are constructed from each data-set. The new draft EFSA Aquatic Guidance Document allows for combining data from fish and aquatic invertebrates to construct one combined taxa SSD. The major advantages of combining the fish and aquatic invertebrate data-sets is the increase in the number of species and a, hopeful, decrease in variability. We will present SSDs for aquatic invertebrates, fish, and the combined data-sets and compare the HC5 values and the upper and lower 95% confidence intervals.

WP199 Development of decision tree model and protein profiling of Heterocypris incongruens exposed to heavy metals and pesticides using SELDI-TOF MS

H. Mo, Division of Environmental Science and Ecological Engineering; Y. Kim, Korea University; S. Lee, Kyungpook National University; K. Shin, Department of Statistics, Hankuk University of Foreign Studies; K. Cho, Korea University / Division of Environmental

Proteomics may help to detect subtle pollution-related changes, such as responses to mixture pollution at low concentrations, where clear signs of toxicity are absent. Also proteomics provide potential in the discovery of new sensitive biomarkers for environmental pollution. We utilized SELDI-TOF MS (surface enhanced laser desorption/ionization time-of-flight mass spectrometry) to analyze the proteomic profile of *Heterocypris incongruens* exposed to several heavy metals (lead, mercury, copper, cadmium and chromium) and pesticides (emamectin benzoate, endosulfan, cypermethrin, mancozeb and paraquat dichloride). Several highly significant biomarkers were selected to make a model of classification analysis. Data sets obtained from *H. incongruens* exposed pollutants were investigated for differential protein expression by SELDI-TOF MS and decision tree classification. Decision tree model was developed with training set, and then validated with test set from profiling data of *H. incongruens*. Machine learning techniques provide a promising approach to process the information from mass spectrometry data. Even thought the identification of protein would be ideal, class discrimination does not need it. In the future, this decision tree model would be validated with various levels of pollutants to apply field samples.

WP200 Dicamba Affect Sex Steroid Hormone Level and mRNA Expression of Related Genes in Adult Rare Minnow (Gobiocypris rarus)

L. Zhu, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences; W. Li, J. Zha, Z. Wang, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences / State Key Laboratory of Environmental Aquatic Chemistry

Dicamba is a benzoic acid herbicide, which has been detected in surface and ground water. It has shown cytogenesis effects and DNA damage as well as organ damage in mammals, whereas little is known about its endocrine disrupting effects in fish. In this study, the histological changes, plasma vitellogenin (VTG) and sex hormone levels, and the expressions of sex steroid hormone related genes mRNA levels were determined from adult rare minnow exposed to environmentally relevant concentrations of dicamba (0, 0.05, 0.5, 5, and 50 μg/L) for 40 days. The results showed inhibition of spermatogenesis in male testes and ovarian degeneration in females. Plasma 17β-estradiol (E2) levels were significantly increased in both genders, and plasma VTG levels were significantly increased in males ($p< 0.05$). These results indicated that sex hormone homeostasis and normal reproduction of fish could be affected by dicamba. Moreover, the mRNA levels of *vtg* were significantly up-regulated in the livers and gonads of both males and females ($p< 0.05$). The down-regulation of cytochrome P450c19a (*cyp19a*) and steroidogenic acute regulatory protein (*star*) mRNA levels, and up-regulation of cytochrome P450c17 (*cyp17*) mRNA levels were observed in livers and ovaries ($p< 0.05$). The results of expressions suggest that steroid hormone related genes could serve as an auto-regulation of sex steroid hormone levels. Overall, dicamba exposure could result in histological lesions, plasma VTG increase, the changes of sex hormone levels and related genes expression. Therefore, dicamba should be considered a potential endocrine disruptor.

WP201 Effect of storage method and associated holding time on nitrogen and phosphorus concentrations in surface water samples

M.T. Moore, USDA-ARS National Sedimentation Laboratory; M.A. Locke, USDA-ARS National Sedimentation Laboratory / Water Quality and Ecology Research Unit

Assessments were conducted to determine the effect of sample storage method and associated holding time on surface water nutrient concentrations from field sites. Six surface water sites and two nutrient spiked, laboratory water samples were evaluated for nitrate, nitrite, ammonium, filtered orthophosphorus, and total orthophosphorus concentrations on four separate days throughout the period of one year. Samples allowed to sit at ambient temperature (23°C) for 24 h prior to nutrient analyses resulted in 18±2% of results being significantly different from controls (which were analyzed immediately upon collection). Samples placed in the cooler (4°C) for 7 d prior to nutrient analyses resulted in 30±1% of values being significantly different from controls. Samples placed in the freezer (-20°C) for 7 d prior to analyses

resulted in 34±12, 44±10, and 28±5.7% of ammonium, filtered orthophosphate, and total orthophosphate, respectively, values being significantly different from controls. This study highlights the challenges facing researchers in efficient collection and nutrient analysis of samples, especially when sites are remote and difficult to access.

WP202 Effects of particulate organic matter on the toxicity of freely dissolved and absorbed bifenthrin to calanoid copepods of the San Francisco Estuary

S.A. Lesmeister, University of California at Davis / Ecology, Veterinary Medicine: Anatomy, Physiology and Cellular Biology / Ecology Graduate Group; E. Parry, University of California, Davis / Agricultural and Environmental Chemistry Graduate Group; S.J. Teh, University of California, Davis / Veterinary Medicine; T.M. Young, University of California-Davis / Dept. of Civil & Environ. Engineering

The calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi* are important prey items for endangered fish species in the San Francisco Estuary (SFE). Recent declines in these zooplankton populations have warranted investigations, including the role of contaminants, on these species. Previous studies demonstrate that *E. affinis* and *P. forbesi* are sensitive to pesticides, especially bifenthrin, at concentrations detected in the environment. In an effort to elucidate the role pesticides play on organism decline in the SFE, this study investigates particulate organic matter (POM) mitigation of the toxicity of bifenthrin to *E. affinis* and *P. forbesi*. Additionally, this study explores the composition of POM and how that influences observed toxicity. Study results demonstrate POM reduces the toxicity of total bifenthrin to *E. affinis* and *P. forbesi* and the particle composition impacts observed mortality. The results also show species specific differences in the sensitivity of total and dissolved fractions of bifenthrin. Understanding the role of POM in mitigating pesticide toxicity to non-target aquatic organisms is important for scientists and managers. Future studies failing to investigate the fractionation of contaminants, composition of POM and the relationship in observed toxicity may lead to a misunderstanding of toxicity witnessed in the environment.

WP203 Endocrine disrupting effect of the herbicide Alachlor on the short term reproduction of Japanese Medaka

J. Lee, Agro-materials safety evaluation division; Y. Park, Rural Development Administry; B. Kim, H. Kwon, A. You, M. Paik, S. Hong, M. Jeong, National Academy of Agricultural Science

Acute toxicity, water resolvability and short term reproduction test on Japanese medaka(*Orizias latipes*) for evaluating alachlor susceptibility to endocrine system were studied. Alachlor is known for suspected endocrine distruptors. As the results of tests, LC_{50}(Median lethal concentration) was determined as 2.365(1.994~2.805) mg/L, and test water replaced at 7 day intervals as its water resolvability was 20% in 15 days. The short term reproduction tests on Japanese medaka(Orizias latipes) were performed with a solvent control group, a treated group(alachlor concentrations of 0.02, 0.04, 0.11, 0.27, 0.68 ppm) and a positive control group(17β estradiol, 0.01, 0.1, 0.5 ppb). The number of spawning and embryo rates were declined as dropping the concentrations and unfertilized eggs rates were increased depending on the concentrations. The adverse effects were partially effected by the concentrations but additional tests were needed to confirm that. Additionally, alachlor was evaluated as a non-vitellogenin by the result of a test of significance of the vitellogenin content test for determination of the effect of estrogen among the endocrine disruptors.

WP204 Fate and transport of atrazine and sulfentrazone in an agricultural field in Central Illinois

J.L. Thorngren, T. Murphy, Southern Illinois University-Carbondale / Department of Zoology; M.J. Lydy, Southern Illinois University Carbondale / Fisheries and Illinois Aquaculture Center and Department of Zoology

Atrazine is one of the most highly used and studied pesticides in the United States. The effects of atrazine to non-target species have been controversial, and its use has been banned in many parts of the United Kingdom. Many mixture toxicity tests have used atrazine as one of the components, but there have been no published studies on mixture toxicity of atrazine and sulfentrazone. Sulfentrazone is a herbicide approved for use on soybeans and other crops. The agricultural field studied had a corn/soybean yearly rotation with the usage of atrazine and sulfentrazone, respectively. The objective of this research was to measure soil, runoff water, and ground water concentrations of atrazine and sulfentrazone and determine if co-occurrence existed. Atrazine and sulfentrazone concentrations were quantified using high performance liquid chromatography with an ultraviolet detector at 214 nm, a C18 scalar column (4.6 x 250 mm, five micron), and external standards. Data from 2011 showed a peak atrazine soil concentration of 281.2 ng/g dry weight in June. Atrazine concentrations quickly decreased in the subsequent months, however, residual levels were detected throughout the 2012 field season. Sulfentrazone was applied in April 2012 and had a peak soil concentration of 199.1 ng/g dry weight in May 2012. The mean concentration of sulfentrazone eight months after application, in December 2012, was still 57.4 ± 25.7 ng/g dry weight. This data indicates that atrazine and sulfentrazone do co-exist, therefore validating the need for mixture tests.

WP205 Frequency of antibiotic resistance genes in Swift Current Creek a small, ephemeral prairie stream located in a rural/urban/agricultural drainage

J.R. Lawrence, Environment Canada / Water Science and Technology Directorate; A. Paule, University of Saskatchewan – Global Institute for Water Security; J. Roy, Water Science and Technology Directorate, Environment Canada; G. Swerhone, Water Science and Technology Directorate, Environment Canada; R. Marti, Agriculture and Agri-Food Canada; D.R. Korber, University of Saskatchewan; E. Topp, Agriculture and Agri-Food Canada; M.J. Waiser, University of Saskatchewan – Global Institute for Water Security

Industrial, medical and sewage treatment plant (STP) effluents have been linked to increased antibiotic resistance in surface waters, thus concerns exist regarding their environmental impact and persistence. Further, there is concern that antibiotic resistance development has been ignored in environmental risk assessments. Effluent-dominated ephemeral water bodies like streams of the central North American prairies represent worst case scenarios and are hotspots for ecological and other changes (e.g., increased development of antibiotic resistance). Swift Current Creek, located in south central Saskatchewan, receives non-point agricultural inputs as well as treated sewage effluent from Swift Current (pop. 15000). In this study, both PCR and Q-PCR analyses were utilized to detect and quantify specific resistance genes in water from effluent and sites upstream and downstream of the STP. The National Antimicrobial Resistance Monitoring System (NARMS) antimicrobial panel was also used to monitor "resistance" to 15 antibiotics. Several antibiotics were routinely detected at (ng L^{-1}) levels in treated effluent, including; ciprofloxacin (1790), clindamycin (124), sulfamethoxazole (110), sulfapyridine (58) and trimethoprim (120). Stream waters also contained mg L^{-1} levels of various metals that may act as co-selective agents for antibiotic resistance. For ciprofloxacin, NARMS screening suggested that although antibiotic resistance was greatest at, and just below, the STP, it declined rapidly with distance downstream. In contrast, resistance determinations (100% NARMS concentration) were frequently found for azithromycin and amoxicillin-clavulanic acid not only at the STP discharge but downstream. PCR screening indicated that resistance genes for sulfonamide, erythromycin, beta lactamase, streptomycin, tetracycline and quinolones could be detected throughout the drainage basin. Integrons, and incompatibility markers, were similarly detected in effluents and at downstream sites. Q-PCR analyses indicated, for example, that *sul I* (coding sulfonamide resistance) was not detectable above the sewage treatment plant, up to 3300 copies mL^{-1} in sewage influent, 630 copies in the river immediately below the STP declining to 1000 copies 51 km downstream but up to 6000 at 93km. Within this agricultural basin frequent occurrence of antibiotic resistance was linked to effluent from the STP, cattle production sites and may be co-selected by the presence of metals.

WP206 General Pesticide Permit Toxicity Study: Monitoring Aquatic Toxicity of Spray Pesticides to Freshwater Organisms

B.M. Phillips, University of California – Davis / Environmental Toxicology / Granite Canyon; B.S. Anderson, University of California – Davis / Department of Environmental Toxicology; J. Voorhees, University of California – Davis / MPSL – Environmental Toxicology; L.L. Jennings, University of California – Davis / Enviromental Toxicology; M. Petersen, University of California – Davis; K. Siegler, R.S. Tjeerdema, University of California – Davis / Environmental Toxicology; D.L. Denton, USEPA Region 9 / c/o SWRCB; K. Larsen, P. Isorena, California State Water Resources Control Board

Pesticides are applied to state and local waterways in California to control invasive aquatic plants and animals, and insect vectors such as mosquitoes. The State Water Resources Control Board is adopting a National Pollution Discharge Elimination System General Permit to address the discharge of pesticides to waters of the United States resulting from adult and larval

mosquito control. Because pesticides used in spray activities have the potential to cause toxicity to non-target organisms in receiving waters the State Water Board funded the current study to determine if toxicity testing provides additional useful environmental risk information beyond chemical analysis in monitoring spray pesticide applications. Monitoring included a combination of aquatic toxicity tests and chemical analyses in agricultural, urban and wetland habitats. Approximately 16% of the post-application water samples were significantly toxic. The toxicity of half of these samples was attributed to the naled breakdown product dichlorvos. Four of the 43 post-application sediment samples were significantly more toxic than their corresponding pre-application sample. In the case of naled in water, analysis of only the active ingredient would have underestimated potential impacts to the receiving system. Toxicity testing provided information that could lead to the inclusion of dichlorvos monitoring.

WP207 How is increased corn production impacting US irrigation?

J.C. Bare, USEPA / MS-466

As a response to the Renewable Fuel Standard, additional corn has been harvested in the Midwestern US. Twelve Midwestern states are currently undergoing significant land cover changes, and are expected to experience 13% land use change between 2001 and 2020. In nearly all of these cases, increased water consumption is expected with increased corn production. Maps will be provided which show the irrigation needs integrated with a water scarcity index.

WP208 Laboratory and field validation of a Bt-Cry1Ab quantitation method in water matrices

K.E. Strain, Southern Illinois University Carbondale / Center for Fisheries, Aquaculture and Aquatic Sciences; S. Mueting, Southern Illinois University Carbondale / Department of Zoology; M.J. Lydy, Southern Illinois University Carbondale / Fisheries and Illinois Aquaculture Center and Department of Zoology

Genetically-modified crops expressing insecticidal crystalline (Cry) proteins derived from *Bacillus thuringiensis* (Bt) now dominate the agricultural landscape throughout much of the world. The wide-spread planting of Bt products has raised questions regarding the environmental fate of Bt toxins. Researchers have developed and validated methods to quantify several Bt proteins in soil matrices using enzyme-linked immunosorbent assay (ELISA), but currently there is no published validated method for the evaluation of Bt-Cry1Ab in water. The objective of this research was to optimize the extraction of Cry1Ab from three water matrices collected from agricultural and forested sites in central and southern Illinois. Two common extraction methods for the concentration of water samples via lyophilization or centrifugal filters were adapted from the literature and optimized for the extraction of Cry1Ab from three water matrices. Percent recoveries were determined and extraction methods were compared using one-way analysis of variance (ANOVA). The results showed significantly higher recoveries using centrifugal filters ($F_{1,16}$= 30.84, P< 0.001), and therefore this method was selected for further validation using a series of ELISA and blotting experiments. Matrix effects were only observed in one of the matrices with greater than 20% deviation from the control. Extraction efficiencies were matrix-dependent and ranged from 42 to 85% with a coefficient of variation (CV) less than 20% between extractions. The applicability of the method was then demonstrated in the quantitation of Cry1Ab in agricultural field samples collected throughout the 2013 growing season. The validated method can also be extended to toxicity testing as a way to measure Cry1Ab concentrations in the water column. The development of a standardized water extraction method bolsters the integrity of research involving the quantitation of Bt proteins in aquatic settings, and provides a foundation for environmental risk assessments.

WP209 Mixture toxicity of multiple herbicides present in commercial herbicide products in Japan

H. Watanabe, S. Ogino, I. Tamura, National Institute for Environmental Studies / Center for Environmental Risk Research; H. Takanobu, National Institute for Environmental Studies; R. Abe, The University of Tokyo / Graduate School of Frontier Sciences; M. Koshio, National Institute for Environmental Studies; N. Tatarazako, National Institute for Environmental Studies / Center for Environmental Risk Research

Pretilachlor is one of paddy herbicides that are widely used in planting season in Japan and its high toxicity to green algae requires an appropriate use and risk management. Since most of herbicide products contain multiple active ingredients (herbicide), one of commercial herbicide products contains dimethametryn, pyrazosulfron methyl, esprocarb other than pretilachlor. Assuming that the mixture toxicity of multiple herbicides in the product can be predicted as the sum of the individual toxicity of herbicide based on a concentration addition approach, we calculated the sum of toxic unit (TU=content rate/NOEC) of each herbicide to predict the mixture toxicity of the multiple herbicides. Pretilachlor contributed 75% of the calculated mixture toxicity; however, it is not always true that the mixture toxicity of the multiple herbicides can be expressed by additive effect of each herbicide. Takeuchi et al. (2012) revealed that pretilachlor and dimethametryn caused a synergic effect in a rapid algal luminescence toxicity test using delayed fluorescence (DF). Therefore, synergic effect of other multiple herbicides used in herbicide products might be observed. In this study, we investigated whether the synergic effect between pretilachlor and dimethametryn was also observed in algal growth inhibition test (OECD TG201) as is the case in the algal luminescence toxicity test using DF. In addition, we evaluated the mixture toxicity of multiple herbicides present in commercial herbicide products using three short-term chronic toxicity tests: algal growth inhibition test (*Pseudokirchneriella subcapitata*), a daphnid reproduction test (*Ceriodaphnia dubia*), a fish short-term toxicity test on embryo and sac-fry stages (*Danio rerio*) to compare with the additive effect of each herbicide. Environmental fate of each herbicide after application in the paddy field was also considered when determining the test concentration of each herbicide.

WP210 Monitoring of Water Quality to Determine Effectiveness of the Best Management Practices for the Larkin Creek Watershed in Northeastern Arkansas

S.K. Vogt, Arkansas State University / Environmental Sciences; T.M. Woodruff, Arkansas State University; J.L. Bouldin, Arkansas State University / Department of Biological Sciences, Environmental Sciences Graduate Program / Biological Sciences

The Larkin watershed, 55,886.76 hectare in total area, is listed as a 305b impaired subwatershed of the L'Anguille River Watershed. The main impairment of the stream is due to sedimentation and fecal coliforms as a possible result from agriculture activities within the watershed. In 2011, several Best Management Practices (BMPs) were initiated that included a sedimentation pond, construction of riparian buffers, sediment removal, and channel restoration. Three sampling locations were chosen based on prior sampling to the implementation of BMPs. Water quality monitoring of Larkin Creek included on-site testing of pH, dissolved oxygen, temperature, and conductivity. Water samples were taken once a week from the three sites and tested for total suspended solids (TSS), turbidity, nitrates, nitrites, and orthophosphates at the Arkansas State University Ecotoxicology Research Facility; State University, Arkansas. Prior to the BMPs in 2010, the Upper site TSS data ranged from 5.03-12.90 mg/L while the Middle site ranged from 7.67-523.47 mg/L and Lower site ranged from 5.10-28.6 mg/L. After the BMPs were initiated, the 2012 data showed the TSS for the Upper site between 1.00-228.83 mg/L while the Middle site ranged between 4.63-404.40 mg/L and the Lower site ranged between 4.47-156.40 mg/L. Ongoing water quality monitoring for Larkin Creek Watershed will help to determine the effectiveness of implemented BMPs.

WP211 Pesticides and predator cues: Multiple stressor interaction patterns through time

J.K. Louder, The Institute of Environmental and Human Health / Environmental Toxicology; G. Qin, Guangxi Center for Disease Prevention and Control; J.D. Maul, Texas Tech University / Environmental Toxicology

Environmental factors and ecological stressors have been shown to modify pesticide toxicity when they co-occur. Typically, interactions are assessed at standardized exposure time points. Less attention has focused on how these interaction patterns may vary over time during a co-exposure period. In a previous study we found that measured toxicity responses varied widely compared to toxic responses estimated from an independent action model across concentration gradients. The patterns were different among several classes of pesticides that had different mechanism of action. Here we report on how bluegill (*Lepomis macrochirus*) chemical cues affect toxicity of bifenthrin, fipronil, malathion, and thiacloprid to *Ceriodaphnia dubia* over a 96-h exposure period in which *C. dubia* mortality was monitored for multiple time points. For those pesticides that were previously found to have potentiation (fipronil) and antagonistic (bifenthrin and thiacloprid) interactions at 96-h, mortality patterns over time varied between the predator cue

and pesticide combination and the pesticide alone. Typically, this variation increased over the exposure period and patterns varied among concentrations tested. The results demonstrate that interpretations of multiple stressor interactions may be dependent on time of monitoring over the co-exposure period. Toxicokinetic mechanisms may be responsible for driving this variation if stressors such as predator cues influence metabolic or uptake and elimination processes. These results also support the idea that monitoring responses to multiple stressors through time and use of integrated approaches will provide a better understanding of risk to aquatic organisms.

WP212 Quality Assurance Program for a Baseline Survey Study of Pyrethroid Pesticides in Publicly-owned Treatment Work Facilities in California

B.H. Van Buuren, van Buuren Consulting / Quality Assurance Research Group; A. Vandervort, van Buuren Consulting; J.C. Markle; A.C. Barefoot, DuPont Crop Protection / Crop Protection Products

Pesticides containing pyrethroid active ingredients have become a highly requested parameter group for low-level chemical analysis in water monitoring programs. This poses a problem to project managers, as there are no standardized methods or commercially available certified reference materials for these analyses. In addition, laboratories may vary in their instrumentation, levels of sensitivity, and techniques for extraction, clean-up, and analysis. In response to the 2006 California Department of Pesticide Regulation data re-evaluation, the Pyrethroid Working Group and Tri-TAC, representing Publicly Owned Treatment Works (POTWs) developed a monitoring project, for eight pyrethroid pesticides in influent, effluent and biosolids. The project surveyed 32 POTWs between January and March 2013. At each location, samples of influent and effluent were collected as consecutive grab samples and at the 19 sites that accommodated sampling of biosolids, this matrix was collected and composited. Two laboratories were used to analyze the biosolids samples as replicates and the effluent and influent samples as consecutive grabs (distinct samples). This poster cover the key steps take to develop a robust quality assurance program for this study including a pre-study inter-comparison study, method modifications to improve robustness, development of a Quality Assurance Project Plan (QAPP), review of the QAPP and method quality objectives by the Surface Water Ambient Monitoring Program (SWAMP), sampling training webinars for each of the POTWs and in-study audits.

WP213 Relative toxicity of bifenthrin to the amphipod Hyalella azteca in 10-d and 28-d exposures

B.S. Anderson, University of California – Davis / Department of Environmental Toxicology; B.M. Phillips, University of California – Davis / Environmental Toxicology / Granite Canyon; J. Voorhees, University of California – Davis / MPSL – Environmental Toxicology; M. Peterson, UC-Davis; L.L. Jennings, University of California – Davis / Enviromental Toxicology; K. Siegler, R.S. Tjeerdema, University of California – Davis / Environmental Toxicology; T.L. Fojut, Central Valley Regional Water Quality Control Board / Environmental Toxicology; M.E. Vasquez, University of California, Davis / Environmental Toxicology

Many watersheds in the Central Valley region of California are listed as impaired due to pyrethroid-associated sediment toxicity. The Central Valley Regional Water Board is developing numeric sediment quality criteria for pyrethroids, beginning with bifenthrin. Criteria are being developed using 10d and 28d toxicity tests with *Hyalella azteca*. Sediment tests have shown that survival LC50s are not significantly different in 10d versus 28d tests with *H. azteca*. Bifenthrin IC25s based on 10d and 28d amphipod growth were variable, particularly in the 28d tests, where no clear dose response relationship was observed. Lack of a clear growth effect in the longer term test may be related to the lack of food adjustment to account for amphipod mortality in whole sediment exposures. Definitive survival LC50s and growth IC25s will be based on measured whole sediment concentrations normalized to sediment TOC and interstitial water concentrations normalized to DOC. This poster will discuss how bifenthrin dose-response data from 10d and 28d exposures will contribute to the validation of a UC Davis Sediment Criteria Method that is currently in development.

WP214 Role of oxidative stress in acetylcholinesterase regulation during organophosphate pesticide exposure

F.J. Rubio-Escalante, Universidad Nacional Autonoma de Mexico / Unidad de Química Sisal; U. Mendoza-Euan, Instituto Tecnológico de Tizimín; E. Norena-Barroso, Universidad Nacional Autonoma de Mexico / Unidad de Quimica-Sisal, Facultad de Química; D. Schlenk, University of California-Riverside / Department of Environmental Sciences; G. Rodriguez-Fuentes, Universidad Nacional Autonoma de Mexico / Unidad de Química Sisal

Cholinesterases are inhibited by numerous pollutants that include organophosphate pesticides (OP), which are, among other pesticides the most toxic to the vertebrates. OP causes the irreversible inhibition of acetylcholinesterase (AChE) in the central and peripheral nervous system resulting in the accumulation of acetylcholine and excessive activation of muscarinic and nicotinic receptors, which may lead to death. Thus, dogma indicates that the target of OP pesticides is AChE, but many authors postulate that these compounds also disturb cellular redox processes, and change the activities of antioxidant enzymes. OP metabolism produces reactive oxygen species (ROS) that are highly reactive molecules, which have one or more unpaired electrons ROS causes lipid peroxidation resulting in the formation of highly reactive stable and unstable hydroperoxides. Interestingly, it has also been reported that oxidative stress plays also a role in the regulation and activity of AChE. The aims of this study were to determine the effect of Chlorpyrifos (CPR) on AChE gene regulation and activity in zebra fish embryos and to evaluate the effect of CPR on oxidative stress alterations on the development of zebrafish embryos. Two hour post-fertilization zebrafish embryos were exposed to CPR, vitamin C (VC, anti-oxidant), t-butyl hydroperoxide (TBH, pro-oxidant) and mixtures of the pesticide with anti or pro-oxidants. AChE, Catalase CAT, Superoxide dismutase SOD and glutathione peroxidase GPx activity and gene expression were evaluated. As indicators or oxidative stress, lipid hydroperoxidation and total glutathione were also evaluated in all treatments. Results indicated that co-exposure of CPR with anti and pro-oxidants modified AChE activity. AChE gene was down-regulated during exposures with anti and pro-oxidants and their mixtures with CPR. Exposures with CPR increased total glutathione and produced differences in GPx expression. Co- exposures with CPR and anti or pro-oxidants resulted in differences in CAT activity and expression. Total glutathione was significant reduced during co-exposures with TBH. Co-exposure with VC and TBH resulted in up-regulation and down- regulation of GPx gene, repectively. Significant differences in production of lipid hydroperoxides were detected in exposures that contained TBH. Results of this work indicate that AChE activity and expression are related to changes in cellular redox system.

WP215 Spatial and temporal variability in fish communities along an agricultural and urban gradient to support a framework for cumulative effects assessment

K. Hicks, University of Waterloo / Department of Biology; G. Tetreault, Environment Canada / Aquatic Ecosystem Protection Research Division; M.E. McMaster, Environment Canada / National Water Research Institute; M.R. Servos, University of Waterloo / Department of Biology

The Grand River watershed, in southern Ontario, is subjected to multiple anthropogenic stressors such as agricultural activities, dams, inputs from municipal wastewater treatment plants (WWTP), and rapidly expanding urban development. Currently in this watershed, only water quality is being monitored regularly, thus the cumulative impacts of these multiple stressors on the biological integrity of the system are unknown. The objective of this study is to assess the cumulative effects of these stressors on fish communities, assessing both spatial and temporal variability along agricultural and urban gradients. A number of sites, stretching a distance of approximately 140 km, were sampled through the spring of 2013. Half the sites were selected in a heavily urbanized area downstream of two secondary treated WWTPs and the other half are located adjacent to agricultural activities. At each site, a 150 m of riffle/run habitat was selected and divided into 15 sub-sites, each measuring 10 m along the bank and extending out 10 m from the bank. Sub-sites were randomly selected out of the 15, where each was sampled for 300 s using a standard backpack electrofisher (for a total of 1800 s/site). An effort to standardize each site includes selecting similar habitat type, standardizing the electrofisher power output, and sampling between dawn to 12 pm. At each site, fish species, length, weight, sex (if possible) and deformities were recorded to estimate biomass and fish condition. Differences in fish community assemblages among sites were assessed in terms of species composition, relative abundance and diversity. Results reveal differences in fish communities among sites located in agricultural reaches, versus sites located in urbanized reaches of the Grand River. Fish abundance at 4 of the 5 agricultural sites is greater (up to 23 fold) than sites located in the urbanized reaches. All agricultural sites are dominated by more vulnerable darter species (up to 92%); whereas most urbanized sites show a shift to the more tolerable and mobile sunfish. Additional sampling periods in summer/

fall will allow the discrimination between natural variability and changes in fish communities in response to cumulative anthropogenic impacts. This research is part of a joint effort to support the development of a regional biomonitoring framework for cumulative effects assessment.

WP216 The effects of bifenthrin exposure on initial phase Thalassoma bifasciatum (bluehead wrasse) brain aromatase (CYP 19A2) expression and aggression

K.A. Burnsed, University of North Carolina, Wilmington / Dept. of Biology and Marine Biology; S.M. Brander, Bodega Marine Lab, University of California, Davis / Environmental Toxicology, University of North Carolina, Wilmington / Department of Biology & Marine Biology

Pyrethroid insecticides, such as bifenthrin, are increasingly used in agriculture, to control the spread of insect-borne disease, and in residential pest-control formulations. Although the intended mechanism of these chemicals is to increase the duration of sodium channel opening, they have been found to also act as endocrine-disrupting chemicals. The precise mechanisms by which they work and their effects are not completely understood and thus the endocrine-disrupting nature of pyrethroids is an area in need of further study. Bifenthrin inhibits estrogen response *in vitro* but is estrogenic *in vivo*, suggesting that metabolites of the compound bind to and agonize or antagonize the estrogen receptor. The effects of bifenthrin also extend to other estrogen-dependent genes. *Thalassoma bifasciatum*, a fish that is a sequential hermaphrodite, may be especially susceptible to estrogenic compounds in the environment due to its already labile sexual characteristics and due to the importance of group dynamics in sex determination. There are two male morphs, the initial phase (IP) and terminal phase (TP) males. This IP male morph is found in higher proportions in large populations than in small populations where it is evolutionarily most advantageous due to their mating strategy of group spawning. Given that aggressive behaviors in *T. bifasciatum* increase in response to decreases in brain cytochrome P450 aromatase expression (*CYP19A2*) during sex change in the bluehead wrasse, it is logical to use those endpoints in order to measure the effects of bifenthrin. It is expected that initial phase (IP) males will display less aggressive behavior and have altered brain aromatase expression following bifenthrin exposure. Bifenthrin may cause positive feedback on aromatase gene expression in the brain and cause the production of more estrogen. Studying what effects bifenthrin might have on the brain aromatase gene expression and aggressive behavior will lead to a better understanding of the nature of bifenthrin as an EDC and promote further study on both its environmental and human health effects.

WP217 Toxicity of a Current Use Insecticide to Resurrected Daphnia pulicaria: Impact of 500 Years of Evolution

A.M. Simpson, Oklahoma State University / Zoology; J.B. Belden, Oklahoma State University / Department of Zoology; P. Jeyasingh, Oklahoma State University / Zoology

This study examined how the natural evolutionary progression in a resurrected *Daphnia pulicaria* population indirectly affected its sensitivity to new anthropogenic stressors, specifically pesticides. Toxicity of the organophosphate insecticide chlorpyrifos was determined through a series of acute toxicity tests quantified by the median lethal concentration (LC50). After comparing LC50 values across clone genotypes, a trend can be seen, demonstrating a decrease in sensitivity to chlorpyrifos as clonal age decreases. This could be explained by a pre-evolved decrease in metabolism in the more contemporary clones, or an indirect resistance acquired through developed tolerance to the increased prevalence of toxic food sources. Ultimately, these results provide insight into preexisting mechanisms that confer developed resistance to new environmental stressors.

WP218 Toxicity of the insect growth regulators on Heterocypris incongruens using alteration of protein patterns and antioxidant enzyme activities

H. Mo, Division of Environmental Science and Ecological Engineering; Y. Kim, Korea University; Y. Lee, Korea Univ.; J. Wee, Korea University; S. Lee, Kyungpook National University; K. Cho, Korea University / Division of Environmental

It is known that insect growth regulators (IGR) have some advantages over conventional insecticides, such as their low toxicity to vertebrates and greater specificity for some target insects. However there have been a few studies about the ecotoxicity of IGR on non-target species, especially living in aquatic ecosystems. *Heterocypris incongruens* Ramdohr belongs to Class Ostracoda, Order Podocopida and Family Cypridae having an almost global distribution. This species is quite common in sluggish streams and rice fields having a high reproductive ability, also takes an important ecological role in nutrient cycle of aquatic ecosystems. But there are few ecotoxicological studies using this species. To assess IGR toxicities on *H. incongruens*, 10 day development, protein-pattern alteration using SELDI-TOF MS (surface enhanced laser desorption/ionization time-of-flight mass spectrometry), and antioxidant activities of CAT, GST, GPx, MDA and SOD were analyzed. The results of this study may help in establishing a useful guideline for IGR.

WP219 Comparative responses of river biofilms at the community-level to common organic solvent and herbicide exposure

A. Paule, University of Saskatchewan – Global Institute for Water Security; V. Roubeix, Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture; G. Swerhone, Water Science and Technology Directorate, Environment Canada; B. Lauga, R. Duran, Institut Pluridisciplinaire de Recherche sur l'Environnement et les Matériaux; F. Delmas, Institut national de recherche en sciences et technologies pour l'environnement et l'agriculture; J. Rols, Laboratoire d'écologie fonctionnelle et environnement – Université de Toulouse; J.R. Lawrence, Environment Canada / Water Science and Technology Directorate

Ecotoxicological experiments were performed at laboratory-scale under controlled conditions to investigate the community-level responses of river biofilms to a chloroacetanilide herbicide (alachlor) and organic solvent (methanol) exposure through the early stages of development referenced to control. Triplicate rotating annular bioreactors, inoculated with river water, were used to cultivate river biofilms under the influence of 1 and 10 µg L^{-1} of alachlor and 25 µg L^{-1} of methanol (0.025% v/v). For this purpose, functional (thymidine incorporation and carbon utilization spectra) as well as structural responses of microbial communities were assessed after 5 weeks of development. Structural aspects included biomass (chlorophyll a, confocal laser scanning microscopy (CLSM) and composition (fluor-conjugated lectin binding, molecular fingerprinting (DGGE) and diatom species composition). The addition of alachlor resulted in a significant reduction ($p< 0.05$) of bacterial biomass (based on CLSM) at 1 µg L^{-1}; whereas at 10 µg L^{-1} it induced a significance reduction of EPS lectin-binding, algal, bacterial and cyanobacterial biomass (by CLSM). However, there were not changes in biofilm thickness or thymidine incorporation. No significant difference between the bacterial community structures of control and alachlor treated-biofilms was revealed by DGGE. However, the methanol treated-bacterial communities appeared different from control and alachlor treated-communities. Moreover, methanol treatment resulted in an increase of bacterial biomass and thymidine incorporation as well changes in dominant lectin-binding suggested changes of the exopolymer and community compostion. Chlorophyll *a* and cyanobacterial biomass (by CLSM) were also altered by methanol. This study suggested that the concentration-dependent effect of alachlor mainly remains limited to biomass and growth inhibition without apparent changes of ecological succession trajectories and functional characteristics. Our work also establishes that the presence of methanol may influence the results of ecotoxicological bioassays by potential direct toxic effects on the river biofilm community or acting antagonistically, synergically or masking the effects of the target compound.

WP220 Effects of sub-lethal malathion exposure to Aedes aegypti larvae: influence of age and temperature on life history traits and population structure

D.E. Dawson, Texas Tech University / TIEHH / Environmental Toxicology; C.J. Salice, Texas Tech University / Environmental Toxicology

Sub-lethal toxicant exposures to developing organisms can have far reaching effects on life history traits such as developmental timing, mortality, and ultimately fecundity. In turn, these types of latent effects can translate to changes in population structure and dynamics. In the case of the Yellow-fever mosquito *Aedes aegpyti* (Family Order: Diptera; Family: Culicidae), sub-acute laboratory exposures to malathion during the 1st instar larval stage have been shown to interact with temperature to influence the weight and fecundity of surviving adult females, with higher temperatures and pesticide concentrations producing fewer, larger and more fecund females. These observed affects were possibly due to reduced competition stress at the higher temperatures and pesticide concentrations. However, recent research indicates that malathion sensitivity in larvae of *Aedes aegypti* also appears to depend upon the stage of development, with first instars (LC50≈0.072 mg/l) showing greater sensitivity than 4th instars (LC50≈0.095 mg/l). Using

different sub-lethal acute toxicity levels for 1st and 4th instar individuals, we conducted experiments which investigated how both temperature and time of larval exposure (immediate or pre-ecdysis) influence stage-specific vital rates, including larval development and mortality, and adult female weight, fecundity, and longevity. We then investigated how these impacts affect population structure over time by incorporating measured vital rates into a stage-based population model. Our experimental results indicate that sub-acute toxicity exposures have similar impacts to1st and 4th instar larvae. Preliminary modeling results suggest that temperature, exposure concentration, and larval population age structure at time of exposure may have significant population-level affects, potentially by altering intra-specific competition pressure. This research demonstrates that exposures to stressors during development can have far-reaching impacts that can manifest later in life and in higher levels of biological organization.

Metals & Selenium

WP221 ATP-binding cassette (ABC) transporters-mediated metal tolerance in the intertidal copepod, Tigriopus japonicus

J. Rhee, Research Institute for Natural Sciences, Hanyang University; C. Jeong, Hanyang Univ.; B. Kim, Hanyang University; J. Lee, Hanyang University / Dept of Chemistry, Hanyang University Graduate School / Dept of Chemistry

The intertidal copepod, *Tigriopus japonicus* shows a strong tolerance to metal exposure, compared to other invertebrates. To better understand its mechanism of metal resistance, we measured the transcript expression profile of whole ATP-binding cassette (ABC) transporters in *T. japonicus*. In this study, we firstly identified whole ABC transporters including P-glycoprotein (P-gp) with conserved motifs/domains and *in silico* analysis from *T. japonicus* genome database. To understand the roles of ABC transporters in metal tolerance, full spectrum of 46 ABC genes was analyzed to understand their potential mode of action on metal-triggered molecular mechanisms. As a result, several ABC subfamilies were strongly modulated by different metal exposures with a strong induction of P-gp. Based on these results, we demonstrated that *T. japonicus* has a strong resistance against metal exposure with modulation of whole ABC transporters as a cellular metal defense system. This finding gives a better understanding on molecular defense mechanisms involved in whole ABC transporters-mediated metal detoxification in marine copepods.

WP222 Bioaccumulation of metals and genotoxic effects on fish from Negro and Solimões-Amazonas tributaries, Brazilian Amazon

M.R. Ferreira, Instituto Nacional de Pesquisas da Amazônia; M.C. Gross, Universidade Federal do Amazonas; Y.G. Goch, R. Peleja, Universidade Federal do Oeste do Pará; E.A. Souza, W.R. Bastos, Universidade Federal de Rondônia; F.X. Domingos, Instituto Nacional de Pesquisas da Amazonia – INPA

The presence of metals, especially mercury, is a cause of concern for animal and human health in the Amazon. In addition to mercury input from mining activities on the Tapajós and Madeira rivers, the natural background levels this metal are known to be high in the Rio Negro basin. Bioaccumulation of metals on muscle (Hg, Cu, Zn, Cr, Fe, Mn, Ni, Pb and Co) and genotoxic effects on blood samples were evaluated in the predator fish *Hoplias malabaricus*, an important food source for riverine communities. The fish were evaluated in tributaries from two hydrological basins with different physicochemical characteristics. Solimões-Amazonas waters contain high levels of suspended solids, neutral pH and low concentrations of dissolved organic carbon. Waters from the Negro basin present acidic pHs and high concentrations of dissolved organic carbon due to the presence of humic substances. The concentrations of chromium in fish muscle exceeded the Brazilian law recommendation of 0,1 mg/kg in two sampling areas from the Solimões-Amazonas river basin. The concentrations of mercury in fish exceeded the Brazilian law recommendation of 1 mg/kg only in one sampling area from the Negro river basin. In contrast the concentrations of Cu, Zn, Fe, Mn, Ni, Pb and Co metals were under the law limits. Nevertheless the occurrence of micronucleus and nuclear abnormalities was observed in both basins, they were significantly higher in fish from the Negro river compared to fish sampled on the Solimões-Amazonas river tributaries. The association among environmental factors and biological effects will be presented and discussed for both basins. These preliminary data can contribute to the discussion on the maximum concentrations of metals allowed in aquatic environments to protect animal and human health in the Amazon.

WP223 Daphnia magna immobilization assay application to toxicity of metal salts and the effect of chelate in medium

T. Abe, Tsuruoka Nat. College of Technology / Dept. of Chemical and Biological Engineering; T. Sugawara, Tsuruoka National College of Technology; S. Takahashi, Tsuruoka National College of Technology / Dept. of Chemical and Biological Engineering

Various metal salts exist in environment, their come from not only natural source but also artificial source. Recently the diffusions of cesium, strontium and iodine induced radioactive environmental pollution because of the Fukushima Daiichi Nuclear Power Station accident in the Great East Japan Earthquake which occurred on March 11, 2011. The cesium-137 which is an isotope of cesium has radioactivity, and generates it by the nuclear fission of uranium-235. Of course radioactive compounds and element affect organisms and disrupt ecosystems. We expect that the metal salts also affect organisms (for example the metal ion). Since metal chloride is one of the forms which are stabilized as for a metal salt. Therefore, the toxic strength of metal chloride is investigated by *Daphnia magna* immobilization assay. Lithium chloride (LiCl), sodium chloride (NaCl), potassium chloride (KCl) rubidium chloride (RbCl) , and Cesium chloride (CsCl) are used as test reagent. *D. magna* immobilization test was carried out based on OECD Guidelines for the Testing of Chemicals (TG202). 20 neonates within 24 hours old were placed into M4 medium (exposure and control) under constant temperature (20±2 degree C). The neonates were observed at 24 and 48 hours after exposure and the number of affected and survived neonates were recorded. The EC_{50} in M4 medium was 61.6 mg LiCl/L, 5100 mg NaCl/L, 769.7 mg KCl/L, 77.8 mg RbCl, and 51.5 mg CsCl/L. The EC_{50} in M4 medium without EDTA was 21.7 mg LiCl/L, 2254 mg NaCl/L, 674 mg KCl/L, 64.5 mg RbCl, and 35.4 mg CsCl/L. These results correlate with chelate formation constant (logKf).

WP224 Ecosystem-Scale Selenium Modeling for the Mountaintop Coal Mining Region of Southern West Virginia and the Phosphate Mining Region of Southeast Idaho

T.S. Presser, US Geological Survey / National Research Program; J.P. Skorupa, US Fish & Wildlife Service / Environmental Contaminants Div

Cross-valley fills associated with waste rock disposal from mountaintop coal mining in southern West Virginia and from phosphate mining in southeastern Idaho are environmental selenium (Se) sources that have the potential to affect reproduction in fish and wildlife. Deformities indicative of Se toxicity recently have been found in fish within these regions. The current national water-quality Se criterion is 5 ppb, but fish tissue Se guidelines in the range of 5-8 ppm (whole-body, dry weight) are being proposed by regulatory agencies. Data is compiled here in food-web diagrams for representative sites to document the progression of Se trophic transfer across water, suspended particulate material, invertebrate, and fish; and to serve as the basis for developing site-specific ecosystem-scale models to predict Se exposure within the hydrologic settings and food webs for these regions. Water-column Se has proven to be an imprecise predictor of Se bioaccumulation in food webs because dietary Se makes up 95% of tissue Se in invertebrates and fish. Intervening links between water-column and fish Se concentrations are needed to account for species-specific Se bioaccumulation among food webs and site-specific Se partitioning and bioavailability at the base of those food webs. If there is a range of vulnerabilities (i.e., predator food web choice, efficiency of biotransfer from invertebrates, and response in toxicity tests) among site-specific fish species to be modeled, then choice of fish species is critical to protection because it determines food web dynamics, and hence the magnitude of biotransfer, through which Se is modeled. We quantify the hydrologic- and species-specificity needed to model maximum observed Se concentrations of 40 to 60 ppm for fish (whole-body, dry weight) in these systems. We also apply the model using a metric describing partitioning of Se between particulate material and dissolved phases (Kd) to link dissolved Se concentrations to fish tissue Se burden in a series of exposure scenarios that illustrate choices of species to be protected, hydrologic settings, and toxicity guidelines. The range of outcomes of these model runs: 1) accounts for critical sources of variability; 2) establishes an understanding of relevant and controlling variables; and 3) illustrates that environmentally safe dissolved Se concentrations will differ among ecosystems depending on the ecological pathways and hydrological conditions in those systems.

WP225 Effects of dietary selenium exposure on swimming performance and energy homeostasis in two juvenile fish species

L. McPhee, University of Saskatchewan / Toxicology Centre, Toxicology Graduate Program; D.M. Janz, University of Saskatchewan / Toxicology Centre

Selenium (Se) is an environmental contaminant known to cause chronic toxicity in aquatic species. It is an essential trace element in fish with a narrow range between essentiality and toxicity. In particular, dietary exposure of fish to selenomethionine (SeMet), the primary form of Se in the diet, is of concern. Recent studies suggest that chronic dietary exposure to SeMet alters swim performance, as well as energy and endocrine homeostasis in adult fish. However, little is known about the direct effects of dietary Se exposure in juvenile fish. The present study was designed to investigate and compare sublethal physiological effects of dietary SeMet exposure in juvenile fathead minnow (*Pimephales promelas*), a warm water species, and rainbow trout (*Oncorhynchus mykiss*), a cold water species. Twenty days-post-hatch (dph) fathead minnow and fifteen dph rainbow trout were exposed for 60 and 37 days respectively to different nominal concentrations (1.0, 3.0, 10.0, 30.0 ug Se/g dry mass) of Se in food in the form of SeMet. After exposure, samples were collected for Se analysis and fish were subjected to a swimming performance challenge (critical swimming speed, Ucrit). A decrease in Ucrit was observed in the 10.0 and 30.0 ug Se/g exposure groups compared to the control group in both test species. Greater oxygen consumption (MO2) and cost of transport (COT) was observed in the 10.0 and 30.0 ug Se/g exposure groups compared to the control in fathead minnow. Energy storage capacity was measured via whole-body glycogen and triglyceride concentrations. Triglyceride and glycogen concentrations in fatigued and non-fatigued fish were significantly different in the 3.0 ug Se/g group relative to controls in fathead minnow. Rainbow trout will be analyzed for the same metabolic and energetic endpoints as the fathead minnow. The stress biomarker cortisol will also be determined in both species. The results from this study will generate a comparative analysis to gain new insights into the sublethal effects of dietary Se exposure on juvenile fish species.

WP226 Effects of maternally derived methylmercury on fathead minnow (Pimephales promelas) embryo neurodevelopment

K. Nielsen, UNT / Department of Biology, Institute of Applied Science; A.P. Roberts, University of North Texas

Mercury is a widespread environmental contaminant released through a variety of natural processes and anthropogenic activities. Upon deposition into aquatic systems, mercury partitions into sediments where it can be transformed by bacteria into methylmercury, a highly bioavailable form that bioaccumulates and biomagnifies through aquatic food chains. Dietary methylmercury may be transferred from adult female fish to eggs during oogenesis. Maternal transfer may induce neurotoxic effects in the offspring, affecting responses necessary for both hatching success and predator evasion responses. The purpose of this study is to investigate the potential neurodevelopmental effects of maternally transferred mercury in a model fish species (fathead minnow; *Pimephales promelas*). Few studies exist which examine these potential impacts under environmentally relevant concentrations and exposure scenarios i.e. maternal transfer. In order to assess the potential neurodevelopmental effects of dietary methylmercury, adult minnows were fed one of three experimental diets spiked with methylmercury. Embryos from exposed fish were collected, counted, evaluated for frequency of movement as well as methylmercury concentrations. At the conclusion of the study, methylmercury concentrations in muscle and ovaries from adult fatheads were determined. Results of the study demonstrate that clutch sizes and spontaneous embryo movements per minute decrease when adults consume flake food spiked with 0.59 ppb methylmercury, whereas time to hatch increased by nearly 50%. Data from this study may be incorporated into risk assessments for methylmercury toxicity in fish.

WP227 Evaluation of DGT for predicting bioaccumulation of Cu in Southern Toads (Anaxyrus terrestris)

R.R. Philipps, Savannah River Ecology Laboratory; S.L. Lance, D.E. Scott, G.L. Mills, University of Georgia / Savannah River Ecology Laboratory

Metals are known to cause both acute and chronic toxicity to a wide range of organisms at low concentrations. The common practice of analyzing water or sediment for total metals does not accurately assess the impact of metals on organisms due to the fact that not all metal species are bioavailable. Several chemical techniques have been employed to measure the bioavailable fraction of metals but none of these methods can be readily done in-situ and thus, extrapolating these results to the natural environment continues to present difficulties. Diffusive gradients in thin-films(DGT) were designed as a passive diffusion, in-situ sampling device with the goal of providing a rapid method for determining the free and labile portions of dissolved metal in surface waters, i.e. the bioavailable fraction. In addition, DGT may provide an alternative to measuring bioaccumulation and bioavailability via tissue analysis of organisms which requires the collection and analysis of large numbers of samples. Thus, passive diffusion samplers that measure the bioavailable metal species are potentially a cost effective option for evaluating environmental risk from metal contamination, but additional studies are needed to validate this approach. DGT have been shown to correlate well with bioaccumulation in plants, but little work has been conducted comparing DGT assessment of bioavailability to aquatic animals. In this study DGT were coupled with bioassays using Southern Toad tadpoles (*Anaxyrus (Bufo) terrestris*) in a controlled laboratory setting (24° C ± 1°, 12 hour dark:12 hour light, soft water mixed according to USEPA, 2002). Three concentrations (0 ppb, 5 ppb, 12 ppb) of Cu were applied to 3.7 liter containers containing 5-10 larval *A. terrestris* and a DGT for set time periods (6, 9, 12, 15, 18, and 21 days). On the final day for each set of replicates, the tadpoles and DGT, along with water samples for total and dissolved (0.45 µm) metals, dissolved organic carbon, alkalinity, and cations were collected. Measurements of pH, temperature(°C), dissolved oxygen, and ORP were also taken. Copper concentrations in *A. terrestris* tissue, filtered and unfiltered water samples, and DGT resin layer eluant were determined using inductively-coupled plasma optical emission spectrometry. The results of this study provide additional data for comparison of the DGT passive diffusion technique to amphibian larvae bioaccumulation of metals and toxicity monitoring.

WP228 Evidence of Mercury Toxicity in Alaskan Yelloweye Rockfish

B.D. Barst, INRS-ETE; P.E. Drevnick, Inrs-Ete, Universite Du Quebec / INRS-ETE

Mercury (Hg), released into the atmosphere through natural or anthropogenic sources, can travel for great distances resulting in increased deposition in even remote areas. Rapidly increasing Hg emissions in Asia, transported across the Pacific, may be causing increased Hg deposition in southeast Alaska, USA. In aquatic environments, bacteria are capable of converting Hg into methylmercury (MeHg), which readily bioaccumulates and biomagnifies in food webs. For this reason, higher Hg concentrations are found in older and larger fish, feeding at higher trophic positions. Yelloweye rockfish (*Sebastes ruberrimus*) are very long-lived fish (up to ~120 years), native to southeast Alaska. The purpose of this study was to determine the effects of Hg bioaccumulation on the health of yelloweye from Prince of Wales Island (POW), AK. Yelloweye rockfish were collected from the east and west coasts of POW and Hg was measured in their muscle, livers, and spleens. Both splenosomatic and hepatosomatic indices were negatively correlated with total Hg in the organs. Additionally, we noted positive correlations between the percent coverage and numbers of macrophage aggregates and Hg concentrations in livers and spleens of these fish. Normal hepatic and splenic tissues, as well as macrophages, were analyzed by laser ablation ICP-MS, revealing a co-localization of Hg within the immune cells. We conclude that Hg is negatively affecting the health of yelloweye rockfish from POW. Future work will focus on the analysis of mercury and lead isotopes in yelloweye rockfish otoliths using microchemistry techniques.

WP229 Examining patterns of selenium bioaccumulation in fish across North America

A.M. deBruyn, A. Atkinson, Golder Associates Ltd; S.A. Hughes, Shell Health – Americas; G. Gilron, Borealis Environmental Consulting

This study is a component of a collaborative initiative aimed at supporting a proposed third-party water quality guideline for selenium for Canada. The specific objective of this analysis was to use available data to characterize selenium bioaccumulation in a range of fish species from sites across Canada and the US. We obtained fish tissue selenium and aqueous selenium concentration data from ten regions across North America. We derived 21 separate bioaccumulation relationships (BARs) as general linear models of log-transformed data. Per data availability, models were derived for 15 fish species, for whole body, muscle, and egg/ovary concentrations, and for lentic and lotic environments. We also examined the influence of aqueous sulphate concentration on selenium bioaccumulation. The most general finding of this analysis was that selenium concentrations in fish are related to selenium concentrations in

water, but this relationship is not linear: all reliable, fitted BAR models had slopes less than unity. The BAR models varied substantially among species, between lentic and lotic environments, and across a range of aqueous sulphate concentrations. Lotic fish tended to have shallower BAR slopes than lentic fish, although there was substantial overlap in the ranges of observed BAR model slopes. Sulphate appeared to inhibit selenium uptake and should be considered as a modifying factor when developing selenium benchmarks.

WP230 Heavy metal content in liver and muscle of Katsuwonus pelamis and Thunnus obesus from the Atlantic Ocean (Azores, Portugal)

P.V. Garcia, University of Azores / Department of Biology, CITA-A – Azorean Biodiversity Group; L.C. Soares, University of Azores / Biology; A.S. Rodrigues, University of Azores / Department of Biology, (CVARG) Center of Volcanology and Geological Risks Assessment

The concentrations of cadmium (Cd), lead (Pb) and mercury (Hg) (expressed in mg kg^{-1} wet weight) were determined in liver and muscle samples of 15 skipjack tuna (*Katsuwonus pelamis*) and 15 bigeye tuna (*Thunnus obesus*) from the Atlantic Ocean (Azores, Portugal). There were significant interspecific differences for Cd and Hg contents in both tissues: Cd concentration was, in average, significantly higher in the liver of *K. pelamis* than in *T. obesus* (11.06 vs. 0.73 mg kg^{-1}), while Hg was significantly higher in the muscle tissue ofbigeye tuna (0.14 vs. 0.04 mg kg^{-1}). No significant association was observed between tuna weight and heavy metal contents, except for muscle Hg concentrations in *T. obesus* that increased with the weight of the individuals. Significant differences were observed for heavy metal concentrations between tissues within species: for skipjack tuna, Cd content was about 70-fold higher (11.06 mg kg^{-1}) in liver than in muscle tissue (0.16 mg kg^{-1}); in bigeye tuna, concentrations of Cd and Pb (0.73 and 0.09 mg kg^{-1}, respectively) were also higher in liver tissue when compared to muscle (0.19 and 0.04 mg kg^{-1}, respectively), while Hg content was about 10-fold higher in muscle tissue (0.14 vs. 0.01 mg kg^{-1}). Mercury concentration in muscle tissue ranged from 0.01 (skipjack tuna) to 0.33 mg kg^{-1} (bigeye tuna) and none of the samples exceeded the EU limit. Lead concentration in muscle tissue ranged from 0.03 (both species) to 0.31 mg kg–1 (bigeye tuna), with only one sample above the EU limit. However, the concentration of Cd in muscle exceed in 53% (bigeye tuna) and 26% (skipjack tuna) of the samples the permissible limits set by the European Union for edible tissues, revealing that the concentration of Cd must be monitored comprehensively with respect to consumers' health. The elevated concentrations of Cd in liver tissue, especially of *K. pelamis,* are also of concern since the viscera are used to produce livestock feed and thus can affect animal and, ultimately, human health.

WP231 How variable is selenium sensitivity among fish species?

E. Costa, A.M. deBruyn, Golder Associates Ltd.

There is a perception in the scientific literature that fish species have similar sensitivity to selenium, with a threshold for reproductive toxicity that consistently occurs between approximately 20 and 25 mg/kg dry weight in eggs. We examined the available toxicity data to evaluate this perception. Our analysis suggests that whereas this range is accurate for some species, it appears to be far from universal. Some of the studies that have been cited to support this narrow range in fact suggest greater tolerance than previously considered. In addition, emerging data indicate relatively high effects thresholds in a range of species. We explore some potential physiological and environmental mechanisms for the observed variability in sensitivity among fish species.

WP233 Modeling the transfer of selenium at the base of an aquatic food web: a novel approach to reduce uncertainty

T.M. Eastham, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment)

Selenium (Se) is a naturally occurring element and an essential micronutrient for many organisms including humans. However, at concentrations only slightly greater than those necessary for biological function it can become teratogenic. Diet is the main route of Se uptake and thus biomagnification must be considered when assessing its potential to cause adverse effects. Currently, the mechanisms underlying its accumulation remain unclear and traditional methods of modelling Se bioaccumulation contain a high degree of uncertainty, especially when predicting the transfer of Se from water to primary producers at the base of the food web. This work provides a novel approach to modelling the uptake and transfer of Se from water to algae in an effort to reduce the overall uncertainty inherent in Se bioaccumulation models. The approach models K_d as a variable, rather than a constant, and considers other aspects of water chemistry, such as sulfate and nitrate, which may affect the rate of uptake. A more thorough understanding of Se bioaccumulation will help regulators better identify wildlife that is at risk of Se toxicosis, and help develop management strategies to mitigate the potential environmental impacts of Se.

WP234 Non-linearity in the bioaccumulation of selenium in aquatic ecosystems

A.M. deBruyn, A. Atkinson, Golder Associates Ltd; T.M. Eastham, Simon Fraser University / School of Resource and Environmental Management (Faculty of Environment)

Various approaches have been taken to modeling the bioaccumulation of selenium, including ratio-based bioaccumulation factors, regression models that relate selenium concentrations in biota directly to those in water or sediment, and multi-step approaches that mimic stepwise uptake and trophic transfer processes as a series of linked ratios or bivariate relationships. A key differentiating factor of these models is that some assume linearity, whereas others do not. Models based on ratios inherently assume that a given factor increase in aqueous selenium concentration will produce the same factor increase in biota. We found that this assumption is not supported – regression models of selenium bioaccumulation consistently exhibit non-linearity. We show that the strongest evidence for non-linearity is in the initial uptake step from water to primary producers, although there is some indication of non-linearity in subsequent trophic transfer steps as well. The inaccuracy in model predictions that arises from incorrectly assuming linearity is potentially large.

WP235 Tisa River Floodplains: Connectivity or Conduit for Contamination?

J.S. Paul, Southern Illinois University Carbondale / Department of Zoology; M.L. Brooks, Southern Illinois University / Dept of Zoology; N. Pinter, Southern Illinois University / Department of Geology

Restoring hydrologic connectivity to severed floodplains, as suggested for increasing flood control capacity of regulated rivers, may inadvertently introduce contaminants to adjacent landscapes. The Tisa River in Eastern Europe has been chronically impacted by heavy metals from industry and mining. In addition, two major environmental disasters in 2000 released tons of sediment and wastewater contaminated with heavy metals into the river. Here we present preliminary data on the transfer of heavy metals from the Tisa River to floodplain food webs. During spring flood stage and lower summer flows in 2013, we evaluated sites on the lower Tisa in Hungary and Serbia across a range of hydrologic connectivity with the main channel. Investigations include metals in water, sediment, detritus, phytoplankton, epiphytic algae, macrophytes, zooplankton, and macroinvertebrates. Trophic transfer of heavy metals were assessed by regressing the ratio of stable nitrogen to stable carbon isotopes ($\delta^{15}N/\delta^{13}C$) against body burdens of metals. Although data analysis is still underway, initial geochemical results indicate that metals concentrations are negatively correlated with the degree of hydrologic connectivity to the main channel Tisa. Thus, disconnected floodplain habitats appear to serve as refugia for aquatic organisms, particularly in the event of environmental accidents affecting the main channel. Reconnection efforts should consider wetland buffer zones and a gradient of hydrologic connectivity.

WP236 Tissue metal concentrations in yellow and silver European and American eels from France and Canada

P. Pannetier, INRS – Centre Eau Terre Environnement; M. Baudrimont, Université de Bordeaux 1 / UMR EPOC – EA; P.G. Campbell, P. Couture, INRS / Centre Eau Terre Environnement

Eel populations are facing a world-wide decline, with the American (*Anguilla rostrata*) and European (*Anguilla Anguilla*) eel stocks reported to have declined between 40 and 80% and 90 to 99% in the last decades for American and European eels, respectively. As a result, the American eel is now classified as threatened in several jurisdictions, whereas the European eel is listed as in critical danger of extinction. Several causes have been identified as likely contributors to this decline, including overfishing, obstacles to migration (hydroelectric dams), parasitism, climate change and habitat contamination. IMMORTEEL is a joint French and Canadian research project that has the overall objective of examining the role of contaminants in the synchronous decline of the two species of Atlantic eels. Because eels live in close contact with sediments, are very long lived (up to forty years or more in some locations), are at the top of the aquatic food chain and contain a high percentage of lipids, especially towards the end of their growth phase preceding their

ultimate reproductive migration, many organic (pesticides, dioxins, furans etc.) and inorganic (metal) contaminants accumulate in their tissues. Contaminant accumulation could severely impact their life cycle, growth, migratory and reproductive capacities. Trace metals like cadmium, lead, mercury and chromium are known for their strong toxicity in eels, which can accumulate them directly through the gill, by contact with sediment or trophically. In order to identify interspecific differences and similarities in inorganic contaminant tissue concentrations and (in other components of this project) their link with biomarkers of toxicity, we measured Ag, As, Cd, Cr, Cu, Hg, Ni, Pb, Se and Zn concentrations in kidney and muscle of both species of Atlantic eels. Generally, renal metal concentrations were significantly higher than those measured in muscle. Eels from the contaminated site in France presented higher concentrations of Ag, As, Cd, Hg, Pb and Se compared to fish from other sites. Surprisingly, in spite of our efforts to sample eels from contaminated and clean sites in Canada, no site was identified as systematically yielding more contaminated fish, except for Hg and Se muscle concentrations that were higher in sites expected to be contaminated. When fish were pooled, they represented a broad gradient of metal contamination, which should allow examination of their links with biomarkers of toxicity.

WP237 Toxicity of mixtures of cadmium, selenium, nitrate and sulphate to Ceriodaphnia dubia

E. Costa, A.M. deBruyn, Golder Associates Ltd.; J.R. Elphick, Nautilus Environmental

The potential for unexpected interactions among constituents to result in increased toxicity has been identified by the scientific community and regulatory bodies as a source of uncertainty in the development of water quality guidelines and objectives that are protective of aquatic life. The purpose of this research was to assess the potential for the toxicity of mixtures of selenium (Se), cadmium (Cd), nitrate (NO_3) and sulphate (SO_4) to differ from the toxicity of individual constituents. Test solutions were evaluated with a *Ceriodaphnia dubia* 7-d reproduction and survival test according to Environment Canada procedures. Three rounds of mixture toxicity tests (nine mixtures total) were conducted with constituent concentrations ranging from 0.02 to 1 µg Cd/L, 4.5 to 52.7 mg NO_3-N/L, 19.5 to 139 µg Se /L and 117 to 934 mg SO_4/L in laboratory water or water from a site in British Columbia, Canada. The sensitivity of *C. dubia* to $NO_3$and SO_4 was also assessed by conducting constituent-specific toxicity tests in a range of water types. No adverse effects were observed in mixture tests containing up to 0.08 µg Cd/L, 17.9 mg NO_3-N/L, 468 mg SO_4/L and 77.5 µg Se/L in combination. Survival and reproduction effects were observed at higher test concentrations and were attributed to NO_3 and SO_4. In one test, observed effects were disproportionate to the individual concentrations of NO_3 and SO_4, suggesting a possible interaction or an effect of elevated total dissolved solids.

WP238 Toxicity of copper sulfate and copper-ethanolamine to Microcystis aeruginosa and Pseudokirchneriella subcapitata at different initial cell densities

K. Tsai, Clemson University; J.H. Rodgers, Clemson University / School of Agricultural, Forest, and Environmental Sciences

Effects of cell density, mass, surface area, and volume of *Microcystis aeruginosa* (prokaryote) and *Pseudokirchneriella subcapitata* (eukaryote) on their relative sensitivities to exposures of copper sulfate and copper-ethanolamine were measured. For these experiments, *M. aeruginosa* UTEX 2385 and *P. subcapitata* UTEX 1648 were cultured in BG11 medium to three initial cell densities (5×10^4, 5×10^5, and 5×10^6 cells/mL). Exposures ranged from 5 to 20000 µg Cu/L and copper concentrations in the treatments were measured using a graphite furnace atomic absorption spectrophotometer. Chlorophyll *a* concentrations and cell densities were measured as response endpoints after 96-h exposures. Cell surface area and volume were estimated by measuring cell dimensions with the hemacytometer and light microscope, and cell mass was measured as dry weight. 96-h EC_{50} estimates were calculated using probit analysis, and potency slopes were calculated from the linearized portions of exposure-response curves. 96-h EC_{50} values of copper sulfate for *M. aeruginosa* at the three cell densities were 5, 67 and 114 µg Cu/L, respectively; and for *P. subcapitata*, were 88, 2844 and 5285 µg Cu/L, respectively. For *M. aeruginosa* and *P. subcapitata* at 5×10^6 cells/mL, the 96-h EC_{50} values of copper-ethanolamine were 103 and 4626 µg Cu/L. For this experiment, *M. aeruginosa* was more sensitive than *P. subcapitata* to exposures of copper sulfate. Copper-ethanolamine was more toxic than copper sulfate to *M. aeruginosa* and *P. subcapitata*. *M. aeruginosa* and *P. subcapitata* had similar cell mass (15 and 16 pg/cell, respectively). In terms of cell surface area and cell volume, *M. aeruginosa* (186 μm^2/cell and 255 μm^3/cell) was about two times smaller than *P. subcapitata* (425 μm^2/cell and 416 μm^3/cell). Thus, cell mass, cell surface area, and cell volume can not explain the difference in sensitivities of *M. aeruginosa* and *P. subcapitata* to exposures of copper in this study.

Nanoparticles

WP239 Biosynthesis of gold nanoparticles by living freshwater diatom Eolimna minima: potential nanofactories or a contamination route in aquatic ecosystems

A. Feurtet-Mazel; L. Charron, University of Bordeaux; N. Mesmer-Dudons, CNRS/UMR EPOC; R. Maury-Brachet, M. Baudrimont, University of Bordeaux

Studies concerning nanomaterials synthesis and those focused on the potential risks incurred by their dispersion in the environment are always tackled separately. In order to consider jointly both aspects of the study of nanomaterials and in a concern for research of nanomaterials more environmentally friendly throughout their lifecycle, this work has therefore pursued a double objective: (i) Test the capacities of biotransformation of living aquatic organisms such as micro algae diatoms to naturally synthetize AuNP from gold salts; (ii) Assess the aftereffects of this biotransformation on the viability status of diatoms by microscope observations. In this study, we conclude to the good capacity of living diatoms to biosynthetize AuNP inside their cellular content. We also point out the risks to spread diatoms full of AuNP through the trophic web of the freshwater ecosystems. These results show the first results of our studies highlighting the role of diatoms in AuNP synthesis while keeping up in a state of cellular viability at different AuNP concentrations, but expelling AuNP outside the cells at higher concentrations as revealed by Tranmission Electron Microscopy.

WP240 Dietary uptake and photoenhanced toxicity of titanium dioxide nanoparticles in Daphnia magna

C.M. Mansfield, University of North Texas / Department of Biology, Institute of Applied Science; A.P. Roberts, University of North Texas

Titanium dioxide nanoparticles (TiO_2-NP) are one of the most abundantly utilized nanomaterials in the world. Studies have demonstrated the mechanism of acute toxicity in TiO_2-NP to be the production of reactive oxygen species (ROS) leading to oxidative stress and mortality in exposed organisms. It has also been demonstrated that the anatase crystalline conformation is capable of catalyzing the cleavage of water molecules to further increase the concentration of ROS in the presence of ultraviolet radiation. This photoenhanced toxicity significantly lowers the toxicity threshold of TiO_2-NP to environmentally relevant concentrations (ppb). The goal of this study was to determine whether dietary uptake and accumulation of TiO_2-NP in the aquatic filter feeder D*aphnia magna* resulted in photoenhanced toxicity. *D. magna* and *S. caprincornatum* were exposed to aqueous solutions of 20ppm and 200ppm TiO_2-NP for 24hrs and then transferred to clean moderately hard water. Samples were taken at at various time points,dried and quantified using ICP-MS. Toxicity assays were run on *D. magna* using three TiO_2-NP (20ppm, 200ppm) exposure protocols and two ultraviolet radiation treatments. The first exposure group was exposed to aqueous solutions of TiO_2-NP for the duration of the test. The second exposure group was exposed to TiO_2-NP for an hour and then transferred to clean water. The third exposure group was fed *S. capricornatum* that had been allowed to adsorb TiO_2-NP. All samples were then placed in an outdoor UV exposure system and exposed to either full spectrum sunlight (with UV) or filtered sunlight (no UV). Here we show that TiO_2 uptake peaked at one hour of exposure likely due to sedimentation of the particles out of suspension thus decreasing bioavailability for the duration of the test. Interestingly, when *D. magna* were moved to clean water, aqueous concentrations of TiO_2 increased as a result of depuration from the gut tract. Data also suggests these excreted particles were bioavailable and re-consumed by *D. magna*. These data will contribute to the understanding of TiO_2-NP environmental fate and toxicity.

WP241 Effect of exposure time on acute toxicity of CuO and ZnO nanoparticles to Daphnia magna

S. Kim, Korea University

This study investigates the effect of exposure time (24h, 48h and 72h) on acute toxicity of CuO and ZnO nanoparticles (NPs) to *Daphnia magna*, and the toxicity results were compared with those of corresponding metal ions (Cu^{2+} and Zn^{2+}). Acute toxicity of CuO NPs was significantly increased

as exposure time increased ($p < 0.05$), whereas acute toxicity of Cu^{2+} ions was not dependent on exposure time. Considering that size of Cu NPs increased with increasing exposure time, the aggregation of Cu NPs largely affected the acute toxicity. In addition, CuO NPs concentration greatly influenced size of aggregates (>5 mm at 10 mg L^{-1} and 400 – 800 nm at 0.312 – 0.625 mg L^{-1}). The micro-sized CuO was coated on the body of *D. magna*, whereas the nano-sized CuO was easy to be ingested. In the case of zinc, exposure time did not influence the acute toxicity of ZnO NPs and Zn^{2+} ions significantly ($p > 0.05$), possibly due to dissolution of ZnO NPs. These findings suggest that exposure time and NPs concentration should be selected carefully, due to aggregation and dissolution of NPs depending on.

WP242 Effects of gold nanoparticles on early development of Oryzias latipes embryo

Y. Shin, Konkuk University / Department of Environmental Science; Y. An, Konkuk University / Department of Environmental Sciences

Gold nanoparticles(AuNPs) have been considered most stable metallic nanoparticles and used in wide applications due to inactivating chemical properties. However, several studies reported ecotoxicity of AuNPs. Among them, all toxicity tests of AuNPs on early development of fish were conducted only using *Danio rerio* (Zebrafish) embryos. Therefore, we investigated the toxicity of AuNPs on *Oryzias latipes*(Japanese medaka) embryos. And we also performed two exposure systems that were embryonic exposure and continuous exposure for comparison of effects by exposure duration. 1) Embryonic exposure; the *O. latipes* embryos were exposed to AuNPs before hatch and then transferred to NPs free test medium and observed until 16th day post-fertilized(dpf). 2) Continuous exposure; the embryos were exposed to AuNPs continuously until 16th dpf. We observed that developmental malformations include blood vessel ordinary (BO), hemostasis (HS), pericardial edema (PE), tube heart (TH), yolk destroy (YD), abnormal body axes, tail malformation, and necrosis and delayed hatching relate to increasing of concentration. Most abnormalities were occurred early developmental stage and most of them led to mortality by exacerbated malformation after or before hatching. Besides, the larvae that were not affected by AuNPs were normally hatched and not shown any effects after hatch, if the exposure was continued. And also, there are no significant different of toxicity of AuNPs by exposure duration. That means that AuNPs can adverse and irreversible affect on early development stage of fish embryo when they are release into aquatic ecosystem. This work was supported by the National Research Foundation Grant funded by the Korean Government (NRF 2011- 0015985). The authors thank the Korean Basic Science Institute for IMP-CLSM and ICP-MS analyses.

WP243 Fate and Toxicity of Titanium Dioxide in Aquatic Ecosystems

J. Coral, Clemson University Institute of Environmental Toxicology; J.L. Brumaghim, Clemson University / Chemistry; S.J. Klaine, Clemson University / Institute of Environmental Toxicology

TiO_2 nanoparticles are a common component for a multitude of everyday items. One common use of these particles is use in sunscreen. As a result of aggressive sunscreen campaigns sponsored by the CDC in the early 2000's, sunscreen use is high. While protecting sunbathers there is concern that the nanoparticles that make these sunscreens effective will wash off and enter aquatic systems. The fate and impact of these nanoparticles in aquatic ecosystems is uncertain. Recent research has shown that these nanoparticles form reactive oxygen species (ROS) that may be harmful to aquatic organisms. The objective of this research is to quantitatively characterize reactive oxygen species produced as a consequence of TiO_2 nanoparticles being exposed to light in fresh water. ROS will be measured by Electron Paramagnetic Resonance spectroscopy. Preliminary results indicate that ROS production is dependent on water quality characteristics such as pH and the presence of dissolved organic carbon. Once quantified and characterized, *Daphnia magna* will be exposed to the TiO_2 solutions in an attempt to correlate organism response with kinds and magnitudes of ROS.

WP244 Histopathology of fathead minnow (Pimephales promelas) exposed to hydroxylated fullerenes

B. Jovanovic, Ludwig Maximilians University of Munich / Fisheries Biology and Fish Diseases; E. Whitley, The College of Veterinary Medicine, Department of Veterinary Pathology, Iowa State University; D. Palic, Fish Diseases and Fisheries Biology, Faculty of Veterinary Medicine, Ludwig Maximilians-University of Munich

Hydroxylated fullerenes are reported to be very strong antioxidants, acting to quench reactive oxygen species (ROS), thus having strong potential for important and widespread applications in innovative therapies for a variety of disease processes. However, their potential for toxicological side effects is still largely controversial and unknown. Effects of hydroxylated fullerenes $C_{60}(OH)_{24}$ on the fathead minnow (*Pimephales promelas*) were investigated microscopically after a 72 hour (acute) exposure by intraperitoneal injection of 20 ppm of hydroxylated fullerenes. Cumulative, semi-quantitative histopathologic evaluation of brain, liver, anterior kidney, posterior kidney, skin, coelom, gills, and the vestibuloauditory system revealed significant differences between control and hydroxylated fullerene-treated fish. Fullerene-treated fish had much higher cumulative histopathology scores. Histopathologic changes included loss of cellularity in the interstitium of the kidney, a primary site of hematopoiesis in fish, and loss of intracytoplasmic glycogen in liver. In the coelom, variable numbers of leukocytes, including many macrophages and fewer heterophils and rodlet cells, were admixed with the nanomaterial. These findings raise concern about *in vivo* administration of hydroxylated fullerenes in experimental drugs and procedures in human medicine, and should be investigated in more detail.

WP245 The influence of exposure route on accumulation and toxicity of silver nanoparticles to the deposit feeder Lumbriculus variegatus (Oligochaeta)

J. Son, School of Biological Sciences; V.E. Forbes, University of Nebraska Lincoln / School of Biological Sciences; J. Vavra, UNL-Lincoln / School of Biological Sciences

With the increasing production and widespread use of silver nanoparticles, there is increasing concern over their potential risk to the aquatic environment, especially to the sediment where the released nanoparticles will eventually be transported and/or deposited. Given the potential routes of exposure by which aquatic species are exposed, it is therefore imperative to compare the relative importance of different exposure routes because they can influence the accumulation and toxicity of silver nanoparticles. In this study, the deposit feeder *Lumbriculus variegatus* was exposed in either water-only or sediment-spiked systems under a range of silver nanoparticle concentrations to compare how different exposure routes affect accumulation and toxicity of silver nanoparticles. The results of this study improve our understanding of how benthic invertebrates are exposed to silver nanoparticles and can be particularly useful for assessing risks of silver nanoparticles to the aquatic environment.

WP246 Toxicity assessment in photocatalytic degradation of organic pollutants by 3D nanofiber membranes decorated with TiO2 nanoparticles

A. Jo, Korea University / Division of Environmental Science & Ecological Engineering; M. Lee, Korea University / School of Mechanical Engineering

Among chlorophenols (CPs), 2,4-DCP and 2,4,6-TCP are found to be highly toxic and hardly biodegradable, making them are difficult to completely remove from industrial wastewater. Thus, advanced oxidation processes (AOPs) including Fenton reaction, UV photocatalysis and ozonation have been studied in treatment of CPs. In this study, photocatalytic degradation of 2,4-dichlorophenol (2,4-DCP) and 2,4,6-trichlorophenol (2,4,6-TCP) were investigated using TiO_2 nanoparticles in 3D nanofiber membranes. Photocatalytic experiments were carried out under UV light (λ = 365 nm and I = 0.6 mW/cm^2), and a TiO_2-decorated nanofiber mat was placed inside an open Pyrex Petri dish containing the aqueous solution. Acute toxicity tests were carried out using *Daphia magna* neonates (≤24 h old) before and after photocatalytic treatments. Acute toxicity of 2,4-DCP (5×10^{-4} M) to *D. magna* was almost disappeared from 2.74 TU after photocatalytic treatment for 3 h. Moreover, this treatment was also effective to remove more toxic 2,4,6-TCP (5×10^{-4} M), dramatically reducing acute toxicity from 9.85 TU to 2.78 TU. These findings suggest that the TiO_2-decorated nanofiber mat photocatalysis is a promising technique to remove toxicity from industrial wastewater containing highly toxic organic pollutants. However, formation of intermediates and their toxicity should be investigated with recalcitrant organic pollutants including high-production-volume chemicals and emerging pollutants.

Environmental Implications of Biochar

RP001 Biochar and soil properties affecting microbial transport through biochar-amended soils

C. Bolster, USDA-ARS; S. Abit, Oklahoma State University; K. Cantrell, USDA-ARS; J. Quijano, University of California at Riverside; S. Walker, University of California, Riverside

The incorporation of biochar into soils has been proposed as a means to sequester carbon from the atmosphere. An added environmental benefit is that biochar has also been shown to increase soil retention of nutrients, heavy metals, and pesticides. We have recently conducted a series of experiments to evaluate whether biochar amendments can also affect microbial transport through soil. We have investigated the role of biochar feedstock type (poultry litter extract and pine chips), biochar pyrolysis temperature (350 and 700 °C), biochar application rate (1, 2, and 10%), soil moisture content (saturated and 50% saturation), soil texture (1 and 12 % clay content), and bacterial surface properties on microbial transport through biochar-amended soils. Under most conditions we found that biochar addition to soils could significantly reduce bacterial transport – in some cases up to five-orders-of-magnitude reduction in transport was observed. In general pine chip biochars were much more effective than poultry litter biochars at increasing microbial retention in our columns. Indeed, in some cases we observed an increase in microbial transport following addition of poultry litter biochars. High temperature biochars were generally more effective at increasing microbial retention in soils than low temperature biochars. Biochar addition to soils was observed to be more effective in partially saturated soils than fully saturated soils. We also found that bacteria with contrasting surface properties responded to biochar additions differently. Our results show that the addition of biochar can affect the retention and transport behavior of bacteria and that biochar application rate, biochar feedstock source, biochar pyrolysis temperature, soil moisture content, soil texture, and bacterial surface characteristics were all important factors determining the transport of bacteria through soil. Our results also suggest that the interactions between biochar and bacteria are complicated and need further investigations to determine the mechanisms involved.

RP002 Effects of biochar amendment on the growth of Canada wildrye (Elymus canadensis) in lead-contaminated soil from an historic industrial site

S.L. Edenborn, Chatham University; H.M. Edenborn, US Department of Energy

Sulfuric acid used to clarify kerosene by petroleum refineries in Titusville, PA was recycled at a nearby factory between ca. 1875-1917. The use of lead distillation pans and tank liners resulted in high total lead concentrations in the soil (up to 50,000 mg/kg), which remains unvegetated. The distribution of lead concentrations at the site is consistent with the location of industrial activities as recorded on old insurance maps. A survey of microbial activity and soil enzyme levels showed that the unvegetated soils were depleted in biological activity relative to surrounding soils. Experiments were done to investigate the potential influence of biochar amendments on the ability of Canada wildrye to become established on unvegetated soil from this site. Biochar was applied in different volumes to test soils using three different application methods: mixed throughout the soil, applied to the soil surface, and alternated with layers of soil. After 61 days of incubation, plant biomass was greatest (shoots and roots) for tests using the largest volumes (20% vol/vol) of incorporated and surface-applied biochar. Biochar-amended soils showed reduced concentrations of lead, consistent with the adsorption isotherms calculated for the biochar. Plants did not effectively incorporate lead, suggesting its exclusion by the plants, or the effective adsorption of bioavailable lead by biochar. The apparent ability of Canadian ryegrass to revegetate the biochar-amended soil could be beneficial in preventing future lead transport from the site via water and wind erosion.

RP003 Use of LiDAR technology to locate historic sources of biochar associated with charcoal iron furnaces in Pennsylvania

J.I. Sams, H.M. Edenborn, US Department of Energy

The location of sites where biochar has been deposited for many years will help in the assessment of its relative longevity in specific climates and provide examples of its long-term impact on soil health and forest ecology. In Pennsylvania, the charcoal iron industry was active throughout the 19th century, and charcoal prepared from hardwood trees provided the bulk of its fuel requirements. Charcoal hearth sites were abundant in the areas near the iron furnaces, but the locations of these sites are now frequently hidden due to land disturbances and the growth of new forest. Data processed in geographic information system (GIS) software from the Pennsylvania light detection and ranging (LiDAR) data acquisition program was used to identify historic charcoal hearths on land near several historic charcoal iron furnaces. For example, at Greenwood Furnace (1834-1904), Huntingdon County, PA, charcoal hearths and associated wagon roads were viewable in shaded relief images of the LiDAR digital elevation model (DEM) as flattened circular features. More than 500 historic charcoal hearths were identified in a relatively undisturbed and heavily-forested 40 square mile area surrounding Greenwood Furnace. Strings of hearths were readily evident along similar topographic contour lines on the adjacent hillsides. Similar charcoal hearth sites were also detectable at several other furnace sites examined, even on land that had been highly disturbed. Site visits confirmed the identity of the charcoal hearth sites and the uniform presence of abundant charcoal.

Point and Non-Point Sources of Organic Pollutants and Plastic Debris in the Marine Environment

RP004 Organic pollutants in the tropical and subtropical global oceans. Initial results from the Malaspina Circumnavigation expedition

J. Dachs, Institute of Environmental Assessment and Water Research -CSIC / Environmental Chemistry; B. Gonzalez, M. Fernandez-Pinos, L. Morales, IDAEA-CSIC / Environmental Chemistry; E. Abad, CSIC / Dioxin Laboratory – Environmental Chemistry Department; B. Jimenez, Institute of Organic Chemistry – CSIC / Instrumental Analysis & Environment; B. Pina, IDAEA-CSIC / Environmental Chemistry, jordi girona

The tropical and subtropical oceans, between 40N and 40 S, account for most of the surface of the global oceans, but they have received less attention in terms of the study of organic pollutants than Polar oceans (Arctic) and some marginal seas (Mediterranean, Baltic). Here, we present the initial results from atmospheric, seawater and phytoplankton samples taken during a 8-months long circumnavigation cruise (Malaspina Cruise) performed in 2011. During the cruise, we sectioned all the subtropical gyres in the North Atlantic and Pacific, south Atlantic, Pacific and Indian Oceans. The objectives of these ambitious study is to determine the spatial distribution of a wide range of organic pollutants such as PAHs, PCBs, perfluorinated compounds, etc, to study the exchange between the atmosphere and seawater and their accumulation in plankton, and finally to determine the potential influence of mixtures of organic pollutants to phytoplankton, especially on its carbon fixation potential. The initial results show for example, Some low MW PAHs are being volatilized from large regions of the ocean, and mid-high MW are being deposited. Concerning PFC, important spatial gradients have been found for acids and sulfonates, but with differences that may reflect different source strength in different regions. The Malaspina circumnavigation cruise is the first global synoptic study of the occurrence, cycling and impact of organic pollutants in the ocean.

RP005 Persistent Organic Pollutants in the Western South Atlantic Ocean

E. Markham, University of Rhode Island Graduate School of Oceanography; R. Lohmann, University of Rhode Island / Graduate School of Oceanography

Persistent organic pollutants, also known as POPs, are synthetic compounds that are distributed globally, have the tendency to persist in the environment, bioaccumulate through the food web, and may cause adverse effects in both humans and the environment. Those found in the environment today broadly fall under two categories – the"legacy" POPs that have been banned for decades, but are still detected at trace levels (e.g., polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs)), and the emerging class of contaminants that have only recently been recognized and may or may not yet be banned (e.g., perfluorinated compounds (PFCs)). The Western South Atlantic Ocean is a region that has gaps in data concerning both of these compound classes and for this reason, samples were taken in the spring of 2013 aboard the *R/V Knorr* from a transect beginning at approximately 37°S, 50°W and ending at 10°N, 55°W. The goals of this sample collection were to (i) gain spatially distributed depth profiles for the more recently recognized perfluorinated compounds (PFCs) and (ii) take high volume surface water and air samples for legacy hydrophobic pollutants such as PCBs, PAHs, and OCPs. Depth profiles of PFCs will reveal how far these compounds have penetrated into the deeper ocean. Sampling of the North Atlantic Deep Water was performed to assess how far the contaminants have

moved away from the Norwegian and Labrador Sea (their entry points into the Deep Atlantic Ocean). Current expectations are that increases in concentrations will be observed in samples taken from waters influenced by the Rio de la Plata (off the coast of Uruguay) and the Amazon River. There may also be trends observed on a latitudinal gradient, with concentrations increasing upon entrance into the Northern Hemisphere.

RP006 Distribution characteristics of micro-plastics in in-situ marine compartments

D. Chae, Department of Marine Science, College of Natural Sciences, Incheon National University; I.S. Kim, Department of Marine Science, College of Natural Sciences, Incheon National University; S. Kim, BK-21, School of Earth and Environmental Sciences, Seoul National University / School of Earth and Environmental Sciences, Department of Marine Science, College of Natural Sciences, Incheon National University / College of Natural Science, Department of Marine Science

The occurrence of micro-plastics (MPs) in the marine environment is an emerging concern. They can clear hydrophobic compounds (HOCs) off surrounding seawaters by sorption process. Thus, they can contribute as an intermediate medium to transport HOCs to organism at higher trophic position through aquatic food-web and to move to other marine environments via ocean currents. On the other hand, MPs can function as a diffuse source of HOCs to the seawater by desorbing HOCs bound as additives in their production. Therefore, the fates of MP-associated HOCs are strongly dependent on the distribution characteristics of MPs themselves including size distribution, composition pattern of ingredients, and weathering extent. Nevertheless, there have been little data for distribution characteristics of MPs observed in in-situ marine compartments. The present aims to determine the size distribution (< 0.1 mm, 0.1-0.5 mm, 0.5-1.0 mm, 1.0-2.0 mm, 2.0-5.0 mm, >5.0 mm) and relative composition pattern (polypropylene, polyethylene, polystyrene and so on) of MPs in marine sediment, subsurface seawater, and air-seawater interfacial layer water. Partition coefficients of HOCs between MP and seawater can be further determined based on observed in-situ MP distribution characteristics, and thereafter the effect of MPs present in marine environments on dynamic fates of HOCs among air, seawater, and sediment can be also assessed.

Risk Assessment of Pesticides to Honey Bees

RP007 Evaluating honey bee (Apis mellifera) exposure to pesticides: a focus on multiple routes of exposure for use in the risk assessment process

C.M. Wendel, USEPA / Environmental Fate and Effects Division; K.V. Garber, USEPA / Environmental Fate and Effects Division; R.D. Baris, USEPA / Environmental Fate and Effects Division

A priori, the question risk assessors must initially address is whether, given the existing or proposed uses of a pesticide, exposure is likely to occur relative to bees. As part of the proposed framework for determining the potential risks of pesticides to honey bees, the US Environmental Protection Agency's (EPA) Office of Pesticide Programs (OPP) in collaboration with Health Canada's Pest Management Regulatory Agency (PMRA) and the California Department of Pesticide Regulation (CA DPR) evaluated potential exposure routes of pesticides to bees, using the honey bee (*Apis mellifera*) as a surrogate species. The evaluation of multiple exposure routes identified for bees are detailed in this poster. The consideration of the different routes of exposure for types and castes of bees (e.g., foraging workers vs. in-hive bees; adult vs. larval) as well as different routes of exposure attributed to the pesticide application method (e.g., foliar spray, treated seed, soil application, trunk injection) are included. The conceptual models for risk assessment are based on various routes of exposure. Exposure routes that were considered include: oral which consists of dietary (nectar, pollen, honey, bee bread, and royal/brood jelly), and drinking water (ponds, puddles, dew and guttation fluid); contact (direct spray, contact with contaminated foliage, and dust); and inhalation (direct spray and vapors). When considering the relative pesticide exposure to bees from all routes, the dietary (oral) and contact exposure routes appear to be the most substantial and are assumed to be protective of other routes of exposure. From our analysis, it was determined that this focused approach on oral (dietary, based on food consumption) and contact exposure is representative and protective of other bee species (e.g., bumblebees). Therefore, the oral (dietary) and contact exposure routes will be used as the initial screening tier of the risk assessment process when evaluating the risk of pesticides to bees.

RP008 Laboratory and Semi-Field Studies demonstrate no effects of Rovral® (iprodione) fungicide on honeybee (Apis mellifera)

S. Mukhi, FMC Corporation, Agricultural Solutions / Agricultural Products, Scientific & Regulatory Support; B. Sharma, FMC Corporation, Agricultural Solutions; G. Mitchell, FMC Agricultural Solutions; A. Eimanis, FMC Corporation, Agricultural Solutions

Rovral®, containing iprodione as the active ingredient is a plant protection product used to control fungi in vegetable, fruits and nuts. The safety of Rovral® and iprodione to honeybee has been evaluated in a series of laboratory studies and in a semi-field (tunnel) test. Technical grade iprodione is practically non-toxic to honeybees, based upon oraland contact LD50 values of>200 and >25 µg/bee (at maximum solubility limit), respectively. Rovral was also determined to be practically non-toxic (LD50 >200 µg/bee) to adult bees. Under semi-field conditions (tunnel assay) conducted in oil-seed rape, Rovral® was sprayed twice at 560 g (active ingredient)/ha; once before flowering (4 days before hive setup) and once after flowering (during bee foraging) with 6 days interval between applications. This experiment demonstrated that Rovral® hasno adverse effects on adult honeybee survival, flight intensity or behavior. Moreover, treatments with Rovral® did not affect the overall brood development after 27 days of exposure. Based on findings from these experiments it is concluded that neither iprodione nor Rovral® affect the survival of adult honeybee or the bee-colony at typical agricultural use rates.

RP009 New Computer Methods for Honeybee Colony Assessments

M. Wang, WSC Scientific GmbH / Dept. Efate & Modelling; L.W. Brewer, Smithers Viscient, LLC / Department of Wildlife Toxicology

Colony assessments are an important method for the evaluation of development and health of honey bee colonies. They are also routinely used to monitor such development, productivity and general health in field trials focusing on effects of pesticides. Currently, colony strength is most often estimated visually, e.g., using the "Liebefelder" estimation method. While this method is quick and easy to conduct in the field, it is only a rough estimate of colony size. Individual variation in estimates among field staff can be highly variable. Due to practical reasons individual counting of honeybees is not feasible. However, recent computer methodologies based on digital images offer less time consuming, yet accurate evaluations. We therefore tested different computer methods for colony strength estimation and compared them to currently used methods.

RP010 Optimization of a laboratory toxicity test method for Bumblebees (Bombus terrestris L.)

N. Hanewald, A. Ufer, BASF SE / Ecotoxicology

Relevant gaps in the current tiered testing systems especially concerning non-*Apis* bees had been identified by the Pellston Workshop. Seeing that non-*Apis* bees are potentially exposed to a range of pesticides and that the toxicity of pesticides are possibly different to non-*Apis* bees than to honey bees the detection of toxic effects on bumblebees and solitary bees are more important than ever. The procedures evolved for rearing bumblebees in the laboratory are based on the established methods for honey bee toxicity testing. Laboratory test methods investigating the effects of PPP´s on bumblebee workers have been developed among others by van der Steen 1994, 1996. The tests allow a determination of an acute oral and contact LD_{50}. Based on the acute oral toxicity study by van der Steen the working group of BASF SE adapted the test system to enhance the continuous quality of the performance. Fasted bumblebee workers are put individually in cages at 25±2°C and 70 % RH, in the dark and fed individually with a range of concentrations of a PPP (dissolved in a 50 % sucrose solution). Given that bumblebees do not practice trophallaxis to share their food, there is a great variation in the amount of consumed test item. Moreover bumblebees have often difficulties to find the food source. The principle of this new method is to house the bumblebees individually in hatching cages (honeybee queen rearing material, Nicot system). In these cages bumblebees are able to ingest the test solution much easier because the shape of the hatching cages lead them directly to the food source The effects on mortality of *Bombus terrestris* L. was investigated through 48 – 72 hours post-preparation using two different handling methods: corresponding to van der Steen (T1) and the improved method by BASF (T2), we studied the efficiency of these methods in assessing the amount of consumed sucrose solution. We detected a significant higher food uptake in bumblebee workers in group T2. The feasibility to achieve the recommended number of bumblebees per replicate

is significantly higher with the improved feeding method. Our results suggested that the adapted method produce more reliable and reproducible results on the toxicity of PPP´s for bumblebee workers. Moreover an additional and more prolonged adult laboratory toxicity test (10 days exposure) as a first tier study requested by the EFSA could be carried out more easily with the improved method.

RP011 Using pollen and nectar residue data to refine the dietary exposure predictions of a fungicide active ingredient to honey bees

R. Singh, BASF Corporation; C.W. Schneider, BASF SE; J.D. Wisk, BASF Corporation / Ecotoxicology

The North American regulatory framework for dietary risk assessment of foliar-applied pesticides to honey bees relies on USEPA's T-REX model using the upper-bound tall grass residue predictions as a surrogate for pollen and nectar residues. The proposed Tier I methods also assume that pesticide concentrations in pollen and in nectar are equivalent. Boscalid is a fungicide active ingredient that can be applied to a number of bee-attractive crops. Several studies have been conducted and published on residues of boscalid in honey bee matrices after applications to blooming crops. Relatively high levels can be found in pollen, although the detected levels are far below those concentrations predicted using USEPA's proposed methodology. However, measured residues in nectar were very low to non-detected. When maximum measured residues in pollen and nectar are used to estimate dietary exposure to foraging adults and larvae according to published food consumption data, results indicate that the refined exposure estimates are far below the Tier 1 screening-level estimates. These results indicate that the Tier 1 dietary exposure estimates for foraging adults and honey bee larvae may be highly conservative, especially for highly lipophilic compounds where concentrations in nectar are far below predicted levels.

Modeling and Interpreting Effects of Metals Mixtures

RP012 Toxicity of binary mixtures of nickel, copper, cadmium and zinc to Daphnia magna

E. Traudt, J.F. Ranville, J.L. Williamson, S.A. Smith, Colorado School of Mines / Department of Chemistry and Geochemistry; M.R. Pastorinho, Aveiro University / Biology Department and CESAM; J.S. Meyer, ARCADIS

Major anthropogenic sources of metals into natural water systems include mining wastes, sewage treatment plants, waste incinerators and power plants. Although potentially toxic metals usually occur in mixtures instead of alone in the water, the toxicity of metal mixtures currently is difficult to predict accurately. As part of a project to provide mixture-toxicity data for development of multi-metal toxicity models, we tested the toxicity of binary Cd-Cu, Cd-Ni, Cd-Zn, Ni-Cu, and Ni-Zn mixtures. To analyze the interactions of these metals, we exposed *Daphnia magna* neonates to the metals alone and in binary combinations in standard 48-h toxicity tests conducted in USEPA moderately hard reconstituted lab water to which 6 mg/L of Suwannee River fulvic acid was added (resulting in dissolved organic carbon concentrations of approximately 3 mg/L). For each combination of metals in the binary mixtures, one metal was held constant at a specified concentration while the second metal was varied through a series that ranged from nonlethal to lethal concentrations; then the roles of the two metals were reversed in a separate series of tests. Sub-lethal concentrations of Cu, Ni, and Zn protected against the toxicity of Cd, with mortality only occurring at considerably higher Cd concentrations than in paired Cd-only tests (i.e., less-than-additive toxicity). In contrast, based on dissolved-metal concentrations, a synergistic (i.e., greater-than-additive) toxicity occurred in binary Cu-Ni and Cu-Zn mixtures, with mortality occurring at concentrations of each of the two metals that were slightly (Cu-Zn) or considerably (Cu-Ni) lower than in the paired single-metal tests. These findings provide evidence for the dominance of competition of metals for complexation to biological ligands or to dissolved organic matter, depending on the metal combination in question. Therefore, geochemical speciation modeling based on inferred metal accumulation on biotic ligands should be a more appropriate way to predict the toxicity of metal mixtures than current methods that predict mixture toxicity based on dissolved-metal concentrations.

RP013 Proteomic analysis in Daphnia magna exposed to As(III), As(V) and Cd heavy metals and their binary mixtures for screening potential biomarkers

T. Le, Chonbuk National University / Dept. of Bioprocessing Engineering; E. Lim, Korea Institute of Toxicology / Environmental toxicology Center; S. Lee, Korea Institute of Toxicology / Ecotoxicology Laboratory; Y. Kim, Chungbuk National University / School of Life Science; J. Min, Chonbuk National University / Division of Chemical Engineering

In this study, the effects of three widespread heavy metals, As(III), As(V) and Cd, and their binary mixtures on the proteomic profile in *D. magna* were examined to screen novel protein biomarkers using the two-dimensional gel electrophoresis method (2DE). Ten 20d Daphnia were exposed to the LC20 concentrations for each of a total of 8 treatments, including the control, As(III), As(V), Cd, [As(III)+As(V)], [As(III)+Cd], [As(V)+Cd], and [As(III), As(V), Cd], for 24h before protein isolation. Three replicates were performed for each treatment. These protein samples were employed for 2DE experiments with a pH gradient gel strip from pH 3 to pH 10. The protein spots were detected by a silver staining process and their intensities were analyzed by Progenesis software to discover the differentially expressed proteins (DEPs) in response to each heavy metal. A total of 117 differentially expressed proteins (DEPs) were found in Daphnia responding to the 8 treatments and mapped onto a 2D proteome map, which provides some information of the molecular weight (MW) and pI value for each protein. All of these DEPs are considered as potential candidates for protein biomarkers in *D. magna* for detecting heavy metals in the aquatic ecosystem. Comparing the proteomic results among these treatments suggested that exposing *D. magna* to binary mixtures of heavy metals may result in some complex interactive molecular responses within them, rather than just the simple sum of the proteomic profiles of the individual chemicals, (As(III), As(V), and Cd).

RP014 Toxicity and uptake of metal mixtures: protective effect of nickel and zinc from cadmium toxicity to adult Daphnia magna

M. Pylypczak, Loyola University Chicago / Department of Environmental Science; A.B. Benson, Emory University / Department of Environmental Science; T.C. Hoang, Loyola University Chicago / Department of Environmental Science, CUERP

In the natural environment metals are present as a mixture of multiple elements. Previous studies that determined the effect of individual metals are not always relevant. Recently, scientists have focused on characterizing the effect of metal mixtures in the aquatic environment; however, the data are scarce. The effects were species and metal mixture dependent. The toxicity mechanism of metal mixtures is not well understood. This research determines the tissue uptake and toxicity of cadmium and zinc (Zn) and cadmium (Cd) and nickel (Ni) mixtures to 6-day-old *Daphnia magna*. Adult *D. magna* were used to increase the sample biomass and the detection limit of Cd, Ni, and Zn in the tissue. The 48-h acute toxicity test method was used in this study. In the first set of tests *D. magna* were exposed to a series of mixtures of a constant Cd concentration (100µg/L) and a variety of Zn concentrations (0-5000µg/L) based on the results of a concentration range finding experiment with adult *D. magna*. In the second set of tests *D. magna* were exposed to a series of mixtures of a constant Cd concentration (50µg/L) and a variety of Ni concentrations (0-8000µg/L). Tests with Cd alone, Ni alone, and Zn alone were also conducted for comparison. This study found that addition of Zn or Ni into the exposure medium decreased Cd toxicity. At 50 and 100µg/L Cd alone, 53.5% and 76% mortality was found, respectively. When Zn and Ni were added to the exposure medium (up to 100 and 3000µg/L, respectively), no significant mortality (< 7%) was found. Increasing addition of Zn into the exposure medium (up to 5000µg/L) resulted in similar mortality with the Zn alone experiments. The results indicate that a Zn concentration of ≤100µg/L would completely protect adult *D. magna* from Cd toxicity and ≤3000µg/L Ni would also convey significant protection. When water concentration of Ni or Zn increased, tissue Cd concentration decreased. This indicates an inhibition of Ni and Zn on Cd uptake and therefore would explain the protective effect of Ni and Zn on Cd toxicity. Tissue Ni and Zn concentration appeared to increase with increasing water Ni and Zn concentrations. Results of this study show a direct relationship between tissue Cd concentration and toxicity of Cd and Zn mixtures. This reveals an implication for the toxicity mechanism and the development of a Biotic Ligand Model for metal mixtures, possibly through competitive binding.

RP015 Quantifying the Effect of Experimental Design on Metal Mixture Acute Toxicity with Daphnia magna: Evaluation of Statistical Power

K.M. Newton, Clemson University Institute of Environmental Toxicology / Biological Sciences; A.C. Ryan, HDR | HydroQual; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX)

Generally, models used to predict metal mixture toxicity to fresh-water organisms assume either dose-additivity or response-additivity. Previous researchers have concluded that responses due to exposure to various metal mixtures can be either "greater than additive" or "less than additive." However, due to high variability these conclusions are often qualitative and not quantitative. The high variability (i.e., error) associated with standard bioassays used in these studies often precludes quantitative discrimination among additivity, synergism and antagonism. The objective of the present research was to quantify the influence of experimental design for acute 48h *Daphnia magna* bioassays on the power of the test to evaluate the toxicity of Cd-Zn mixtures. Standard acute toxicity bioassays with *D. magna* were conducted using 3 replicates per treatment and 5 organisms per replicate. These results were compared to those generated using a modified experimental design of 10 replicates per treatment and 10 organisms per replicate. This design was intended to provide much greater statistical power in the bioassay. In single metal bioassays, the higher power experimental design resulted in LC50 values that were not statistically different from those generated in the lower power standard experimental design but had smaller confidence intervals. Further, when these bioassays were repeated, LC50 values among bioassays using the higher power experimental design were much more consistent than those using the lower power experimental design. Preliminary mixture studies using a background concentration of 8 µg/L Cd resulted in LC50 values for Zn of 913 µg/L (793.1, 1053) and 1138 µg/L (1034, 1253) for the lower power experimental design and 966.6 µg/L (911.5, 1025) and 1087 µg/L (1035, 1142) for the higher power design. These preliminary studies indicate that the higher power experimental design resulted in greater accuracy and precision when determining point estimates such as LC50 values. Further research will quantify the influence of the statistical power of the experimental design on the ability to discriminate additivity from either synergism or antagonism.

RP016 Evaluating Acute and Chronic Cadmium and Zinc Metal Mixture Toxicity to Daphnia magna

K.M. Newton, Clemson University Institute of Environmental Toxicology / Biological Sciences; A.C. Ryan, HDR | HydroQual; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX)

While most metal toxicity assessments in fresh water aquatic organisms focus on single metal exposures, actual environmental exposures occur with multiple metals. The present study characterized the acute and chronic toxicity of cadmium and zinc exposures to *Daphnia magna*. Further, the study evaluated the response of these organisms to single and multiple metal exposures. Cadmium was more acutely toxic to *D. magna* than Zn in single metal exposures. Initial metal mixture bioassays tested Zn from 50 µg/L -1500 µg/L in the presence of 8 µg/L Cd. Preliminary results indicated that low levels of Zn had a protective effect on Cd toxicity to *D. magna.* At higher concentrations, Zn became toxic in a dose-dependent manner. These results will be useful for evaluating models that have been developed to predict metal mixture toxicity on the basis of dissolved metals and on the basis of bioavailable metal using the biotic ligand model framework.

Wastewater Effluents: Chemical and Ecotoxicological Characterization

RP017 Biodegradation study of a recalcitrant azo dye in a pilot scale reactor anaerobic-aerobic

E. Franciscon, Campinas State University / Laboratório de Ecotoxicologia e Microbiologia Ambiental "Prof. Dr. Abílio Lopes"; D. Mendonca, S. Seber, Campinas State University; G.J. Zocolo, M.B. Zanoni, Paulista Júlio de Mesquita Filho State University; M.J. Grossman, L.R. Durrant, G.A. Umbuzeiro, Campinas State University

Azo dyes are characterized by the presence of one or more azo bonds (N=N) and among them Disperse Red 1 has been widely used for polyester fibers. A commercial sample of this dye has been chemically characterized. It contains 60% of dye component, 20% subsidiary colorants with similar structure of the main dye and 20% of a dispersing agent as a dyeing auxiliary. Disperse red 1 is considered highly toxic according to GHS and also has mutagenic properties. The biodegradation of Disperse Red 1 was studied in an anaerobic-aerobic pilot scale reactor during long term treatment (168h). The bacterial strains inoculated in the reactor were previously isolated from a textile treatment plant and characterized. To follow the process of degradation and its efficiency, chemical and toxicological analyzes were employed. The bacterial consortium was able to decolorized and degrade the azo dye forming different aromatic amines which were characterized by Liquid Chromatography-Electrospray Ionization-Mass Spectrometry (LC-ESI-MS/MS Qtrap). Acute toxicity to *D. similis* (OECD, 2004) decreased in 12.5 times and to *H. attenuata* (Trottier, 1997) in 1.6 times. Mutagenicity, evaluated with the Salmonella microsome assay YG1041 with and without S9, also decreased after treatment. Others studies are being conducted to optimize treatment in order to obtain the complete mineralization of the dye and removal of the residual toxicity and mutagenicity. We acknowledge financial support from FAPESP-SP–BRAZIL- Process n° 2010/13003-0 and Thematic Project n° 2008/10449-7.

RP018 Enhanced Biodegradation Tests; Application to Persistency Evaluations

C. Mead, Harlan Laboratories Ltd / Ecotoxicology Dept.; N. Clarke, Harlan Laboratories Ltd

With the onset of REACh renewed focus was placed on the biodegradability of substances and the application of the Persistent criteria in PBT assessment. Standard ready biodegradation tests have been designed to be stringent screening tests with limited potential for biodegradation to occur such that a positive result obtained in such a test is unequivocal. However failure to pass the stringent conditions imposed in a ready biodegradation test does not preclude biodegradation of the substance under relevant environmental conditions and hence a fail in a ready biodegradation test should not automatically be taken as evidence of persistency. To help in persistency evaluations, a number of enhancements to the ready biodegradation test methods have been identified. These enhancements aim to improve the environmental relevance of the biodegradation test and to allow the results of such a test to be used in persistency evaluations without the requirement for expensive and time-consuming simulation testing. Here we describe some of the potential enhancements that can be employed in biodegradation tests and present results of various enhanced biodegradation tests that have been conducted.

RP019 Modeling the fate of selected pharmaceuticals and personal care products in aerated lagoons

F. Cloutier, Universite Laval / Département de génie civil et de génie des eaux; E. Hoque, Environmental & Resource Studies; C.D. Metcalfe, Trent University / Environmental & Resource Studies; P.A. Vanrolleghem, ModelEAU – Université Laval / Département de génie civil et de génie des eaux

Aerated lagoons and stabilization ponds are widely used bioprocesses to treat municipal wastewaters around the world. Notable advantages of such system are their high efficiency, low running cost and maintenance, as well as the low frequency and amount of sludge removal (e.g., sediment removal once every several years). They also allow extensive exposure of the wastewater to sunlight which provides, compared to other treatment technologies, an additional degradation pathway for photo-sensitive chemicals. Moreover, the long sludge retention time offers the benefit of a more diversified and efficient biomass. Meanwhile, literature indicates a growing concern about the occurrence and impact of chemicals of emerging concern (CEC) in aquatic environments and studies have shown that wastewater treatment plants (WWTPs) are a main contributor. Consequently, wastewater discharges in receiving waters contribute significantly to the contamination of sources of drinking water. Dynamic models of the fate of CECs in wastewater treatment systems were developed as part of the European SCORE-PP project in view of studying and reducing their release in the environment. However, aerated lagoons systems were not included in this project. Literature shows that several studies have been undertaken focusing on CEC removal in aerated lagoons, but to our knowledge, no publication exists on modeling their fate in those systems. In this study, a model to predict the fate of traditional pollutants and CECs in aerated lagoons was developed with the software tool WEST® (www.mikebydhi.com). Kinetic expressions were derived from the River Water Quality Model No. 1 and its extension for pesticides. The fate of nine selected pharmaceuticals and personal care products (carbamazepine, ibuprofen, gemfibrozil, sulfamethoxazole, thimethoprim, triclosan, and the synthetic musk compounds HHCB and AHTN) is modeled and

compared with data collected in different seasons at the Lakefield Sewage Lagoon in Peterborough, Ontario. Physicochemical properties of the compounds (aerobic, anoxic and photolysis half-lives, partition coefficient, Henry's constant) were found in the literature and used as initial estimates for model calibration.

RP020 Removal of Selected Pharmaceuticals and Personal Care Products in a Sewage Lagoon

E. Hoque, Environmental & Resource Studies; C. Arcieri, M. McInnis, C. Murray, Trent University; F. Cloutier, P.A. Vanrolleghem, Laval University / modelEAU, Département de génie civil et de génie des eaux; C.D. Metcalfe, Trent University / Environmental & Resource Studies

Many pharmaceuticals and personal care products (PPCPs) have been detected in surface waters around the world, and these chemicals are entering the environment primarily through discharges from wastewater treatment plants (WWTPs). In small municipalities, sewage lagoons are often the only systems used to treat wastewater prior to discharge into receiving waters. Study on removal of PPCPs in sewage lagoon during different seasons and prediction of their fate using lagoon modeling are not well known yet. In this study, a sewage lagoon serving the small municipality of Lakefield in Ontario, Canada was monitored in the summer, fall and winter to determine removals of carbamazepine, trimethoprim, sulfamethoxazole, ibuprofen, gemfibrozil triclosan and the synthetic musk compounds, HHCB and AHTN. Concentrations of these compounds in untreated and treated wastewater were estimated by deploying POCIS and SPMD passive samplers at three points in the sewage lagoon. Passive samplers were also deployed in the summer and fall at several points upstream and downstream of the point of discharge from the lagoon into the Otonabee River. LC-MS/MS and GC-MS were utilized to determine the concentrations of PPCPs. Among compounds sampled by POCIS, the highest estimated concentration in untreated wastewater was ibuprofen sampled during the fall, at an estimated concentration of 60.3 ng/L. Similarly, concentrations of triclosan, HHCB and AHTN in SPMDs were high during fall season, at 30, 1677 and 109 ng/L, respectively. For all compounds except gemfibrozil and carbamazepine, removals were highest in the summer (83.0 to 98.8%) relative to removals in the fall (48.4 to 91.4%) and winter (14.0 to 78.3%). Only carbamazepine, HHCB and AHTN were detected in the Otonabee River, but estimated concentrations were very low (i.e., < 1 ng/L) and probably do not pose a threat to the ecosystem or aquatic organisms. This small sewage lagoon was as effective at removing PPCPs as many conventional WWTPs, but removals were better during the summer. With the collected data, lagoon modelling is conducted to predict the fate and/or removal pathways of the selected PPCPs in this sewage lagoon.

RP021 Isolation of endocrine active chemicals in wastewater effluents in the Grand River, Ontario, Canada

B.M. Smith, University of Waterloo / Department of Biology; K.D. Oakes, Cape Breton University / Department of Biology; H. Engelhardt, L. Bragg, P. Marjan, University of Waterloo / Department of Biology; G. Tetreault, Environment Canada / Aquatic Ecosystem Protection Research Division; M.R. Servos, University of Waterloo / Department of Biology

Adverse reproductive effects have been observed in fish exposed to municipal wastewater effluents (MWWEs) from highly urbanized areas throughout the world. These effects have included altered reproductive success, somatic indices, and gonad morphology as well as perturbed metabolic pathways indicative of exposure to endocrine disrupting compounds (EDCs). However, identifying EDCs that cause these effects remains difficult, and with different remediation technologies, each effluent has the potential for unique chemical profiles or novel emergent chemicals of concerns. The MWWEs in the Grand River watershed of southern Ontario have been associated with a variety of effects in fish including altered reproductive performance and high incidence of intersex (ova-testes). To elucidate the potential chemical toxicants in these effluents, an Effects-Directed Assessment (EDA) approach was applied to two effluents for endocrine disrupting compounds using recombinant yeast cell assays (e.g., Yeast Estrogen Screen). Although each effluent had different whole effluent estrogenicity (YES) and chemical profiles, estrogenic EDCs identified consisted of the natural hormone, estrone, 17b-estradiol, and environmental contaminants, 17a-ethinylestradiol, and bisphenol A. Each of the effluents were also tested for their ability to elevate circulating plasma vitellogenin (VTG) in immature rainbow trout after 14 d exposure. Despite the effluents having elevated total estrogenic equivalents and wild fish demonstrating clear signs of endocrine disruption, the effluents did not significantly elevate circulating plasma VTG in rainbow trout. This is inconsistent with much of the reported literature where effluents often cause a very large induction of VTG. Additional testing is being performed on these effluents to explore the potential causative agents and links among contaminant profiles, treatment processes and effects observed in wild and experimentally exposed fish.

RP022 A comparative study of membrane bioreactors and conventional activated sludge systems for the elimination of musk fragrance residues from wastewater

R. Reif, University of California Riverside / Department of Environmental Sciences; J.M. Lema, F. Omil, University of Santiago de Compostela / Chemical Engineering

Polycyclic musk fragrances (PMFs) are chemicals included in the category of emerging contaminants. They are used in a variety of household products such as detergents and cosmetics, and can be frequently found in the aquatic environment. PMFs gain entrance into the hydraulic cycle and can become pseudo-persistent due to a lack of efficient design in current sewage treatment plants, not specifically devoted to the elimination of these substances. PMFs are also hydrophobic compounds, being largely transferred to the sewage sludge during treatment, with potential implications for the use of biosolids or its safe disposal. The use of filtration membranes for wastewater applications, the so-called membrane bioreactor (MBR), is gaining increased attention during the last years. The feasibility of this innovative technology for the removal of emerging pollutants from wastewater have been studied and compared with conventional units. However, data available are scarce and show a high variability range since, in many cases, the treatment units compared are substantially different in terms of size (pilot vs full-scale) and/or operating regime. This work compares the performance of 2 parallely-operated units, a conventional activated sludge and a MBR, eliminating 3 musk fragrances from sewage: galaxolide, tonalide and celestolide. The set-up of this study was placed at the premises of a sewage treatment plant located in NW Spain. Operating parameters (hydraulic retention time and sludge retention time), and working conditions (mixed liquor suspended solids, pH, temperature, dissolved oxygen) were maintained at similar values in both systems and their influence on PMFs removal was assessed during 6 different operational periods. Mass balances were calculated for each PMF entering and leaving the MBR and CAS, in order to estimate total eliminations considering both liquid and solid phase data. Likewise, the amount of PMF sorbed onto the biological sludge was used for calculating solid-water distribution coefficients (Kd). Interestingly, CAS sludge showed a slightly higher tendency to sorb PMFs, since Kd values were moderately higher compared with those obtained with MBR sludge. For example, log Kd values for galaxolide ranged from 3.2 to 3.5 for the MBR and 3.4-3.8 for the CAS. Consequently, the CAS system always achieved higher eliminations of the considered PMFs, being this tendency maintained throughout the different operational periods.

RP023 Relationship between sorption kinetics and hydrodynamic condition in passive sampling of hydrophobic organic chemicals with polyethylene film

W. Lao, Southern California Coastal Water Research Project; D. Tsukada, K.A. Maruya, Southern California Coast Water Research Project Authority

Low-density polyethylene (PE) film is a passive sampling material that shows great promise for equilibrium measurement of ultra-low concentrations of aqueous phase hydrophobic organic chemicals (HOCs). However, correction for non-equilibrium conditions under a wide range of hydrodynamic conditions is a non-trivial exercise that may limit the widespread acceptance and thus practical application of PE in situ. The goal of this study was to determine kinetic uptake (k_1) and release (k_2) rate constants and equilibrium partitioning constants (K_{pew}s) for 96 individual HOCs (e.g., pyrethroids, PBDEs, organochlorine pesticides, PAHs and PCBs) using time-series seawater exposures. Estimated log K_{pew} values showed good correlation with the logK_{OW}. Similar k_2 ratios for C^{13} labeled performance reference compounds (PRCs) and their native analogs in lab and pre-loaded, field exposed PE samplers suggested that a universal relationship for prediction of non-equilibrium correction factors is feasible. A linkage among k_2 values and water flow speeds in the laboratory and field exposures was established by applying boundary layer and convection mass transfer theories. These results were used to generate a generic non-equilibrium correction equation, which was applied to estimate seawater concentrations of pre-calibrated HOCs using PE samplers deployed off the coast of southern California.

RP024 Transcriptomic profiling permits the identification of pollutant sources and effects in ambient water samples

M. Hasenbein, Department of Anatomy, Physiology and Cell Biology, School of Veterinary Medicine; I. Werner, Swiss Centre for Applied Ecotoxicology / Department of Anatomy, Physiology and Cell Biology; E.B. Holland Fritsch, University of California / Molecular Biosciences; A. Javidmehr, Aquatic Toxicology Program, School of Veterinary Medicine; L.A. Deanovic, UC Davis; N.A. Fangue, University of California, Davis / Wildlife, Fish, & Conservation Biology; J. Geist, Technische Universität München / Aquatic Systems Biology Unit, Department of Ecology and Ecosystem Management; R.E. Connon, University of California, Davis / School of Veterinary Medicine, Aquatic Toxicology Laboratory

The delta smelt (*Hypomesus transpacificus*) is an endangered fish species endemic to the Sacramento-San Joaquin Estuary, California; populations of which have significantly declined over the past three decades. Contaminant exposure is one of many likely contributors to the decline. We investigate transcriptional responses in larval delta smelt resulting from exposure to water samples collected at the Department of Water Resources Field Station at Hood, a site of concern situated upstream of known delta smelt spawning habitats, and downstream of the Sacramento Regional Wastewater Treatment Plant (SRWTP). Microarray assessments indicate impacts on the energy metabolism, DNA and RNA processing, the immune system, development and upon activity. Transcription responses of fish exposed to water samples from Hood were compared with exposures to 9% effluent samples from SRWTP, water from the Sacramento River at Garcia Bend (SRGB); upstream of the effluent discharge, and SRGB water spiked with 2 mg/L total ammonium; 9% effluent equivalent. Results indicate that transcriptomic profiles (microarrays and qPCR) from Hood are similar to SRWTP effluent and ammonium spiked SRGB water, but significantly different from SRGB, however, SRGB samples were also significantly different to laboratory controls, suggesting that SRWTP effluent is not solely responsible for the responses determined at Hood, that ammonium exposure likely enhances the effect of multiple-contaminant exposures, and the observed mortality at Hood is due to the combination of effluent discharge including contaminants arising from upstream of the tested sites. This study shows that transcriptomic responses of fishes can be valuable endpoints for identification of sources of toxic compounds in surface waters that occur at sublethal concentrations.

RP025 Pushing the limits of organic matter characterisation in wastewater: a quantitative approach to technique complementary

E. Plant, University of Adelaide / Soils Group, School of Agriculture, Food and Wine; R. Smernik, Soil and Land Systems, School of Earth and Environmental Sciences, The University of Adelaide, Waite Campus; J. van Leeuwen, University of South Australia; P. Greenwood, University of Western Australia; L. Macdonald, CSIRO; D. Leverett, WCA-Environment Ltd

Characterisation of organic material (OM) in WW comes with considerable challenges. The inability of any analytical technique to provide complete characterisation is now understood and common practice now involves characterisation with more than one analytical technique, in order to avoid missing chemical information. This is particularly important when organics such as pharmaceuticals are of interest, yet are detected by few techniques. Until now, there have been few attempts to highlight the ability of techniques to provide complementary information. This study aims to provide advanced characterisation of a suite of UK sewage WW samples at various stages of treatment, as well as a quantitative assessment of the ability of the techniques used to provide redundant and unique information provided. Characterisation involved the widely used analytical techniques of solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy, flash pyrolysis-gas chromatography mass spectrometry and high performance size exclusion chromatography (HPSEC). The methods used here were developed to study OM through a pulp and paper mill WW treatment plant in South Australia, and to identify any organics of previous WW discharge in the sediments of the receiving environment. Many chemical similarities were seen in the sewage WW organics: NMR identified carbohydrate in all samples, pyrolysis identified fragments indicative of pharmaceuticals in the majority of samples, and HPSEC showed the apparent molecular weight of organics to be relatively consistent in each sample. The use of non-parametric multidimensional scaling (nMDS) ordination plots for each technique helped to identify sample similarities. Further to this, a mantle-type test called RELATE measured a significant ($P< 0.05$) similarity between the NMR and HPSEC data, with approx. 60% of sample discrimination common to both analytical techniques and approx. 40% technique specific information. There was no such relationship identified between these techniques and pyrolysis, thus pyrolysis provided further unique information. The complementary nature of NMR and HPSEC provides assurance for the conclusions drawn by these techniques. The ability for all three techniques to provide unique information is important and highlights the importance of using multiple techniques together to characterise OM, especially as these are often chosen based on time and money constraints.

RP026 A university campus´s contribution to municipal wastewater toxicity and receiving water quality

A.H. Mounteer, D.S. Aquino, Universidade Federal de Vicosa

Brazilian legislation does not permit discharge of effluents that cause or possess potential to cause toxic effects to aquatic organisms but municipal wastewaters are not generally considered potential sources of aquatic toxicity and are therefore not required to monitor toxicity. However, only about half of all municipal wastewaters generated in Brazil are treated before discharge and little is known about the toxic potential of these untreated wastewaters. The city of Viçosa, Minas Gerais (population 72 000) is home to the Federal University of Viçosa (19 000 students, staff and professors). The bulk of Viçosa´s and the Federal University´s wastewaters are combined and transported for discharge without treatment to the São Bartolomeu Creek. No studies have been published to date on the toxic contribution of the University to the city´s wastewater and only a few studies have been undertaken to evaluate the impact of discharge of the city´s raw wastewaters to receiving waters. The objective of this study was to evaluate toxicity of the wastewaters from the Federal University and the city center and to evaluate the impacts caused by discharge of these wastewaters to the São Bartolomeu Creek and its sediments. Wastewater samples were collected bimonthly over the course of a year at the ends of the University´s two sewer mains, at the discharge point of the mixed University-city wastewaters and surface water and sediment samples in the São Bartolomeu Creek upstream and downstream of the discharge point. Samples were characterized by measuring pH, dissolved oxygen, total /dissolved organic carbon (DOC), suspended solids, ammonia and metals. Acute toxicity was evaluated using the *Daphnia similis* immobilization assay. Samples that didn´t present EC50 in *D. similis* were tested for chronic toxicity using the *Ceriodaphnia dubia* survival and reproduction assay. Wastewater discharge decreased surface water quality downstream, by increasing ammonia, suspended solids, DOC and Zn. All wastewater samples collected at the point of discharge and five of the eight University wastewater samples presented acute toxicity (EC50=23-87%) however surface water and sediment elutriate collected downstream was toxic on only one of four occasions. Wastewater sample toxicity correlated significantly with Cr, Cu and Ni while sediment elutriate toxicity correlated with ammonia, DOC, Cu, Pb and Zn.

RP027 TIE/TRE Case Studies for Industrial Wastewater Effluents

L.T. Heise, ENVIRON International Corp; S. Hall, ENVIRON International Corp / Ecotoxicology

Laboratory Toxicity Identification Evaluations/ Toxicity Reduction Evaluations (TIEs/TREs) are designed to characterize and identify the constituents toxic to aquatic life and reduce the effects of the constituents in the waste stream. The USEPA has a step-wise approach for use in determining the key constituents, however they also allow the use of non-conventional treatments, such as granular activated carbon (GAC), specialty resins, modeling mock effluents, and ferric chloride addition. The goal of these investigative methods is to reduce toxicity, via end-of-pipe solutions (TRE) or identify the toxicant for removal or replacement (TIE). Presented herein are case studies where successful non-conventional laboratory treatments were integrated on-site to remediate toxins from the effluent.

RP028 The Chronic Toxicity of Chloride, Sulfate and Bicarbonate to Ceriodaphnia dubia

K.A. Johnson, Clemson University / Institute of Environmental Toxicology; S.J. Klaine, Clemson University / Institute of Environmental Toxicology (CU-ENTOX)

Total Dissolved Solids (TDS), a natural entity of aquatic systems, have become increasingly abundant through human activities. Effluents, in particular those from coal-fired power plant flue gas desulfurization systems, may contain large quantities of TDS and these additional ions present a potential hazard to aquatic organisms in receiving streams. Freshwater organisms must use energy to maintain the ion balance between their

bloodstream and the environment. When the ionic strength of their surrounding environment becomes too elevated, the energy required by freshwater organisms for ionoregulation decreases their ecological fitness. While research exists on the acute toxicity of elevated TDS, few evaluations of chronic effects have been reported. The overall objective of this study was to characterize the chronic toxicity of major anions to *Ceriodaphnia dubia*. Through a series of 8-day static renewal assays, *C. dubia* were exposed to chloride, sulfate and bicarbonate individually and in mixtures; mortality and reproduction were quantified. The IC_{50} values for chloride were 621.11 mg/L (567.86-677.25) and 526.13 mg/L (482.37-587.57), and sulfate IC_{50} values were 1023.95mg/L (953.565-1091.43) and 1076.54mg/L (1016.09-1136.978). These values are further supported by previous studies. Single anion bicarbonate, as well as binary and tertiary mixture tests will also be conducted. Results from this study will facilitate better management practices for coal-fired power plant effluents.

RP029 Addressing Artifactual Toxicity in Ion Imbalanced Wastewaters

B.A. Rabe, M.A. Baker, S.P. Rewa, J. Brinks, ERM

The artifactual toxicity resulting from pH drift in whole effluent toxicity tests is well documented for pH dependent toxicants such as ammonia and certain metals. Various test procedure modifications have been developed to minimize this pH drift and thus reduce the artifactual toxicity from these constituents. Ion imbalanced wastewater with relatively low ammonia and metal concentrations has demonstrated toxicity under similar pH drift testing conditions. The use of a carbon dioxide (CO_2) enriched testing environment has been successfully used to reduce toxicity to two freshwater test species (*Ceriodaphnia dubia* and fathead minnow) in exposures to treated groundwater and reverse osmosis wastewater.

RP030 Evaluating the genotoxic potential of ciprofloxacin and its transformation products after photolysis treatment

A. Toolaram, Leuphana Universitat Luneburg / Institute for Sustainable and Environmental Chemistry; T. Haddad, M. Schneider, K. Kuemmerer, Leuphana University Lüneburg / Institute for Sustainable and Environmental Chemistry

It was found that the main source of Umu C induction in hospital wastewater comes from the presence of ciprofloxacin. Ciprofloxacin is a broad-spectrum antibiotic and it can be found in concentration ranges in µg/L in various environmental compartments. Therefore, it is necessary to investigate the ability of wastewater treatment techniques like UV photolysis to degrade ciprofloxacin and to verify that their resultant transformation products (TPs) do not amplify the inherent Umu C genotoxicity of ciprofloxacin. In this study, ciprofloxacin (20 mg/L) was subjected to UV photolysis for varying treatment periods. The resultant mixtures were assessed for Umu C induction at non-cytotoxic concentrations using the ISO 13829 protocol. Identification of the transformation products and estimation of their relative proportions in the mixtures were analyzed with liquid chromatography coupled to multiple stage ion trap mass-spectrometry ($LC–MS^n$). No induction of the Umu C gene was found for untreated Ciprofloxacin up to a concentration of 0.0013 mg/L without S9 mix. Metabolic activation (+S9) resulted in lower levels of induction without any DNA damaging potential up to 0.005 mg/L. After photolysis, at least seven transformation products were identified. Umu C genotoxicity was not apparent for mixtures after 32 minutes of irradiation without the presence of S9 mix and at non-cytotoxic levels. LS-MS analysis confirmed that the level of Ciprofloxacin tested with an irradiation time greater than 32 min was much lower than the concentration of untreated Ciprofloxacin that resulted in a positive response. Therefore, our conclusion is that the mixture of TPs from photolysis does not enhance the genotoxic nature of ciprofloxacin. Therefore, the key to reducing the genotoxicity of ciprofloxacin enriched wastewater is to degrade the parent compound. Further studies would be conducted with other treatment methods such as ozonation, chlorination, and photo-catalysis to verify that treatment of ciprofloxacin does not result in the formation of Umu C inducing genotoxins.

Environmental Stereochemistry Then and Now: A Tribute to Wayne Garrison

RP031 GC-MS Analysis of Chiral PFOA

J.E. Naile, USEPA / Ecosystems Research Division; J. Washington, USEPA; A.W. Garrison, Private Citizen. retired from the USEPA / NA

Perfluoroalkyl substances are ubiquitous in commercial, industrial, and consumer products. They impart anti-wetting and anti-staining properties to a wide range of products including clothing, upholstery, food packaging, and carpeting. Perfluoroalkyl pollutants are globally distributed in both urban and remote environments, and are routinely detected in wildlife, humans, and the environment. One of the most prominent and routinely detected perfluoroalkyl substances is perfluorooctanoic acid (PFOA), which has been shown to be toxic to both humans and animals. PFOA exists as both linear and branched isomers; some of the branched isomers are chiral. A novel GC-MS method was developed to allow for isomer/enantiomer separation, which was achieved using two columns working in tandem; a 30 meter BGB-172 Analytik column and a 30 meter DB-5MS column. Soil and sediment samples from various locations around the United States were extracted and derivatized to form methyl esters of the PFOA isomers, and allow for GC-MS analysis. In some samples, as many as 8 PFOA isomers were detected, of which 2 were chiral and sufficiently seperated to allow for enantiomer fraction calculations. Non-racemic ratios were observed for several enantiomers. This method should be useful for source identification, and for highlighting stability differences among enantiomers. Currently this method focuses on PFOA, but preliminary results suggest that it should be broadly applicable to other chiral and achiral perfluoroalkyl acids.

RP032 Sorption and Enantiomerization of Malathion and Metalaxyl by Mineral Surfaces

A. Hall, Nutra Manufacturing; C.M. Lee, Clemson University / Environmental Engineering and Earth Sciences Department

Sorption experiments with racemic and individual enantiomers of malathion and metalaxyl and bentonite, calcite, diatomaceous earth, kaolinite, montmorillonite, and seashells, were conducted and analyzed by achiral and chiral high performance liquid chromatography (HPLC). Chiral analysis of sorption experiments with racemic malathion indicated nonenantioselective sorption, resulting in racemic enantiomeric fractions (EFs) for all sorbents. However, sorption of the individual enantiomers of malathion, *R*-(+)- and *S*-(-)-malathion, resulted in enantiomerization to racemic EFs after contact with the mineral sorbents. Construction of sorption isotherms revealed that the malathion racemate and enantiomers have different isotherm shapes, possibly indicating different sorption mechanisms. Sorption of racemic metalaxyl was also found to be nonenantioselective with racemic EFs for all sorbents. However sorption of metalaxyl-M (consisting of 97% *R*-(-)-metalaxyl and 3% *S*-(+)-metalaxyl) resulted in an increase of EF with bentonite and montmorillonite indicating the possibility of enantioselective sorption. EFs also increased for calcite, and at one concentration level EF decreased for kaolinite, suggesting that enantiomerization is occurring for metalaxyl-M when in contact with these sorbents, since sorption on calcite and kaolinite was not observed. Although enantiomerization of chiral pesticides has been observed during degradation and in aprotic solvents, this research presents the first evidence of enantiomerization during sorption for two current-use chiral pesticides.

RP033 Enantioselective Bioaccumulation of Chiral Polychlorinated Biphenyls in Lotus Root

S. Dai, Institute of Quality Standard & Testing Technology for Agro-Products, Chinese Academy of Agricultural Sciences; C.S. Wong, University of Winnipeg / Richardson College for the Environment; J. Qiu; M. Wang, L. Fan, S. Shuming Yang, Institute of Quality Standard & Testing Technology for Agro-Products, Chinese Academy of Agricultural Sciences

There are 19 polychlorinated biphenyls (PCBs) displayed stable chirality at ambient temperatures out of a total of 209 PCBs congeners, their enantiomers exhibited different biochemical processes including bioaccumulation, metabolism, and toxicology in previous reports. Thus, chiral PCBs may present enantioselectivity by biota although they were originally released to environment as racemates. As a kind of aquatic vegetable with bigger cultivated area in China, lotus root has become very important economic crop and can be used as food and also possess higher medicinal value. However, their major growth environment is sediment in pond or lake, and these

places are easy to be contaminated by pollutants including PCBs, pesticides, and heavy metal etc. This study investigates the enantioselective bioaccumulations of chiral PCBs including PCBs 95, 91, 136, 149, 176, and 183 in aquatic plant. The lotus root was exposured in the flowerpot filled with sediment that was spiked with six chiral PCBs in whole growth period. The analytical results of PCB enantiomers in root, stem, leaf, sediment, and water showed that the enantioselectivity on accumulation of six chiral PCBs in lotus root were quite different. A preferential accumulations of the E1-PCB 95, E2-PCB 91, and (-)-PCB 136 were observed in root, stem and leaf, while the accumulations of PCBs 149, 176, and 183 were non-enantioselective, and all chiral PCBs always held nearly racemic in sediment and had not been observed in water after 30 d. These indicated that the enantioselectivity firstly occurred through translocation of chiral PCBs from sediment to root because only less PCBs are present in the water, and can further produce in their transmission from root to stem and leaf because of different protein and DNA combination, enzyme metabolism or other actions between two enantiomers of chiral PCBs. Also, the enantioselectivity might be explained by the relationship of the six congener structures because they have different amount and places of substituted chlorine atom chlorine on benzyl ring of PCBs which may lead to very different spatial structure. The concentrations of chiral PCBs in root were the highest at 60 d, but the total concentrations in whole plant were the highest at 120 d, suggesting continual accumulation of PCBs by lotus root during whole experimental period. This work was funded by National Natural Science Foundation of China (No. 20907073).

RP034 Stereoselective Formation of Hydroxylated Polychlorinated Biphenyls by Rat Cytochrome P-450 2B1 Isozyme

Z. Lu, University of Manitoba; I. Kania-Korwel, H. Lehmler, University of Iowa; C.S. Wong, University of Winnipeg / Richardson College for the Environment

The atropisomer enrichment of chiral polychlorinated biphenyls (PCBs) by rat cytochrome P-450 2B1 (CYP2B1) and their hydroxylated metabolites were investigated *in vitro*. Rat CYP2B1 could stereoselectively biotransform chiral PCBs to generate 5-OH-PCBs as the major metabolites after 60 min incubations. Non-racemic enantiomer fractions (EFs) of 5-OH-PCBs were detected as 0.17, 0.20, 0.85, 0.77 and 0.41 for incubations with PCBs 91, 95, 132, 136 and 149, respectively. Minor products included 4-OH-PCBs. CYP-mediated stereoselective formation of dihydroxylated PCBs (diOH-PCBs) was observed for the first time. Both 4-OH-PCB 95 and 5-OH-PCB 95 were stereoselectively biotransformed, the latter more rapidly with EFs of OH-PCBs changing after 60 min from racemic (i.e., 0.5) initially to 0.62 and 0.46, respectively. These transformations generated 4,5-diOH-PCB 95, with EFs of 0.53 and 0.58 for 4-OH-PCB 95 and 5-OH-PCB 95, respectively. 4,5-diOH-PCB 91 and 4,5-diOH-PCB 136 were also observed from PCBs 91 and 136 biotransformation. These *in vitro* results were consistent with that observed for stereoselective PCB biotransformation by rat microsomes and *in vivo*. The biotransformation interference between two atropisomers of PCB 136 was first time elucidated in this study. The biotransformation process of (-)-PCB 136 was significantly disrupted by the presence of (+)-PCB 136, but not the other way around. Thus, stereoselective metabolism of chiral PCBs and OH-PCBs by CYPs is a major mechanism for atropisomer enrichment of PCBs and metabolites in the environment, with the degree of enrichment dependent, at least in part, on stereoselective interference of atropisomers with each other at the enzymatic level.

Animal Alternative Methods for Evaluating Toxicity: Methods, Endpoints, andNew Testing Strategies

RP035 High-content screening assay for identification of chemicals impacting spontaneous locomotion in zebrafish embryos

T.D. Raftery, G.M. Isales, K.L. Yozzo, University of South Carolina / Environmental Health Sciences; D.C. Volz, University of South Carolina / Department of Environmental Health Sciences

Although cell-based assays are available, there is currently a lack of rapid and cost-efficient high-throughput screening assays within intact animals to support prioritization for developmental neurotoxicity testing in fish and mammals. During zebrafish embryogenesis, spontaneous tail contractions represent the first sign of locomotion and result from innervations of primary motoneuron axons to target axial muscles. As this behavior commences during late-segmentation (~19 hours post-fertilization, hpf) and continues through early-pharyngula (~29 hpf), spontaneous locomotion is an endpoint amenable for detecting the potential for developmental neurotoxicity within < 30 h following test initiation. Moreover, as a single zebrafish embryo develops normally within 50 µl of water, the potential effects of chemicals on spontaneous locomotion can be evaluated within 384-well plates, increasing throughput while minimizing the need for large quantities of test material. Therefore, we have developed and optimized an automated 384-well-based high-content screening assay that quantifies spontaneous locomotion within single zebrafish embryos after exposure to vehicle or test chemicals in concentration-response format. For this assay, we rely on transgenic zebrafish (*fli1:egfp*) that stably express eGFP within vascular endothelial cells, as this strain begins expressing eGFP at ~14 hpf, allowing us to accurately track spontaneous locomotion in the absence of shading effects observed under transmitted light. Following manual loading and static exposure from 5-25 hpf at 28°C, live embryos (one per well) are acclimated at room temperature for 1 h, and imaged for 6 s each under fluorescent (FITC) light using a 2X objective and automated image stream acquisition procedure within our high-content imaging system. After converting time-lapsed images to AVI files, spontaneous locomotion within individual videos are quantified using an automated procedure within our behavioral analysis software. Control embryo survival under these conditions is consistently >95%, and we find that spontaneous locomotion responds to reference developmental neurotoxicants. Therefore, we are currently finalizing assay validation and screening a subset of teratogenic chemicals within EPA's ToxCast Phase-I library. Compared to existing locomotion-based zebrafish assays conducted later in development, this method provides a simpler discovery platform for identifying potential developmental neurotoxicants.

RP036 Comparative probabilistic hazard assessment of in vitro estrogen agonist activity

D.A. Dreier, Baylor University / Department of Environmental Science; K.A. Connors, Baylor University / Institute of Biomedical Studies; B.W. Brooks, Baylor University / Department of Environmental Science

Environmental and human health implications of endocrine disrupting chemicals, particularly xenoestrogens, have received extensive study. However, a critical question remains, how does one select an in vitro model for estrogenicity when multiple model systems exist? ToxCast is an effort launched by the EPA that seeks to prioritize chemicals requiring additional toxicity testing. The primary objective of the present study was to examine the comparative sensitivity of three in vitro assays for estrogen agonists representing a diverse group of industrial chemicals and pesticides. We specifically utilized chemical toxicity distributions to perform probabilistic hazard assessments, which tested the hypothesis that no differences in sensitivity will exist among the three in vitro assays for estrogenicity. When all available data from ToxCast for each of the three in vitro assays were examined, we predict that there was a 10% probability of detecting a compound that will elicit an estrogenic response at or below concentrations 1.906 mg/L (HepG2 Reporter Gene Assay, ATG_ERa_TRANS), 0.802 mg/L (HepG2 Reporter Gene Assay, ATG_ERE_CIS), or 0.181 mg/L (HEK293H Reporter Gene Assay, NCGC_ERalpha_Agonist), which suggests that the NCGC_ERalpha_Agonist assay is most sensitive for estrogenicity. When ToxCast data with only common chemicals for each of the three in vitro assays were considered, we predict that there was a 10% probability of detecting the same estrogenic response at or below 0.160 mg/L (ATG_ERa_TRANS), 0.062 mg/L (ATG_ERE_CIS), or 0.201 mg/L (NCGC_ERalpha_Agonist), indicating that the ATG_ERE_CIS assay is the most sensitive assay in this assessment. Future studies should examine comparative sensitivity of these assays for specific chemical classes.

RP037 Long-term effects of early life exposure to methylmercury and undernutrition in Daphnia pulex

D. Doke, UAB / Environmental Health Sciences; S. Hudson, UAB; J.M. Gohlke, UAB / Department of Environmental Health Sciences

Human populations are exposed simultaneously to chemical stressors and differing nutrition. Understanding how early life nutrition plays a role in modulating chemical toxicity as measured by later life outcomes is less documented. The high cost and time required to test interacting stressors (eg. chemical and malnutrition) in traditional animal models, makes the development of novel model systems of critical importance. This study evaluated *Daphnia pulex* as a tool for assessing effects of early life exposures to coupled stressors. As a well-known ecotoxicological model organism, *Daphnia* offers several advantages in human health research. Here we examined the toxicity

of methylmercury, a chemical which is a known developmental toxicant, under optimal and sub-optimal nutrition. We hypothesized that: Early life exposures in a low food regime will increase methylmercury toxicity as measured by reproduction and lifespan in *Daphnia pulex*. These endpoints provide information on the effect of early life stage exposures to MeHg and long-term effects on overall health. *D. pulex* will be exposed to two feed rates (half and full nutrition of algae) and exposed in early life to varying concentrations of methylmercury (5 dose levels ranging from 0 to 1600ng/l of methylmercury (II) chloride). Initial data in the different treatments suggest that there are significant differences in time to first reproduction. It is expected that in addition to methylmercury toxicity, health outcomes will be significantly different under the different nutrition regimes. We expect this research to serve as a basis for further work in using alternative model systems in human health research and in setting safe limits of exposure to chemicals that account for the wide range of nutrition experienced in the human population.

RP038 Expanding the evaluation of the chemical activity hypothesis for toxicity assessment

J.A. Arnot, ARC Arnot Research & Consulting / University of Toronto Scarborough / Department of Physical and Environmental Sciences; J. Armitage, University of Toronto Scarborough / Department of Physical and Environmental Sciences; A. Orazietti, ARC Arnot Research & Consulting / University of Toronto; T.N. Brown, Helmholtz centre for environmental research – UFZ / Department of Analytical Environmental Chemistry; L.S. McCarty, L.S. McCarty Scientific Research & Consulting; D. Mackay, Trent University

Regulations are being applied to evaluate the potential hazard, exposure and risk of chemicals to ecological receptors and humans. Evaluating the potential hazard and risk for all chemicals using traditional monitoring and animal testing data is not feasible. Consequently, there is value in maximizing the applicability of existing measured data for toxicity and risk assessment through the development and evaluation of models and Quantitative Structure-Activity Relationships (QSARs). The chemical activity hypothesis (model) has shown promise for characterizing toxic effects for acute lethal baseline narcosis for certain chemicals and test organisms. The hypothesis has been evaluated for a relatively limited range of species and taxa and the focus has been on the acute lethal endpoint and neutral organic chemicals. This project seeks to expand the evaluation of the chemical activity hypothesis to a broader range of organisms, effect endpoints and chemicals. A large aquatic ecotoxicity database has been compiled from publicly available sources and the peer-reviewed literature (ca. 1949 to 2012). The initial database includes more than 100,000 fish toxicity test entries for approximately 2,500 organic chemicals with molar mass ranging from 27 to 1356 g/mol and octanol-water partition coefficients (log K_{OW}) ranging from -7.3 to 11.4. Screening-level data quality assessment methods were developed and applied using a 1-compartment toxicokinetic model and chemical properties to identify test results considered to be either "unreliable" or "conditionally acceptable". The general approach of the data quality screening is to identify key sources of possible experimental error realizing that it is impossible to identify and address all possible sources of experimental uncertainty in the database. Relationships between chemical activity and predicted mode-of-action classifications are examined. A subset of well documented toxicity tests is used as a case study of "acceptable quality" data to examine the applicability of the activity hypothesis to neutral and ionogenic organic chemicals (accounting for the presence of the chemical in neutral and dissociated forms).

RP039 Selenium in otoliths of creek chub and green sunfish from a coal mining-impacted reach of the Mud River, West Virginia

L. Friedrich, Stantec Consulting; M.C. Arnold, Duke University / Nicholas School of the Environment; T. Lindberg, Nichloas School of the Environment; R.T. Di Giulio, Duke University / Nicholas School of the Environment; N. Halden, University of Manitoba / Geological Sciences; V.P. Palace, Department of Fisheries & Oceans Canada, Stantec Consultants Ltd.

Establishing the exposure histories of wild fish to trace elements is often difficult for mobile species. Chemical analyses of muscle or visceral tissues can provide information on recent exposure, but depuration, metabolic transformation, and tissue redistribution precludes temporal resolution. Otoliths, the calcified structures in the inner ear of teleost fish, are considered to be metabolically stable and therefore may serve as continuous records of exposure to trace elements in the environment. Otoliths are composed of layers of aragonite in a protein matrix deposited annually throughout the lifetime of the fish, thus providing a time scale. Both the inorganic portion and protein matrix have the capacity to incorporate a wide range of trace elements, and, as such, otoliths may be used to determine a fish's history of contaminant exposure in the wild. The Mud River ecosystem in West Virginia has been impacted by selenium (Se) emanating from mountain top removal/valley fill coal mining. Otoliths from creek chub (*Semotilus atromaculatus*) and green sunfish (*Lempomis cyanellus*) were analyzed using laser ablation inductively coupled mass spectrometry (LA-ICP-MS) to determine Se concentrations. As part of a larger study examining trophic transfer and bioaccumulation of Se, each fish's capture location and concentration of Se in their otoliths are described in relation to adjacent coal mining activity. This study represents the first determinations of Se in otoliths of creek chub and green sunfish. Preliminary results indicate that Se is present in otoliths of fish captured from mining-impacted sites to a greater extent than in fish from reference sites.

Molecular Toxicology, Toxicogenomics, and Biomarkers

RP040 Development of Circulating miRNAs as Biomarkers for mTBIs

D. Dobbins, East Carolina University / Biology; X. Pan, East Carolina University / Department of Biology

Although highly undiagnosed and untreated, mild traumatic brain injuries (mTBIs) are extremely prevalent and often result in neurological deficits including cognitive and emotional impairments. Severe traumatic brain injuries are easily detectable with modern imaging techniques, but mTBIs often lack proper diagnosis. This study aims to identify circulating miRNAs as novel biomarkers for blast-overpressure induced TBI and related neurological and systemic damages, especially for mild TBIs, which currently lack effective diagnostic strategy. First, animal models of mTBIs have been generated through the subjection of Sprague Dawley (SD) rats to above ambient pressure waves (5-15 psi) generated from compressed air. Next comparison and optimization of different RNA extraction procedures for circulating miRNA analysis were performed. Influential factors including anticoagulants, temperature, and sample storage duration have been examined utilizing both whole blood and serum specimens. Finally, gross pathological observations have been performed in SD rats subjected to 10 psi overpressure 7 days after injury. There is no significant lesion and hemorrhage observed in brain and other organ tissues. Detection of histological and subtle biochemical changes is ongoing. Gene expression analyses will be performed to identify aberrantly expressed circulating microRNAs (miRNAs). Bioinformatics approaches will be used to identify miRNA-regulated pathways that are related to neurological deficits and other biochemical changes.

RP041 Effects of Bisphenol A on Reproduction and Neurological Functions of Caenorhabditis Elegans

Z. Flood,

Bisphenol A (BPA) is a chemical component that widely exists in polycarbonate plastics. In this study we assessed the effects of BPA on reproduction and behaviors of the model organism *Caenorhabditis elegans* (*C. elegans*). Worms at larvae stage 1 (L1) were exposed to 0.1 and 1 μM of BPA until adulthood, and then transferred daily to BPA containing plates for five consecutive days. Reproduction evaluations were done using a light microscope, where the number of eggs and larvae were counted daily. Our results indicated that chronic exposure to BPA inhibits both brood size the egg hatching success. Behavioral analysis showed that the locomotion behaviors were altered following BPA exposure, indicating neurological disorders. Ongoing experiments are investigating the gene expression changes associated with observed reproductive and neurological effects.

RP042 Germ cell apoptosis is implicated in crude oil/dispersant induced reproductive toxicity in Caenorhabditis elegans

J.R. Polli, Y. Zhang, X. Pan, East Carolina University / Biology

One of the most detrimental environmental disasters in US History was the Deepwater Horizon (DWH) oil spill. Chemical dispersants were utilized to clean up the crude oil which led to further environmental impacts. However, health risks related to crude oil-dispersant toxicity remains unknown. We have employed *Caenorhabditis elegans* (*C. elegans*) to investigate the impacts of crude oil-dispersant exposure on reproduction and underlying molecular mechanisms. *C. elegans* were exposed to a range of different concentrations of oil-dispersant (oil-dis) mixtures. Previously, we have determined growth

and reproduction are inhibited by specific doses. Recent experiments determined that oil-dis mixtures affect germline apoptotic pathways resulting in increased apoptosis, especially at low dosages, at the molecular and cellular levels. Results from the experiments showed changes in several genes indicated in the apoptosis pathway. These expression changes may be regulated by microRNA-mediated mechanisms.

RP043 Lead-induced physiological changes and stress-responsive miRNAs expression in cotton (Gossypium hirsutum L.)

Q. He, East Carolina University / Department of Biology; B. Zhang, East Carolina University

The presence of heavy metals in the agricultural soil constrains the crop productivity and even exhibits hazardous influences on human health. MicroRNAs (miRNAs) are a class of endogenous small RNAs that regulate plant growth and development by silencing gene expression at post-transcriptional level. Recently, many literatures have shown that miRNAs are the regulators of plant response to environmental stresses. Also, some miRNAs are in response to heavy metals are reported. But there is few study has been performed the miRNA expression profile on the effect of lead-induced in cotton. Therefore, to determine the abiotic stress responses mediated by miRNA, the cotton (*Gossypium hirsutum L.*) seeds, which is one of the important fiber crops in the world, were exposed to different concentrations of plumbum (Pb) 0, 250, 500, and 1000mg per liter medium and then the toxicological effects were investigated. The effect of plumbum on seed germination ratio, changes in the root and shoot length, plant dry weight and moisture content were studied. The results suggested that, exposure of plumbum affected the seed germination, growth rate, biomass content and antioxidant enzyme activities in the root, shoot and leaves of the cultivars. Quantitative real-time PCR will be used to identify the expression fold change of miRNAs and miRNA targets in leaves and roots under heavy metal stress treatments. These results would help defining the potential roles of miRNAs in plant adaptation to heavy metal stress and further understanding of miRNA regulation in response to abiotic stress.

RP044 Linking germline apoptosis to reproductive success in Caenorhabditis elegans following exposure to ZnO Nanoparticles

B. O'Donnell, Department of Biology; X. Pan, East Carolina University, East Carolina University / Department of Biology

The ZnO nanoparticles (NPs) are widely used in personal care products such as toothpaste, sunscreens, cosmetics or beauty products. There is a data gap regarding effects of chronic exposure to low concentrations of manufactured ZnO NPs on reproduction and development. Our data indicated that *Caenorhabditis elegans* (*C. elegans*) exposed to ZnO NP (5 µg/L, 50 µg/L, 500 µg/L, and 5000 µg/L) from Larvae stage 1 to adulthood display more apoptotic germ cells. qRT-PCR analysis showed that several genes involved in the apoptosis pathway are expressed aberrantly. Ongoing experiments are conducted to explore the relationship between germ cell apoptosis and reproductive success using different mutants. The potential development defects resulting from ZnO NP exposure will also be investigated.

RP045 Potential cytotoxicity and genotoxicity of wastewater from alcoholic beverage industry in Southeastern Nigeria

A.C. Udebuani, Federal University of Technology / Department of Biotechnology; A.C. J, Federal University of Technology / Dept of Biotechnology; I.N. Onwurah, University of Nigeria / Dept of Biochemistry

The potential cytotoxic and genotoxic effects of wastewater from alcoholic beverage industry in Southeastern Nigeria was evaluated using *Allium cepa* Linn assay in a soil slurry microcosm experiment and standard routine analysis of water properties. The soil slurry microcosms were prepared using different dilutions (0.1 1.0, 10.0 and 20.0 % v/v) of the effluents with distilled/deionised water. Onion seedlings (four) were planted in each soil slurry microcosm and allowed to grow for four weeks and measurements taken at weekly intervals. The experiment was repeated with different soil types and the mean root growths (± SD) of the onion seedling evaluated. The least growth was observed in soil slurry-microcosms treated with 20.0% dilution. The decrease in mitotic index relative to control ranged from 69.00 to 58.00 and the differences in growth of the seedlings were significant ($p < 0.5$). Genotoxicity of the effluent was based on the diverse chromosomal aberrations observed in the exposed root tips of onion and they include stickiness, vagrant, bridges, multi polar deviation, and laggard. Stickiness and bridges were most frequent and the polar deviation was observed in microcosms having higher concentrations of the effluent. Since effects at higher levels of organization (i.e., populations and communities) represent the net sum of effects on individuals that resulted from alterations in cellular and molecular responses, the corollary holds for the result of this experiment. Therefore this model has the potential to enhance ecological risk assessment. It also reduced some uncertainties usually extrapolated from individuals to populations.

RP046 Small RNA sequencing uncovered the important roles of switchgrass miRNAs in response to drought and salinity stress as well biofuel production

F. Xie, East Carolina University / Biology; B. Zhang, East Carolina University

MicroRNAs (miRNAs) are a class of important small RNAs negatively regulating gene expression either by degrading mRNAs or by inhibiting mRNA translation. miRNAs are identified to play key roles in fundamental biological processes, including cell proliferation, development, metabolism, and stress response. In this study, three small RNA libraries were constructed from control, salt-, and drought-treated switchgrass seedlings and then were sequenced. 16-17 million short reads were generated in each library, resulting in a total of 51,953,577 reads and 16,289,218 unique sequences. Of them, a total of 84,206 unique sequences were detected to be conserved with known miRNAs, representing 670 known miRNA families. miRDeepFinder was employed to search miRNA precursors from the switchgrass genome in JGI. A total of 273 miRNA' precursors were identified, including 126 conserved miRNAs and 147 novel miRNAs. 265 of 273 miRNAs could be found to have miRNA* at least in one library. To further verify miRNA precursors with transcriptome evidence, we also aligned miRNA precursors against the switchgrass EST database and its assembled transcriptome sequences from 31 high-throughput RNA sequencing datasets. 194 miRNAs were detected in switchgrass transcriptome sequences. 11 miRNA clusters were also identified, which contained 29 miRNAs. These miRNAs were similar to those found in rice and Arabidopsis. Deep sequencing uncovered a series of miRNAs expressed only in plants treated with water- and salt-stress. Nonetheless, many miRNAs had no significant expression change among control, salt, and drought treatments, indicating their importance for broad gene regulation in swichgrass. A total of 28,549 genes were identified to be miRNA targets, including various transcription factors, stress-response proteins, cellulous-biosynthesis-related proteins, and other important protein. Furthermore, GO and KEGG analysis showed that the identified miRNAs and their targets were classified to 3,779 GO terms including 1,534 molecular functions, 1,851 biological processes, and 394 cellular components and were enriched to 147 KEGG pathways. Interestingly, 195 miRNA families and 450 their targets were involved in the biosynthesis pathways of carbon, glucose, starch, fatty acid, and lignin and in xylem formation. These results allow us to better understand the roles of miRNAs in stress response and improving biomass yields for biofuel production in switchgrass.

RP047 Systematic Alteration in MicroRNA Expression Profiles in response to Chronic Nicotine Exposure during Post-embryonic Stages in C. elegans

F.A. Taki, East Carolina University / Biology; B. Zhang, East Carolina University

Tobacco smoking is associated with many diseases, addiction being of the most notorious. The tobacco dependence is majorly attributed to nicotine, which is considered one of the most addictive chemicals. Though smoking is decreasing in developed countries, the situation is reversed in developing countries where the percentage of teenage smokers remains high. Early developmental stages have been reported to be more sensitive to different stresses. In our study, we chose *C. elegans* as a biological model to study the effect of chronic nicotine exposure. Nicotine treatment (20µM and 20mM) was limited to the post-embryonic stage from L1-L4 (~31 hours) period after which worms were collected for systematic miRNA profiling via qRT-PCR. Our results showed that nicotine significantly altered the expression patterns of 40 miRNAs. The effect was proportional to the nicotine dose and was expected to have an additive, more robust response. Based on pathway enrichment analysis coupled with nicotine-induced miRNA patterns, we inferred that miRNAs as a system mediates "regulatory hormesis", manifested in biphasic behavioral and physiological phenotypes. As conclusion, exposure to nicotine during the post-embryonic stage was indeed associated with significant changes in miRNA profiles. Thus, our study offers new insights for a better understanding of the sensitivity of early developmental stages to nicotine.

RP048 The microRNA mechanism in Crude Oil -Dispersant induced on Reproductive toxicity in Caenorhabditis elegans

Y. Zhang; X. Pan, East Carolina University

We employed the model organism *Caenorhabditis elegans (C. elegans)* to investigate impacts of crude oil/dispersant exposure on reproduction and underlying molecular mechanisms. In previous study, we found the crude oil and/or dispersant induce growth inhibition and offspring reduction in *C. elegans.* However the underlying molecular mechanism in response to oil/dispersant is poorly understood. Here we have tested various germline phenotypes after exposure to oil-dispersant mixture. Various abnormal germline phenotypes including immature spermatocytes, disordered germ cell arrangement, and increased apoptotic cells were observed. Also importantly, many microRNAs (miRNAs) implicated in reproduction and development processes are differentially expressed. Ongoing experiments are exploring the miRNA-mediated mechanisms in spermatogenesis defects and oocyte meiosis maturation process that lead to less progeny production.

RP049 Indirubin-3′-(2,3 dihydroxypropyl)-oximether (E804) is a potent modulator of LPS-stimulated functions and a weak AhR agonist in macrophages

A.L. Anderson, Clemson Univeristy / Biological Sciences; C.D. Rice, Clemson University / Biological Sciences

Indirubin is a deep-red bis-indole isomer of indigo blue, both of which are biologically active ingredients in Danggui Longhui Wan, an ancient Chinese herbal tea mixture used to treat neoplasia and chronic inflammation and to enhance detoxification of xenobiotics. Multiple indirubin derivatives have been synthesized and shown to inhibit cyclin-dependent kinases (CDKs) and glycogen-synthase kinase (GSK-3β) with varying degrees of potency. Several indirubins are also aryl hydrocarbon receptor (AhR) agonists, with AhR-associated activities covering a wide range of potencies, depending on molecular structure. Previous work in our lab examined the effects of indirubin-3′-(2,3 dihydroxypropyl)-oximether (E804), a novel indirubin derivative with potent effects on STAT3 signaling, on LPS-stimulated inflammatory profiles in RAW264.7 murine macrophages. Most genes, proteins, and biological functions up-regulated by LPS treatment were suppressed by E804, including LPS-induced expression and secretion of pro-inflammatory cytokines. However, RAW264.7 cells show only modest CYP1A1 induction following treatment, thus at this time it is not clear if E804 modulates inflammatory responses through AhR signaling. To further explore the possible effects of E804 on AhR signaling, THP-1 human monocytes were differentiated to macrophages by a 48 hr treatment with phorbol-12-myristate-13-acetate (PMA), then treated with E804 and select other indirubin derivatives and PCB-126, a well-characterized AhR ligand and potent inducer of CYP1A1. CYP1A1 gene and protein expression was significantly induced by 7-bromoindirubin-3'-oxime (7-BIO), another novel indirubin, and PCB-126, but not E804. Furthermore, E804 suppressed IL-6 secretion in LPS-stimulated THP-1 macrophages, thus confirming that AhR signaling is not a requirement for the anti-inflammatory properties of this compound.

Ecotoxicology and Risk Assessment of Aquatic Primary Producers

RP050 Effects of copper in Pistia stratiotes physiology in different concentrations of phosphorus

D.D. Schneider, H. Lobo, EESC – USP / Center for Water Resources and Applied Ecology; E.G. Espindola, University / Center for Water Resources and Applied Ecology

Copper in high concentrations is extremely toxic mainly to aquatic freshwater organisms, causing oxidative stress and impairing the photosynthetic, enzymatic and respiratory functions of the plants. In addition to this problem, there has been constant changes in the nutritional status of aquatic systems, due to the increased supply of nutrients, causing changes in biotic communities, including the aquatic macrophytes that are environmental bioindicators. Given this fact, one toxicity test was conducted to evaluate the effects caused by copper (73μgCu/L) in the physiology of *Pistia stratiotes*, taking into consideration three treatments with different concentrations of total dissolved phosphorus (A=16μg/L, B=36μg/L e C=90 μg/L), with and without copper, all in triplicate, by evaluation of parameters such as: final biomass, root length, height and width of leaves, number of sprouts, chlorophyll and number of leaves with chlorosis. The toxicity test lasted 10 days and was conducted in a laboratory fully controlled. The statistical results indicated that there was significant difference (significance level = 5%) for the variables: final biomass (ranging between 2.71g and 2.25g in treatments B and C respectively without copper and 1.16g and 0.75g in treatments B and C respectively with copper), root length (ranging between 9.52cm and 13.36cm in B and C respectively without copper and 2.5cm and 2.86cm in A and C respectively with copper), growth in leaves height (range between 0.14cm and 0.44cm both in B without copper and of 0.12cm in B to 0.20cm in A and C with copper), and number of yellow leaves (having minimum 2 in B and C without copper and maximum of 13 in C with copper). To the variable chlorophyll, there was no significant results caused by the action of copper. In general, there were very significant differences between treatments with and without copper, but there was no significant difference between the different phosphorus treatments (AxBxC). The results show that copper metal interferes with the physiology of macrophyte *Pistia stratiotes*, mainly on root development and final biomass, regardless of the amount of nutrients observed in this assay. However, the concentration of copper in question did not alter significantly the rates of chlorophyll held by this macrophyte, this fact may perhaps be related to the bioacumulator potential of this species or the amount of nutrients available in the treatments.

RP051 Ecological Risk Assessments with Primary Producers: Influences of Nutrients on Aquatic Toxicity Thresholds

B.W. Brooks, Baylor University / Department of Environmental Science; B. Fulton, ARCADIS; M.L. Hanson, University of Manitoba / Department of Environment and Geography

Nutrient concentrations and stoichiometries often vary among a number of growth media formulations commonly employed for aquatic toxicity assays with algae and macrophytes. Subsequently, nutrient availability and limitation during algae and macrophyte toxicity tests can significantly influence control growth rates, which ultimately alter point estimates of toxicity thresholds and thus introduce uncertainty in ecological risk assessments. Further, nutrient concentrations and stoichiometries in these various standardized growth media are often not reflective of environmental concentrations or stoichiometries of N and P. This observation is critical because nutrients are common co-occurring stressors with other contaminants in non-point (e.g., agricultural runoff) and point (e.g., wastewater effluent discharge) source scenarios. Here we critically review available literature pertinent to such observations, highlight lessons learned from our recent related studies, and present a framework for future assessments with aquatic macrophytes and algae.

RP052 Effects of exposure period duration on the response and recovery of Lemna minor exposed to the herbicide metolachlor

P.C. Wilson, University of Florida / IFAS / IRREC- Soil & Water Science; Y. Lin, University of Florida / IRREC

Non-target aquatic organisms in the environment may be exposed to pesticides in pulses associated with application and runoff/drainage events. The amount of time that the organism is exposed to the pesticide, and the pesticide concentration, are critical for determining potential toxicity. This study evaluated the effects of exposure time and concentration on the toxicity of metolachlor to the aquatic macrophyte, *Lemna minor*. *L. minor* fronds (10 fronds/replicate, 3 replicates/treatment) were exposed to metolachlor at the following concentrations for three or six days: 0, 2, 4, 8, 15, 30, 60, 125, 250, 500, and 750 μg/L. Frond production was counted every day during the exposure periods. Following exposures, 10 fronds from each replicate were transferred to non-spiked media and frond production was counted every two days. Root length and fresh weights were also measured at the end of each exposure period, and at the end of the recovery periods. Following 3 d exposure, no differences in frond production or fresh weights were observed, but root lengths were significant reduced at ≥15 μg/L. Significant reductions in frond production occurred by 7 d after treatment (dat)(4 d recovery) at ≥15 μg/L metolachlor. No differences in frond production, root length, or fresh weights were observed by 15 days after treatment. For the 6 d exposure treatment, significant reductions in frond production began at 4 dat (≥60 μg/L), peaking 10 dat (≥8 μg/L). Root lengths and fresh weights were also significantly reduced at the end of the 6 d exposure period at concentrations ≥15 μg/L. Frond production, root lengths, and fresh weights were similar to controls by 18 days after treatment. Results indicate a delayed onset of effects on frond production after 3 d exposure, but effects are reversible as indicated by restoration of frond production, root length, and fresh weights. In both cases it took approximately 12 d for growth responses to return to control levels.

RP053 Root structure: A potential mechanism of exclusion for gold nanoparticle uptake in aquatic vascular plants

B. Glenn, Clemson University / Clemson Institute of Environmental Toxicology; S.J. Klaine, Clemson University / Institute of Environmental Toxicology

Roots of *Azolla caroliniana* and *Myriophyllum simulans* have been found to absorb spherical, citrate capped gold nanoparticles (AuNPs) in a size dependent manner, while *Egeria densa* roots have been shown not to absorb AuNPs from suspension. To determine a possible mechanism of size and species dependent AuNP absorption, an electron microscopy study of the root structure was preformed to identify structural differences. It is routinely observed that the highest tissue concentrations result when exposed to the smallest AuNPs (3.5-5 nms). From the micrographs, it was determined that each size AuNP did associate with each of the root types, however only the 4-nm AuNPs were seen within the plant apoplast for *M. simulans* and middle lamella layer of *A. caroliniana*. The 18- and 30-nm AuNPs were only adsorbed to the root surface. Because *A. caroliniana* resulted in the highest tissue concentrations, a published solute exclusion technique was utilized to estimate root pore size, or spaces in the cellulose microfibril matrix, and further document the structure of the roots with regards to AuNP exclusion. The solute exclusion technique indicated that the roots were unable to absorb solutes larger than 5- to 6-nm. Electron micrographs also indicate the roots of *Azolla caroliniana* are the least dense, and thinnest of all species tested. To determine if 18- and 30-nm AuNPs could be absorbed through root wounding, each species was exposed to 4-, 18- and 30-nm AuNPs in a root wounding study. Although results indicate that root structure is an important factor in screening AuNP size, wounded roots did not increase tissue concentration revealing it was not a major pathway to increase AuNP absorption. This study also indicated that root structure is a significant factor in explaining the size dependent uptake observed.

Contaminants of Emerging Concern for Fish: Assessing Exposure and Effects Across Biological Scales

RP054 Linking Bioavailability to Bioactivity of Persistent Contaminants in Fish

V.D. Dang, K.J. Kroll, University of Florida / Physiological Sciences; S.D. Supowit, Arizona State University / Department of Civil and Environmental Engineering; R.U. Halden, Arizona State University; N.D. Denslow, University of Florida / Physiological Sciences, Center for Environmental and Human Toxicology

Persistent organochlorine contaminants in sediments are bioavailable to aquatic organisms and prone to bioaccumulate at higher trophic levels. These contaminants may also activate estrogen and androgen receptors in fish particularly at early life stage. Attention has been paid to muck farms located on the north shore of Lake Apopka, FL as they are historically contaminated with organochlorine pesticides (OCPs) from heavy agricultural application from 1940-1970. Sediments and soils in the muck farms have high levels of OCPs (e.g., 252 ± 164 and 1720 ± 383 ng/g dry wt for dieldrin and *p,p'*-DDE, respectively, among other OCPs) which are known to impair the nervous system and reproductive processes in fish in the wild. Here, we performed a bioaccumulation experiment to gain insight into the linkage of bioavailability to bioactivity of contaminants in fish. Control soils featuring a high total organic carbon (~ 36 % w/w) and background levels of OCPs were obtained from the Duda muck farms of the north shore of Lake Apopka, FL. Individual chemicals or a mixture of *p,p'*-DDE, dieldrin, triclosan, triclocarban, and fipronil were spiked into a slurry of soil and water. The slurry was agitated continuously for 28 days and then stored for one month prior to bioaccumulation experiment. The bioaccumulation study was accomplished by exposing *Lumbriculus variegatus* (blackworm) to contaminated soils for 2, 7, 14, 21, and 28 days. In addition, worms were exposed only to spiked water and then fed to *Pimephales promelas* (fathead minnow). Worm and fish tissues collected at different time intervals were analyzed for chemical concentrations. Preliminary data show that body burdens of chemicals reached their maxima faster in worms exposed to spiked water than in worms exposed to spiked soils. Since *p,p'*-DDE behaves as both a weak estrogen and an anti-androgen, tissue extracts with detectable *p,p'*-DDE concentrations are being subjected to *in-vitro* toxicity assays to specifically measure these activities. Application of both chemical and *in-vitro* assays to study bioaccumulation and bioactivity will be useful for both risk assessment of contaminated sediments and decision making for site remediation.

RP055 Effect of co-exposure DDT and pyrethroids or surfactants on steroidogenic and catabolic cytochrome P450 enzymes male hornyhead turbot

J. Crago, UC-Riverside / Environmental Sciences; F. Jia, University of California, Riverside / Department of Environmental Sciences; J. Gan, University of California, Riverside / Department of Environmental Science; D. Schlenk, University of California-Riverside / Department of Environmental Sciences

The Palos Verdes (PV) Superfund Site offers an opportunity to examine the simultaneous effect of both legacy compounds, such as DDT and PCBs, and emerging compounds (CECs) such as pyrethroids and surfactants on endocrine endpoints in benthic fish. Benthic fish found within the PV Shelf, such as Horyhead Turbot have high concentrations of DDT metabolites (µg/g tissue wgt), and are continually exposed to low-level concentrations of CECs from the outfall site.The goal of this study was to determine the effect on endocrine disruption in male turbot fed worms exposed to PV sediment containing high levels of DDT, and then to exposure to the pyrethroid bifenthrin and the surfactant nonylphenol. The first part of this study determined the uptake and metabolism of DDT metabolites from PV Shelf sediment in exposed polychaete worms and male hornyhead turbots fed those exposed worms over a 7- and 28-d time period. The second part of the study demonstrated that male turbot fed 30mg of sediment-exposed polychaete worms/d for 28-d showed an increase in plasma E2 and LSI as compared to the control. The next part of the study examines the effect from co-exposure to environmentally relevant concentrations of the pyrethroid bifenthrin or the surfactant nonylphenol on steroidogenic and catabolic cytochrome P450 enzymes in the liver and plasma hormone levels in male hornyhead turbot. (NIEHS Grant No: R01ES20921)

RP056 Bifenthrin uptake, depuration and potential metabolism in Coho Salmon

J. Teerlink, USGS / California Water Science Center; K.L. Smalling, K.M. Kuivila, US Geological Survey; C.A. Laetz, NOAA / Northwest Fisheries Science Center; D.H. Baldwin, N.L. Scholz, NOAA Fisheries / Northwest Fisheries Science Center; J.D. Stark, Washington State University / Dept. of Entomology

Directly measuring pesticide residues in the tissue of non-target organisms allows quantification of body burden responsible for non-lethal toxicological endpoints. Recent advances in analytical capabilities allow for such measurements and thus, the linkages between tissue concentrations and effects are just beginning to be described for current-use pesticides. Bifenthrin is a third-generation synthetic pyrethroid insecticide characterized by increased environmental persistence and toxicity to aquatic species, including fish used in standard toxicity testing. Although the lethal toxicity has been described for several fish species, much less is known about any sub-lethal effects of both the parent pesticide and its metabolites. In this study, the uptake and depuration of bifenthrin in juvenile Coho salmon (*Oncorhynchus kisutch*) was investigated in a water-exposure laboratory experiment. Bifenthrin concentrations were analyzed using gas chromatography tandem mass spectrometry and the presence of bifenthrin metabolites were investigated using forensic software on full scan chromatograms. After exposure at two aqueous concentrations (20 ng/L and 200 ng/L), bifenthrin uptake was quantified (4 time points, 6 fish at each time point). To study depuration, salmon were exposed to 20 ng/L bifenthrin concentrations for 96-hours. Salmon were then placed in clean water and individual fish subsampled for tissue concentration over the course of 7 days (8 time points, 6 fish at each time point). Detectable concentrations of bifenthrin were still observed at the end the 7-day depuration experiment. Linking sub-lethal effects of bifenthrin exposure (e.g., changes to smoltification, outmigration, ocean survival, weight, predator/prey response) to concentration in animal tissue is needed to better understand the impact of this pesticide.

RP057 Toxicological effects of perfluorooctanesulfonate (PFOS) on embryonic and early life stages of zebrafish Danio Rerio

C.E. Greenfield, Rutgers University / Environmental Science; K.R. Cooper, Rutgers University

Perfluorooctanesulfonate (PFOS) is a highly persistent chemical that has been detected world-wide and is known to accumulate in both the receiving environments and in animals. This chemical has been used in both industrial applications such as solvent stabilizers as well as in commercially aspects such as water-repellent clothing. It is hypothesized that chronic sub-lethal embryonic exposure to PFOS in Zebrafish (*Danio rerio;* strain A-B) will display developmental defects and reduced long-term survivorship. Preliminary

studies have indicated that embryonic zebrafish exposure to increasing levels of PFOS (control 0 ug/mL, 0.1 ug/mL, 1 ug/mL, 10 ug/mL, 25 ug/mL) resulted in no significant increase in death up until 3 days post fertilization. Incidence of pericardial edema increased with the level of exposure (control = 4/15, 0.1 ug/mL = 3/15, 1 ug/mL = 9/15, 10 ug/mL = 5/15, 25 ug/mL = 14/15). Zebrafish from the highest exposure group (25 ug/mL) showed hyper-pigmentation at 48 hours when compared to the control group, but at time of hatching 4 of the surviving fish showed hypo-pigmentation. Additionally, fish from the two higher exposure groups (10 ug/mL, 25 ug/mL) showed hyper-activity in the tail flexure within the egg prior to hatching. Based on these sub-lethal observations, future studies will examine the survival post-hatch as well as use QPCR to analyze the PPARα, CAR, and PXR nuclear receptors. (NJAES Rutgers AD421).

RP058 Multi-Tiered Effects-Based Evaluation of Contaminants of Emerging Concern In The Maumee Area of Concern

J.P. Berninger, USEPA; J.E. Cavallin, USEPA, ORISE / National Health and Environmental Effects Research Laboratory; K.E. Stevens, USEPA Mid-Continent Ecology Division, ORISE; M. Hughes, USEPA; E. Eid, USEPA / MED; D.L. Villeneuve, USEPA / Mid-Continent Ecology Division; G.T. Ankley, USEPA / National Health and Environmental Effects Research Laboratory

Great Lakes Areas of Concern (AOC) have been defined by traditional pollutants (e.g., persistent organics, metals), but newer contaminants of emerging concern (CECs) contribute to the overall pollution profile and likely impacts. The Maumee River, near Toledo, OH is one such AOC, where CEC contributions come from urban and agricultural runoff and waste water treatment plant (WWTP) outflows. To evaluate the role of CECs in this AOC, a multi-tiered effects-based methodology was employed over two years. This approach uses analytical chemistry, cell bioassays, and caged fathead minnows for the assessment of CECs. Eight different sites in the Maumee AOC were evaluated. Sites were selected to cover the entire AOC, with a focus on WWTPs as a major source for CECs. Chemical analysis showed the largest numbers of CECs near the Toledo WWTP, including pharmaceuticals, pesticides, plasticizers, surfactants, and estrogenic compounds. Some CECs were also present at the mouth of Swan Creek, small, urban tributary. Two cell-based bioassays, T47D and MDA-kb2, were used to evaluate grab and composite water samples. Estrogenic activity, measured using the T47D assay, was elevated above baseline in grab samples from areas near the WWTPs, and in most 4-d composite samples. Androgenic activity, measured using the MDA cells, was significantly elevated at the Swan Creek, and an MDA anti-androgenic response was seen in samples from near the Toledo WWTP. Fathead minnows (n=24 per site; 12M, 12F) were deployed for 4 days at all eight sites. Tissues from the exposed fish were used for several measurements including metabolomics (urine, liver, gonad), plasma steroid and vitellogenin analysis, ex-vivo steroid production (gonad), transcriptomics and targeted qPCR (liver, gonad). The ex-vivo assay (estrogen-females) and qPCR vtg mRNA (male-liver) showed significant increases over baseline at Swan Creek sites. Expression of the hepatic transcripts for the xenobiotic metabolizing enzymes cyp1A1 and 3A were significantly increased at sites near the Toledo WWTP and Swan Creek. While more data are pending, the available information thus far from our multi-tiered analysis points to potential adverse effects resulting from CECs. With efforts underway to clean up and eventually delist AOCs, poorly regulated and constantly incoming CECs (e.g., from WWTPs) contribute to the contaminant load in the Maumee AOC, presenting a challenge to those involved in remediation at the site.

RP059 Determining the effect of municipal waste water effluent on the spawning behaviour of male rainbow darter (Etheostoma caeruleum) in the Grand River

A. Crichton, University of Waterloo; M. Fuzzen, M. Servos, University of Waterloo / Department of Biology

The Grand River, which runs through the Kitchener-Waterloo region in Ontario, Canada, has multiple input sites of municipal wastewater effluent (MWWE). Rainbow darter (*Etheostoma caeruleum*) have been shown to have very high incidence of intersex (ova-testes) downstream of the MWWE outfalls. Previous studies have also reported decreased gonadal 11-ketotestosterone (11-KT) production in male rainbow darter at sites downstream of these outfalls. Since 11-KT is associated with the expression of secondary sexual characteristics and aggression in fish species, it is hypothesized that spawning behaviour differs between populations of rainbow darter living above and below MWWE outfalls. To test this, fish were collected from sites directly upstream and downstream of the Kitchener wastewater treatment plant, and at a reference site outside of city limits. Size matched males (one from each site) were placed into a tank with a female and a spawning substrate. Interactions between males (chasing, guarding) and with the female (spawning) were video recorded and quantified. While no significant differences in these measures were observed between collection sites, fish with intersex condition (oocytes present in testes) displayed male behaviours less frequently than fish with normal testes. These findings have serious implications for the reproductive success of rainbow darter in the Grand River.

RP060 Measured and modeled toxicokinetics in cultured fish cells and application to in vitro – in vivo toxicity extrapolation

J. Stadnicka-Michalak, Eawag,Swiss Federal Inst. of Aquatic Science&Techn; K. Tanneberger, Ecosens AG / Environmental Toxicology; K. Schirmer, Eawag, Swiss Federal Institute of Aquatic Science and Technology / Environmental Toxicology; R. Ashauer, University of York / Dept of Environmental Toxicology

In assessing the environmental risk of chemicals, fish play a very important role, being the most frequently tested vertebrate representative for freshwater systems.Integrated Testing Strategies include consideration of use of computer (*in silico*) and *in vitro* methods to allow *in vivo* toxicity extrapolation. The integration process often starts with the lowest units of thebiological organization, the molecular and cellular levels. However, the question is how to link cellular responses to whole organism effects because if it is possible,*in vitro* fish cell assays might be considered as an alternative to fish bioassays to replace or reduce the use of fish in toxicological testing.Effect concentrations in the toxicity assessment of chemicals with fish and fish cells are generally based on external exposure concentrations. External concentrations as dose metrics, may, however, hamper interpretation and extrapolation of toxicological effects because it is the internal concentration that gives rise to the biological effective dose. Thus, we need to understand the relationship between the external and internal concentrations of chemicals in fish and fish cells.The objectives of this study were to: (i) measure the time-course of the concentration of chemicals with a wide range of physicochemical properties in the compartments of an *in vitro* test system, (ii) derive a general, predictive model for toxicokinetics in the *in vitro* test system, and (iii) test the hypothesis that internal effect concentrations in fish (*in vivo*) and fish cell lines (*in vitro*) correlate. We measured time-dependent amounts of organic chemicals in medium, cells (RTgill-W1) and the plastic of exposure wells. We investigated the relation between uptake and elimination rate constants for cells and log K_{OW}. We also used our toxicokinetic model to predict internal effect concentrations in cells, which we compared with internal effect concentrations in fish gills predicted by a Physiologically Based Toxicokinetic model. Our model could predict concentrations of non-volatile organic chemicals with log K_{OW} between 0.5 and 7 in cells. In addition, the significant correlation (R^2> 0.7, p = 0.0008, F-test) between modeled internal effect concentrations in fish gills and a fish cell line is a very promising step to predict lethal effects on fish based on *in vitro* data.

RP061 Screening and modeling neuroendocrine perturbations along the hypothalamic-gonadal-pituitary axis to predict adverse outcomes in fish

B.M. Armstrong, C.A. Murphy, Michigan State University / Fisheries and Wildlife; N. Basu, McGill University / Department of Environmental Health Sciences; J. Head, University of Michigan / Cooperative Institute of Limnology and Ecosystem Research; R. Goetz, NOAA / Northwest Fisheries Science Center; M.J. Carvan, University of Wisconsin-Milwaukee / School of Freshwater Sciences; A. Arini, School of Public Health; D. Nam, Chonnam University / Department of Environmental Health Sciences; E. Smigielski, K. Chopra, Michigan State University

Many environmental contaminants cause adverse reproductive effects through disruption of the endocrine system and there is a pressing need to develop techniques that are able to simultaneously screen thousands of chemicals and to predict individual/population level effects . Previous efforts that modeled the fish hypothalamic-pituitary-gonadal (HPG) axis simulated the individual production of vitellogenin as an end product that could be linked to population relevant outcomes. These models were driven by the production of gonadotropin and did not incorporate components that influenced gonadotropin release. Many end products of the hormonal cascade, such as vitellogenin, that occur within the HPG axis are controlled by the upstream release of neurotransmitters that modulate gonadotropin releasing hormone. Neurotoxicants have been shown to impair electrical

conduction, alter synaptic transmission and cause actual physical damage to the neural cells, and can alter gonadotropin release. We suggest that inclusion of a neurotransmitter compartment into an HPG model can increase its predictive power and accuracy and allow for mid-throughput testing through cell-free assays. We adapted a current fish vitellogenesis model and incorporated gamma-aminobutyric acid and dopamine dynamics to better estimate reproductive effects caused by neurotoxicant exposure. We calibrated and validated our model using adult female yellow perch that were raised in a controlled laboratory environment and exposed to concentrations of methylmercury, a known neurotoxin, throughout the period of gonadal growth. During the experiment, fish from each concentration were sampled at several time points and several molecular endpoints were measured. As part of an adverse outcome pathway approach, we also integrated results from cell-free mid-throughput bioassays for various other neurotoxicants into the model to predict adverse reproductive outcomes at the individual level. Our model results indicate that exposure to toxicants which interfere with neurotransmission can potentially cause harmful reproductive effects with population implications that can be detected through mid-throughput cell free assays.

RP062 Contaminants of Emerging Concern and Their Effects to Fish and Wildlife in the Great Lakes Basin

Z. Jorgenson, US Fish and Wildlife Service / Aquatic Toxicology Laboratory / Environmental Contaminants; L. Thomas, St. Cloud State University; K. Lee, US Geological Survey; S. Choy, US Fish and Wildlife Service; J.N. Moore, US Fish and Wildlife Service / Dept. of Animal Science / Ecological Services; J.A. Banda, US Fish & Wildlife Service; D. Gefell, US Fish and Wildlife Service; H.I. Schoenfuss, St. Cloud State University

In recent years, the Great Lakes basin has seen an increase in the detection of contaminants of emerging concern (CEC) from multiple studies. CECs include organic chemicals used in pharmaceuticals, fragrances, surfactants, plastic components, natural and synthetic hormones, and other products that are often unregulated by state or Federal water quality programs. Although CECs have been detected, their environmental fate or effects are not well known, and available information is generally inadequate to address the risks from these chemicals on wildlife. Failure to identify and understand the effects of CECs on fish and wildlife may result in deleterious impacts to ecosystems of the Great Lakes that could in turn result in adverse ecological, economic and recreational consequences. The US Fish and Wildlife Service has been working with multiple partners (US Geological Survey, US Environmental Protection Agency, West Virginia University, and St. Cloud State University) since 2010 to investigate the occurrence and concentrations of approximately 150 CECs and the potential impacts on fish and wildlife resources. This investigation has included the collection of sediment, surface water, and resident fish at multiple locations throughout the Great Lakes basin. Additionally, bluegill sunfish have been caged at water and sediment sample locations, and additional water at these sites has been collected for a predator avoidance assay. Resident fish were weighed, measured, bled and necropsied in the field. Biological measurements include plasma vitellogenin concentrations, reproductive hormone concentrations, liver and gonadal histological endpoints, and gene expression analyses. Data analyses will include determination of geographic differences in CEC occurrence and concentrations and observed effects in fish responses. The potential sources of CECs will also be explored. Results will contribute to our understanding of how different landscapes influence CECs occurrences across the Great Lakes basin and how exposure to these compounds may affect fish and wildlife.

Application of Bioavailability in Risk Analysis and Remedial Decision-Making

RP063 Impact of Zn, Cu, Al and Fe on the partitioning and bioaccessibility of 14C-phenanthrene in soil

I.S. Obuekwe, Lancaster University; K.T. Semple, Lancaster University / Lancaster Environment Centre

This investigation considered the effects of Zn, Cu, Al and Fe (50 and 500 mg/kg) on the loss, sequential extractability, using calcium chloride ($CaCl_2$), hydroxypropyl-ß-cyclodextrin (HPCD) and dichloromethane (DCM) and biodegradation of 14C-phenanthrene in soil over 63 d contact time. The key findings were that the presence of Cu and Al (500 mg/kg) resulted in larger amounts of ^{14}C-phenanthrene being extracted by $CaCl_2$ and HPCD. Further, the $CaCl_2$+HPCD extractions directly predicted the biodegradation of the PAH in the presence of the metals, with the exception of 500 mg/kg Cu and Al. The presence of high concentrations of some metals can impact on the mobility and accessibility of phenanthrene in soil, which may impact on the risk assessment of PAH contaminated soil

RP064 Examining Temporal Changes of Speciation and Bioaccessibility of Heavy Metals in Soils by a Flow-through Column Experiment

Y. Shen, S. Shdo, Harvard School of Public Health; J.P. Shine, Harvard School of Public Health / Department of Environmental Health

Heavy metal contamination in soil is a widespread problem due to past and current mining activity, smelting, and other industrial processes. Incidental oral ingestion of metal-contaminated soils is considered an important pathway of human exposure to heavy metals. The bioaccessible fraction of metals can be highly variable, and some of the variability which is often ignored comes from temporal change due to soil aging and fluctuating field conditions. Soil aging experiments were conducted for As, Zn, Pb and Cu in some of their frequently occurring forms in the soil (mainly sulfide, carbonate, and oxide minerals). Each column contained clean soils with 500 ppm of metal in one of its mineral forms and was under advective flow using site-specific water. Flow velocities upward through the column were maintained at about 20 cm/d (pore water velocity). Effluents were monitored monthly for pH and metal content. At 0,1,2,6,12 mon, columns were sacrificed and soil samples were air-dried and subject to six-step sequential extractions and Simple Bioaccessibility Extraction Test (SBET). A huge increase of bioaccessibility of metal sulfides was observed over the one year period probably due to slow oxidation process: 6.7% to 70.7% for Galena (PbS), 1.2 to 26.9% for Sphalerite (ZnS), 3.5% to 55.0% for Covellite (CuS) and 1.9% to 39.6% for Chalcopyrite (CuFeS). There was also corresponding metal redistribution to more mobile forms. In comparison, no substantial increase of metal lability occurred for metals in more oxidized mineral forms within one year. However, after six months these metals in more oxidized mineral forms tended to move to the residual fraction. Metal concentrations in the effluents manifested multimodal pattern which might indicate various binding sites for metals in the soil matrix. Multi-linear regression models were put forward to incorporate temporal rate of metal lability as well as other important factors to assess the risk of heavy metals in soil through the ingestion pathway.

RP065 Incorporating Bioavailability into Sediment Assessments in the St. Louis Area of Concern

N.W. Johnson, X. Zhu, University of Minnesota Duluth / Civil Engineering

The state of Minnesota, in partnership with federal agencies, has devoted significant resources to characterizing sediment contamination across the St. Louis River Area of Concern (Lake Superior near Duluth, MN). However, outside of heavily contaminated areas, very little consideration has been given to the bioavailability of sediment-associated contaminants. A review of existing data has shown that sediment characteristics at some sites with moderate levels of PAH and mercury contamination have non-standard geochemical conditions which could make contaminant bioavailability critically important. This ongoing project seeks to ascertain the most important mechanisms controlling contaminant bioavailability in the working harbor in order to enhance the utility of existing sediment chemistry data for restoration-potential site assessment. Preliminary results based on the use of predictive bioavailability based techniques (organic carbon normalization and equilibrium partitioning) have shown little improvement in predictive ability of lethal or sub-lethal endpoints over those based on bulk sediment (screening values). Beginning with moderately contaminated, high-priority restoration sites, as defined by the goals of local partners, gaps in existing sediment chemistry data are being filled by targeted sample collection and analysis. Porewater PAHs are monitored through SPME passive sampling and total- and methyl- mercury are monitored in both the solid phase and pore waters to quantify the long term potential for methyl mercury production and the immediate potential for transport away from the location of production. Though sample collection and analysis are still ongoing, these direct analyses of bioavailable contaminants have shown promise in predicting sublethal endpoints in areas of moderate levels of contamination.

RP066 How Sampling and Handling of Anoxic Sediments Can Result in False-Positive Assertions of Metal Bioavailability Due to Metal-Sulfide Oxidation

B. Tease, ENVIRON International Corp; M.T. Sorensen, ENVIRON International Corp; J. Occhailini, J. Kowalski, Alpha Analytical Inc.

The oxidation of insoluble metal sulfides as a result of aggressive sampling and/or handling practices can contribute to the over estimation of metal bioavailability in anoxic sediments. Such "false positives' can lead to the implementation of unnecessary and costly remedial actions, as well as instill uncertainty in the evaluation of metal bioavailability as a risk assessment tool. A 2012 study was conducted to assess the extent to which the accuracy and precision of the acid volatile sulfide (AVS) and simultaneously extracted metal (SEM) method results are affected by typical sample collection and handling methods. Discrete and composite samples were prepared from river sediment cores to assess the extent to which metal sulfides were oxidized during sample collection. Full and half-filled containers of composited material were also prepared to evaluate the potential for metal sulfide loss attributed to sample head space and holding time. The average sum of the SEM concentrations represented by cadmium, copper, lead, nickel and zinc (∑SEM) in the discrete samples was 86% of the average sum of the total metal concentrations (∑Total Metal) for these elements. In comparison, the ∑SEM concentrations in the full container composite samples were 54-63% of the ∑Total Metal concentrations, implying a loss in ∑SEM of 23-32% attributed to sediment oxidation. Sediment from the half-filled containers exhibited 34-40% less AVS compared to sediment from the full containers, which was attributed to head-space induced oxidation of the weaker iron and manganese sulfide complexes. Significant differences in AVS content due to holding time were not apparent. Total iron, %TOC and %solid measurements were shown to correlate well with AVS concentrations suggesting their beneficial use as monitors of potential metal sulfide oxidation during sample collection and/or handling. The concentration of 1-2%TOC was demonstrated to be a threshold value above which sufficient AVS was present indicating the suitability of implementing the SEM-AVS method at the study site. Comparison of individual total metal to SEM concentrations were also helpful in evaluating whether the variations in AVS and SEM concentrations detected were representative of heterogenic site conditions or artifacts of sample handling. Recommendations for preferred sample handling and analytical protocols directed at minimizing metal sulfide oxidation are presented.

RP067 Considering Maximum Solubility and Apparent Solubility in the Regulatory Assessment of Organic Chemicals

M.A. Bonnell, Environment Canada / Ecological Assessment Division

When conducting the ecological assessment of organic chemicals, maximum equilibrium solubility is routinely reported and considered as a function of measured water solubility limits or vapour pressure at standard temperatures and pressure. However, there are factors that can influence the maximum thermodynamic solubility in water or air such as temperature and ionic strength (for ionizing chemicals) that are not routinely considered in assessment. Maximum solubility limits can also be calculated for other media such as sediments, soil or biota and these can depend on organic carbon contents or lipid contents of the target medium. Water solubility can also be measured beyond thermodynamic limits due to the presence of co-solvents or micelles that produce an apparent solubility greater than the thermodynamic limit in the dissolved phase. In risk assessment, understanding maximum solubility and apparent solubility becomes particularly important when dealing with very poorly soluble substances in water and other phases, such as some dyes, pigments or high $\log K_{ow}$ compounds. Risk assessors continue to be challenged with interpreting the realism of physical-chemical property, fate and toxicity data for these substances when exposure concentrations are beyond the maximum solubility limit, when there are no effects at saturation or when solvents have been used to artificially increase bioavailability in the exposure medium. Here we discuss maximum solubility in various media as well as its domain of applicability in risk assessment. We then apply this concept to the interpretation of traditional aquatic toxicity tests for poorly soluble (but perhaps reactive) substances and introduce concepts such as critical body burden, fugacity capacity and chemical activity as tools to help interpret assessment toxicity data exceeding solubility limits. Ultimately the goal of such discussion is to prevent false negative and false positive conclusions that can arise from basing assessment decisions on data for the wrong exposure medium or due to the possibility of test interferences.

Assessing Contaminant Effects in Multi-stress Ecosystems

RP068 Interactions of single compounds and mixtures of PAHs in the presence of UV in larval zebrafish and implications for other environmental contaminates

A.M. Willis, Toxicology Excellence for Risk Assessment (TERA) / Zoology; J.T. Oris, Miami University / Department of Biology

This study examined photo-induced toxicity and toxicokinetics for acute exposure to selected polycyclic aromatic hydrocarbons (PAHs) in zebrafish. Photo-enhanced toxicity from co-exposure to ultraviolet radiation (UV) and PAHs enhanced the toxicity and exhibited toxic effects at PAH concentrations orders of magnitude below effects observed in the absence of UV. Because environmental exposure to PAHs is usually in the form of complex mixtures, this study sought to examine the photo-induced toxicity of both single compounds and mixtures of PAHs. In a sensitive larval lifestage of zebrafish, acute photo-induced LC_{50}s were derived for four PAHs (anthracene, pyrene, carbazole, and phenanthrene) to examine the hypothesis that phototoxic (anthracene and pyrene) and non-phototoxic (carbazole and phenanthrene) pathways of mixtures could be predicted from single exposures. Anthracene and pyrene were phototoxic as predicted; however, carbazole exhibited moderate photo-induced toxicity and phenanthrene exhibited weak photo-induced toxicity. Individual toxicity for each chemical was then used to compare the toxicity of mixtures in binary, tertiary, and quaternary combinations of these four PAHs, and was examined using a Toxic Unit approach. Toxicity was predicted to be additive for all mixtures, most likely due to toxicity caused through similar adverse outcome pathways. The results indicated that the predictions of acute toxicity of PAH mixtures were additive in phototoxic scenarios, regardless of severity of photo-enhancement. More information is needed on other compounds that may exhibit photo-enhanced toxicity, and the relevance to other phototoxic components of environmental mixtures such as pesticides and dyes.

RP069 Understanding climate induced toxicant sensitivity: exposure history influences temperature and cadmium tolerance

D.A. Kimberly, C.J. Salice, Texas Tech University / Environmental Toxicology

The Intergovernmental Panel on Climate Change (IPCC) projects that global climate change (GCC) will have significant impacts on environmental conditions including potential effects on sensitivity of organisms to environmental contaminants. Because of the projected increase in regional variation of climate change parameters including temperature, and because many environmental contaminants also vary temporally, acclimation to one stressor may come at the cost of being more sensitive to additional, novel stress factors. The objective of this study was to evaluate how climate induced changes in temperature influences subsequent sensitivity to toxicants in the freshwater snail, *Physa pomilia*. Adult snails were acclimated to one of two temperatures, including 22°C (near optimal) or 28°C (marginally stressful), for 28 days. After 28 days, snails were then exposed to either a low (150µg/L) or a high (300µg/L) cadmium challenge at their acclimation temperature (22 or 28°C) or where temperatures were switched (22 to 28°C or 28 to 22°C) and mortality was observed twice daily for 14 days. We found that acclimation temperature did not have a strong influence on cadmium sensitivity except at the high cadmium challenge concentration. However, throughout all treatments, snails that were exposed after a switch in temperature (from acclimation temperature to cadmium challenge temperature) exhibited the highest mortality. Interestingly, snails acclimated at 28°C and then exposed to high cadmium at 22°C exhibited significantly less mortality than those snails acclimated to 22°C and then exposed at 28°C. This suggests that there was a higher cost associated with acclimating to a higher temperature compared to if snails were abruptly exposed to that higher temperature as a novel stress. While clearly beneficial in the short term, acclimating to one stressor could leave a population vulnerable to the next and this vulnerability may persist even if the previous stressor is relieved. Thus, as GCC continues to have far reaching global implications, it is increasingly important to understand impacts at the regional scale, where increased variation in parameters like temperature in concert with other stressors may be a significant driver of population vulnerability.

RP070 Leveraging Existing Data Resources with Spatial Eco-epidemiology for Screening-Level Ecological Risk Assessment: Case Studies in the US and the UK

K.E. Kapo, C.M. Holmes, Waterborne Environmental, Inc.; S.D. Dyer, The Procter & Gamble Company / Central Product Safety; L. Posthuma, RIVM / Lab. for Ecological Risk Assessment / Centre for Sustainability, Environment and Health; D. De Zwart, RIVM / Centre for Sustainability, Environment and Health; M.J. Whelan, Cranfield University / Natural Resources / School of Applied Sciences, University of Leicester / ES&T Department; R. Williams, Centre for Ecology and Hydrology; V. Keller, Centre for Ecology & Hydrology; A. Young, Wallingford HydroSolutions; S. Marshall, Unilever; G. Burton, University of Michigan / School of Natural Resources & Environment and Cooperative Institute for Limnology & Ecosystem Research; J. Murray-Bligh, Environment Agency

Identification of potential stressor-response associations based on retrospective analysis relating and integrating spatial patterns of chemical mixture toxicity, water chemistry, physical habitat, land use and biological data over broad geographies can provide a useful screening-level approach as part of an ecological risk assessment. Leveraging and enhancement of existing monitoring program data and other geospatial data resources provides a cost-effective means for enhancing the risk assessment process with additional information which can guide strategies for higher-tier components of an ecological risk assessment, such as site selection and refinement of stressor hypotheses. This poster presents case studies applying statistical and spatial data modeling approaches to existing data resources to delineate quantitative screening-level stressor hypotheses for decision-support in the design of subsequent higher-tier risk assessment. In the first case study, we demonstrate the use of spatial analysis to predict riverine macrofaunal community condition in terms of a range of potential stressors such as water chemistry, metals and pesticide mixture toxicity, wastewater effluent, flow regime and land use variables (as surrogates of diffuse pollution) in a study area comprising 307 catchments in England and Wales. In another case study, we apply the approach to the prediction of fish community and trophic level status for three ecoregions of Ohio for nearly 2000 catchments using a similar, but more comprehensive integration of environmental data. The outcomes of the case studies highlight a strong association of factors related to water chemistry, land use and physical habitat with the geographic distribution of biological impacts. The case studies demonstrate the utility of these types of approaches across different geographic areas as well as the importance of data availability and quality for optimizing screening-level analyses.

RP071 Barring Success: Identifying barriers to accelerate post-industrial habitat recovery of intertidal biodiversity in Howe Sound, BC, Canada

S. Bard, Keystone Environmental Ltd.; V.L. Marlatt, University of Fraser Valley

Studies conducted in 1990-1993, 2004 and 2012 showed that biodiversity of intertidal species in the mid- to low tide zone decreased significantly as exposure to pulp mill effluent increased along pollution gradients in Howe Sound. Diversity was also assessed adjacent to the Britannia Beach before and after successful treatment of mine drainage effluent. During the earliest Howe Sound surveys two pulp mills (Woodfibre and Port Mellon) were operational, and since the 1990s successful pollution abatement was undertaken to decrease the toxicity of marine effluent. This research describes the recovery of intertidal biodiversity at the most highly impacted sites, Darrel Bay proximal to the currently decommissioned Woodfibre pulp mill and Britannia Mine beach. At Darrel Bay, intertidal biodiversity increased from 6 species in 1990s to 12 species in 2004 post-pollution abatement, to 15 species in 2012 post-mill closure. Britannia Beach had zero macroscopic intertidal species during 1990 surveys, post-effluent treatment 4 species were observed in 2004, and in 2012 over a dozen species were present. Experiments investigating whether recolonization and recovery are accelerated at the Darrel Bay site by providing additional rocky intertidal habitat through rock transplantation to the mid-low tide zone are underway. Recruitment of several species to naïve rock was detected by 4 weeks, but mortality of some of these species was observed by 6 weeks. These studies indicate increases in species diversity following pollution reduction, however further time and potentially remediation of historical contamination with habitat augmentation are likely necessary to accelerate recovery and achieve recolonization to the level of reference sites.

RP072 A Hazard Scoring Method for the Prediction of Benthic Impacts in the Detroit River

K. McPhedran, Great Lakes Institute for Environmental Research; K.G. Drouillard, University of Windsor / Great Lakes Institute for Environmental Research; E. Szalinska, University of Windsor; A. Grgicak-Mannion, University of Windsor / Great Lakes Institute for Environmental Research; D. Haffner, University of Windsor / GLIER

The Detroit River is an international connecting channel between the United States and Canada and is a listed Great Lakes Area of Concern (AOC). The river has continually received contaminants from a wide-variety of sources resulting in a spatially-diverse region of contaminated sediments. The current study was a river-wide assessment of sediment contamination and benthic community structure completed to address cause-effect linkages between sediment pollutants and benthic community abundances. Multiple benthic habitats were identified using environmental attributes via a multivariate approach including: silt/high TOC, low velocity/sand, high velocity/sand and high velocity/gravel. Individual sites were assigned into generated habitats and categorized by degree of contamination using a novel hazard scoring system. The hazard score was developed for multi-chemical mixtures using sediment quality guidelines (SQGs) for 14 priority pollutants using the Lowest Effect Level (LEL) and Probable Effect Level (PEL) SQGs to create a fitted sigmoidal toxicity curve, where LEL is equivalent to a 5% toxic effect and PEL a 50% toxicity effect. Contaminant concentrations from each sampling site (n=150 sites in total) were used to determine a hazard score representing a cumulative contaminant effect level which is generated by summing predicted effects of each individual pollutant. These cumulative scores were developed using SQGs from two jurisdictions (Michigan and Ontario) and contrasted versus other previously developed multi-chemical hazard metrics (e.g., PEC-quotient variants). After the development of site hazard scores for each individual habitat, the habitat-matched hazard scores across sites were compared with respect to benthic community structure using a breakpoint analysis of benthic abundances using a dose/response curve developed between hazard score (dose) and relative abundances (response). Significant differences in benthic community composition were observed based on the toxicity versus abundances for sites in the silt/high TOC and low velocity/sand habitats but not in the other two habitats. Chironomidae and Hexagenia were the most sensitive groups with strong dose/response relationships. Although the various scoring approach methods produced comparable evaluations, the hazard score approach using Ontario SQGs yielded site designations providing the strongest statistical power for habitat-specific comparisons.

RP073 Combined Effect of Cadmium and Elevated CO2 on Immunity of Eastern Oysters (Crassostrea virginica) and Hard Shell Clams (Mercenaria mercenaria)

A. Ivanina, I. Sokolova, UNC Charlotte / Biology

Innate immunity of marine bivalves is sensitive to environmental stressors which can thus affect their susceptibility to diseases, pathogens and parasites. We studied the interactive effects of elevated P_{CO2} (hypercapnia) and metals (Cd) on the immune cells (hemocytes) of bivalves *Crassostrea virginica* (eastern oyster) and *Mercenaria mercenaria* (hard shell clam). Bivalves were exposed for 28 days to clean seawater (control) or 50 μg L^{-1} Cd at one of the three CO_2 concentrations – ~400, 800 and 2000 ppmv representative of the present-day conditions and moderate scenarios (Intergovernmental Panel on Global Climate Change) for the year 2100 and 2250, respectively. Cell density and immune-related functions of circulating hemocytes (including total and mitochondrial respiration, adhesion, and phagocytosis) were assessed. In hard clams, total hemocyte respiration increased under 2000 ppmv CO_2 in the absence of Cd while Cd exposure suppressed respiration under elevated CO_2 conditions. Elevated CO_2 had no effect on hemocyte mitochondrial respiration. In oysters, both total and mitochondrial respiration declined by 40-46% at 800 ppmv CO_2 in the presence of Cd. Hemocyte counts of clams were not affected by Cd or elevated CO_2 levels but cell adhesion and phagocytic activity declined at the highest CO_2 concentration (2000 ppmv) in the presence of Cd. In oysters, individual stressors had no effect on the density or functions of the circulating hemocytes, while combination of elevated CO_2 (800-2000 ppmv) and Cd exposure led to a significant increase in the hemocyte counts as well as reduction in hemocyte adhesion and phagocytic activity. These data indicate that exposure to elevated CO_2 levels (such as expected in the case of the global climate change) may compromise

immune function of mollusks in metal-polluted estuaries and that immune function of oysters will be impacted at lower CO_2 levels than in clams in the presence of Cd. Supported by NSF IOS-0951079 and UNC Charlotte Faculty Research Grant.

RP074 Isolated and combined effects of hypoxia and nickel contamination in the Asiatic clam Corbicula fluminea

M. Baudrimont; P. Dranguet, P. Gourves, University of Bordeaux; P. Gonzalez, CNRS UMR EPOC; A. Legeay, University of Bordeaux

Since the beginning of industrial era, most aquatic ecosystems are threatened by many types of pollution such as trace metals. The Gironde estuary, historically polluted by polymetallic contamination originating from Zn ore mine treatment in its catchment basin, also suffers of the presence of the urban area of Bordeaux, located on the middle part of the Garonne river. This sensitive zone is periodically submitted to increasing periods of high temperatures, outflow reducing and hypoxia in summer, jointly to discharges of urban, industrial and agricultural pollutants in the river bed. Among metals, nickel (Ni) is highly bioaccumulated by organisms leaving in this area. In order to understand the combined effect of hypoxia and Ni exposure on the Asiatic clam *Corbicula fluminea*, we experimentally exposed these bivalves in controlled conditions under realistic conditions encountered in the Garonne river. *C. fluminea* were exposed to 4 and 40 µg/L of Ni during 21 days, and one period of hypoxia during the second week of exposure was applied to half of the organisms at a pressure of 4 kPa (21 kPa for normoxic conditions). Ni accumulation, metallothionein concentrations and quantitative gene expression of catalase, glutathion-s-transferase, metallothionein, cytoplasmic superoxide dismutase, cytochrome c oxidase subunit 1 and 12S were studied in gills and visceral mass of bivalves. The results suggested that Ni at low doses is regulated in the same way of an essential metal in gills and visceral mass of *Corbicula fluminea*. The application of a period of hypoxia during 7 days, after one week of Ni exposure, lead to a decrease of the capacities of metals regulation by the bivalves and a lower transfer of the metal from gills to visceral mass. Moreover, Ni disrupts the metallothionein synthesis in these conditions suggesting a weakening of the organisms. At the gene level, the presence of Ni leads to a decrease of the mitochondrial metabolism and the generation of an oxidative stress in visceral mass. These responses seem to mimic those observed under hypoxia conditions without any contamination. In the presence of the combined effect Ni/hypoxia, all these effects are amplified, suggesting a deleterious impact of the Garonne river conditions for aquatic organisms living in this hydrosystem.

RP075 Previous exposure of predatory fish to a pesticide alters palatability of larval amphibian prey

S. Hanlon, M. Parris, University of Memphis

Amphibians breed and persist in a variety of habitats, ranging from ephemeral ponds with short hydro periods, to ponds that can permanently sustain aquatic life. Amphibians that persist in predator-rich ponds must either be able to avoid predators through anti-predatory defense mechanisms, or through mechanisms that would make them less desirable to potential predators. Previous studies have shown that amphibians, specifically anurans in the obligate aquatic tadpole stage, vary in their palatability to predators. In natural habitats, both tadpoles and their predators are exposed to anthropogenic pressures. Specifically, contaminants such as pesticides are especially prevalent in aquatic systems, and non-target aquatic organisms (e.g., fish and tadpoles) are at risk of exposure through runoff, spray drift, or direct overspray. Moreover, pesticides can affect predator-prey relationships. We investigated how bluegill fish (*Lepomis macrochirus*), previously exposed to the pesticide carbaryl (via the commercial formulation Sevin®), varied in their ability to capture and consume *Anaxyrus fowleri (*Folwer's toad) and *Hyla versicolor (*gray treefrog) tadpoles. We tested the hypothesis that exposure to carbaryl would alter the fishes' sensitivities to unpalatable species (i.e., toads). As predicted, survival was significantly higher in *A. fowleri* compared to *H. versicolor*, with survival reducing over time in both species. Most importantly, bluegill exposed to the high concentration of Sevin consumed more *A. fowleri* tadpoles over time compared to bluegill in the water control and low treatments. In *H. versicolor,* only an effect of trial was observed with survival decreasing over time. The results of this study suggest that carbaryl can reduce the unpalatability of *A. fowleri* tadpoles to bluegill. Our study shows that tadpoles can experience the adverse effects of pesticides without actually being exposed to such pesticides. Most importantly, prey defense mechanisms such as noxious toxins, may not be useful in situations where aquatic contamination occurs. Future studies must examine how dual exposure of predators and prey (specifically those that have developed chemical anti-predator defenses) to pesticides alter predator-prey relationships, taking into consideration that factors other than behavioral alterations may play a role in such interactions.

RP076 Effects to metabolome profiles in Japanese medaka (Oryzias latipes) exposed to combined stressors

S. Uno, Education and Research Center for Marine Resources and Environment, Faculty of Fisheries; K. Nakayama, Ehime University / Center for Marine Env. Studies; E. Kokushi, J. Koyama, Kagoshima University / Faculty of Fisheries

In the environment, aquatic organisms are received several stress such as changing temperature and water qualities, exposures of chemicals, and others. The mechanism of effects from multiple stressors is generally complicate and this explore is difficult. Recently, we have continuously evaluated negative impacts in fish with metabolomics approach especially aiming on establishing evaluation method with combined stressors. In this study, we try to establish the new stress evaluation in Japanese medaka (*Oryzias latipes*) with metabolomics approach. Here, 1) dissimilar stressors as fasting and cold shock, 2) similar stressors as exposure of phenanthrene and pyrene, and 3) multiple stressors as exposure of whole extracts from coastal sediment. The metabolites with significantly different levels among treatment group with either or both stress were screened by one-way analysis of variance (ANOVA). Principal component analysis (PCA) was then applied to the screened metabolites data, and principal component (PC) scores for each sample were obtained. In the exposure of dissimilar exposure, the group exposed to both stressors was clustered away from the groups exposed to either fasting or cold shock on two dimensional PCA score plot and almost placed at the sum of two vectors for groups individually exposed to fasting or cold shock. Especially, the metabolic effects to fasting were serious. In the exposure of similar stressors, each individual exposure group of phenanthrene and pyrene clustered at similar place on PCA score plot. This result suggests that the effects to individual exposure of phenanthrene and pyrene were similar in metabolite profiles of medaka. On the other hand, the group exposed to both of phenanthrene and pyrene was clustered away from individual exposures. This suggests that the combined exposure of both PAHs induced some different effects to medaka metabolites from those of individual exposure. Finally, medaka was exposed to extracts from Subic bay, near Manila, Philippines, and Osaka bay, near Osaka and Kobe, Japan. The differences of effects with exposure were explained the distance from control group along PC1 on PCA score plot. As a result, sediment in Subic bay more seriously affected to medaka than that in Osaka bay.

RP077 Physicochemical and toxicological properties of anthropogenic organic matters from sewage, industrial and livestock effluents

J. Yoo, J. Jung, Korea University / Division of Environmental Science & Ecological Engineering; B. Lee, J. Hur, Sejong University / Department of Earth and Environmental Sciences

Organic matters in aquatic environments are complex heterogeneous mixtures that can be distinguished in two main groups: natural organic matters (NOMs) derived from the weathering and decay of plant materials, as well as anthropogenic organic matters (AOMs) consisting of discharges from various wastewater treatment plants. Rapid development of industry has led to large amounts of AOMs being released into water bodies, which greatly influence the composition and property of dissolved organic matters. In this study, three kinds of AOMs (sewage, industrial and livestock wastewater treatment effluents) and Suwannee River NOM were isolated into hydrophobic (HPO), transphilic (TPI) and hydrophilic (HPI) fractions by DAX-8 and XAD-4 resins (Amberlite, USA), and characterized by fluorescence excitation emission matrix (FEEM) and specific ultraviolet absorbance (SUVA) analysis. In addition, acute toxicity of each fractionated samples was conducted using *Daphnia magna*. As a result, AOMs showed similar characteristics to microbially derived organic matters having low hydrophobicity, and acute toxicity to *D. magna* was largely dependent on fraction types of AOMs. These findings suggest that AOMs have different physicochemical and toxicological properties compared with those of NOMs, which needs to be further studied with various sources and different fractions of AOMs.

RP078 Particulate accumulations in the vital organs of wild Brevoortia patronus after the Deepwater Horizon oil spill

D.R. Millemann, Rutgers University / Environmental Science; K.R. Cooper, Rutgers University

An estimated 4.9 million barrels of Louisiana light crude oil were released into the northern Gulf of Mexico following the Deepwater Horizon oil spill (DHOS). It has been estimated that 50% of the oil from the wellhead reached the surface and drifted towards the northern shores of the gulf, contaminating areas inhabited by *Brevoortia patronus* (gulf menhaden). Gulf menhaden are an obligate filter feeding fish found in the northern Gulf of Mexico and make up the second largest fishery in the United States. Their feeding mechanism and high lipid content make them especially susceptible to bioaccumulation of toxic polycyclic aromatic hydrocarbons (PAHs) associated with crude oil spills. It was hypothesized that histological evaluation of wild gulf menhaden inhabiting areas affected by the DHOS would show an initial occurrence of acute lesions followed by repair and chronic lesions in fish collected in later years. Fish were collected from Louisiana waters in 2011 and 2012 for histopathological evaluation. In gill tissue, inflammation, hyperplasia, and scar tissue formation were representative of an acute and then chronic response to contaminants. Abnormal black particulates (emboli) were present in heart and stomach blood vessels from both the reference and the exposure sites. These particulates were present in 60% of fish heart ventricles collected in 2011 and 70% of fish heart ventricles collected in 2012, regardless of site. Particulates were also present in the stomach muscularis of 67% of fish from 2011 and 89% of fish from 2012. Necrotic tissue was associated with the presence of black particulates in both the heart and stomach samples. Based on these observations, it is believed that the particulates act as emboli that block capillary blood flow causing ischemia and focal necrosis in gulf menhaden tissue. The origin of these particulates is not currently known but preliminary chemical analysis is consistent with weathered hydrocarbons which aggregate to form "mini tar ball" emboli. Samples grouped by site show no statistical differences, which may be expected due to the large area affected by the spill, migratory patterns of individual schools of fish, and recurring natural oil seeps. Menhaden collected from the northern Gulf of Mexico exhibited acute lesions that were in the process of repair or have led to permanent tissue damage. (LDWF-LSU contract 69670; NJAES-Rutgers)

RP079 Seasonal proteomic profile in seagrass from the Gulf Islands National Seashore at the time of the Deepwater Horizon oil spill

J. Corrales, University of Mississippi; M. Dailey, University of Mississippi / Environmental Toxicology; S. Ankisetty, University of Mississippi; M. Slattery, University of Mississippi / Department of Pharmacology; K.L. Willett, University of Mississippi / Environmental Toxicology Research Program

Understanding how seagrass seasonal biomass changes are affected by environmental factors is important both from an environmental and economic standpoint. While thirteen seagrass-specific ecological roles in coastal ecosystems are recognized (for example: provision of grazing and nursery grounds, support of detrital food webs, wave protection and sediment stabilization), seagrass beds continue to be decimated worldwide due to anthropogenic pollutants and climate change effects including ocean acidification, temperature increase, salinity fluctuations, erosion, and UV-B radiation. The seagrass *Thalassia testudinum* is a dominant climax species, while *Halodule wrightii* is a pioneer species, and both are abundant in the northeastern Gulf of Mexico. Biomass production peaks in ~July following the flowering (reproductive) stage. Anthropogenic impacts such as the Deepwater Horizon oil spill (DWHOS; the largest marine oil spill in the history of the petroleum industry with over 200 million gallons of crude oil spilled into the northern Gulf of Mexico) put at risk seagrasses and, in turn, all other species that rely on them for survival. Here we present a temporal proteomic (bottom-up shotgun approach) analysis in the seagrasses *T. testudinum* and *H. wrightii* from May to October 2010 at the Gulf Islands National Seashore, N 30 18 18.6; W 87 24 26.4. Moreover, sediment samples collected in July and October were analyzed for polycyclic aromatic hydrocarbons (PAHs) using gas chromatography mass spectrometry (GC/MS). PAHs decreased from 111 to 21 ng/g dry weight from July to October. Protein identification against both the *Oryza sativa* and *Arabidopsis thaliana* databases was done using the Protein Lynx Global Server software. Stress response proteins such as heat shock proteins, 14-3-3 proteins, glutathione and superoxide dismutase were consistently expressed. Metabolic and photosynthesis related proteins (RuBisCO oxygenase, chloroplast photosystem I reaction center subunit II protein) varied in expression through time. This type of proteomic profiling serves as a tool to monitor stress biomarkers while considering constitutive seasonal fluctuations in protein expression. Supported by Northern Gulf Institute 191001-306811-02 / TO 002 and NIUST NA07OAR4300494.

Fate and Effects of Metals: Geochemical Perspective

RP080 Aluminum: Development of Comparative Toxicity Potentials for Freshwater Archetypes to be used in Environmental Impact Assessments

N. Gandhi, University of Toronto / Dept of Earth Sciences; M.L. Diamond, University of Toronto / Department of Earth Sciences

Despite the importance of aluminum (Al) emissions to natural environments, the characterization factor (CF), which ranks its hazard relative to other chemicals after release into an environment, is not yet available due to its complex geochemistry and mechanisms of toxicity. We used the modelling method of Gandhi et al. (2010, 2011) to characterize ecotoxicity and the influence of various water quality parameters on the geochemical speciation, fate and toxicity of Al in several freshwater archetypes. The method uses (1) USEtox^{TM} model for environmental fate, (2) WHAM for metal partitioning and speciation in aquatic systems, and (3) Biotic Ligand Model (BLM) to calculate average toxicity (HC_{50}). Aluminum CFs vary over 7 orders of magnitude for seven archetypes mainly due to changes in ecotoxicity resulting from differences in water chemistry. The largest contributor towards this variability was toxicity (vary 8 orders of magnitude) followed by bioavailability (~200X) and then fate (~2X). Next, we explored the sensitivity of CFs to critical chemistry parameters such as pH, dissolved organic carbon (DOC), concentrations of major cations (Ca and Na), and water temperature. Freshwater pH is by far the most critical parameter affecting Al speciation and especially toxicity, followed by temperature, DOC and Ca (and thus water hardness).

RP081 Utility of fluorescence quenching to determine metal (Cu, Zn, Pb, Ni) speciation for saltwater solutions containing dissolved organic matter

S.D. Smith, Wilfrid Laurier University / Department of Chemistry; T. Tait, Wilfrid Laurier University; M. Ashoka, Wilfrid Laurier University / Chemistry; J. McGeer, Wilfrid Laurier University / Department of Biology

The speciation of metals play a strong role in determining bioavailability and potential toxicity of metal exposures in aquatic environments. Specifically, natural organic matter (NOM) can complex with metals, influencing speciation and decreasing bioavailability. For regulatory and risk assessment purposes bioavailability models need good estimates of metal speciation in the presence of variable amount and source of NOM. Fluorescence quenching (FQ) is a technique that has been used to determine speciation of metals in freshwater but has had limited application to saltwater environments. The basis of FQ is that a metal ligand complex will be less fluorescent than the corresponding free ligand. Using this assumption FQ data measured during a titration at fixed salinity and pH can be fit to an equilibrium model and the resultant binding constants (K) and capacities (LT) can be used to estimate free metal for any exposure conditions. The basis for the equilibrium model is the assumption that the quenching is via the so-called "static" mechanism. If the quenching is dynamic (i.e., due to collision and not complexation) then quenching will be overestimated and not representative of the true speciation. Here, FQ is tested in salt water using two approaches (1) measurements in model system (tryptophan in artificial sea water) (2) measurements of speciation by multiple techniques in real systems. In the tryptophan tests, equilibrium models based on standard certified K values for inorganic and the organic ligand are compared to measurements of fluorescence and agreement with measured fluorescence was best for copper titrations. Similarly, for Cu, nine different samples from variable estuarine sources were titrated and binding sites ranged from one to three ligands per sample. Binding was relatively strong for all sites, with logK values ranging from 9.33 to 11.22 and binding capacities at multiple fluorophores ranged from 4 to 1614 nmole mg C^{-1}. Free copper was calculated using the FQ data for these samples and the results agreed with the cupric ion selective electrode data within ± 0.3 pCu. These results confirm the applicability of fluorescence quenching techniques, especially for Cu, in marine water.

RP082 Bioavailability of uranium to Chironomus dilutus exposed to a range of physicochemical properties of uranium-spiked formulated and field sediment

S.E. Crawford, University of Saskatchewan – Toxicology Centre; K. Liber, University of Saskatchewan / Toxicology Centre

Sediments act as a reservoir for many metals; however, our knowledge and understanding of how physicochemical characteristics of sediment alter the toxicity and bioavailability of some metals is incomplete. In particular, there is limited information regarding the bioavailability and toxicity of sediment-associated uranium (U). Thus, the goal of this research is to quantify sediment characteristics that influence bioavailability and hence toxicity of sediment-associated U to a model freshwater benthic invertebrate. The freshwater midge, *Chironomus dilutus*, were exposed to both field-collected and formulated sediment spiked with U for 10 d to determine differences in U bioaccumulation from sediment with varying physicochemical properties. The physicochemical characteristics of sediment that were examined in this research include particle size distribution and organic matter (OM) content, as they are predicted to alter the partitioning and bioavailability of U and other metals associated with sediment. Formulated sediments were prepared to mimic the physical and chemical properties of field-collected sediment in order to quantify the influence of different sediment physicochemical characteristics on U bioavailability without test artifacts associated with field sediment. Tests were conducted with control sediment and sediment spiked with sub-lethal U concentrations of 5 to 200 mg U/kg dry weight (d.w.) in formulated or field sediments, aged for 20 d. Test endpoints and measurements included midge survival and growth, and U concentrations in whole organisms, whole-sediment and water (both overlying water and pore water). In all 10-d experiments, *C. dilutus* survival was above 80% regardless of sediment type, U concentrations, or physicochemical characteristics. No statistical difference in U bioaccumulation occurred in organisms exposed to field or peat moss-amended formulated sediment (p>0.05) spiked with the same U concentration. Additionally, increasing concentrations of OM demonstrated a significant decrease of U bioaccumulation in the exposed organisms. An increase from 3 to 6% OM content (d.w. basis) in field sediment spiked with 50 mg U/kg d.w., resulted in a reduction in U bioaccumulation from 19±1.5 to 13.4±2.0 mg U/kg d.w. in the exposed midge (p=0.038). Similar results were observed with clay content, suggesting that physicochemical characteristics of sediment, such as particle size and organic matter, play an important role in modifying U bioavailability.

RP083 Investigating the relationship between bioturbation rate of Lumbriculus variegatus and lead distribution in freshwater microcosms

E.R. Blankson, University of Louisiana at Lafayette / Biology; P.L. Klerks, University of Louisiana at Lafayette / Department of Biology

Bioturbation-driven metal distribution in sediment, worm tissue and the water column (particulate and dissolved phases) was investigated in two experiments. Both experiments used lead-spiked sediment in laboratory microcosms with *Lumbriculus variegatus* at densities of 0, 2093 and 8372 ind./m², for a period of 14 days. In one of the experiments, luminophores were added to estimate bioturbation rates with a one-dimensional biodiffusion model. The results showed that the bioturbation rate affects transport and distribution of metals in sediments. Bioturbation rate was strongly correlated with lead transfer from sediment to the overlying water. This lead was mainlypresent in the particulate form. Bioturbation rate was not significantly correlated with lead in the worms, and in the sediment. The results also show that the population density of the worms influenced bioturbation rates and lead had a strong affinity for sediment.

Communities, Ecology, and Health: Making the Connection

RP084 Assessment of Changes in Potential Contamination Exposure in a Karst Aquifer Using GIS and Temporal Analysis

N.I. Torres Torres, University of Puerto Rico at Mayaguez / Civil Engineering and Surveying; I.Y. Padilla, M. Mudarra, University of Puerto Rico, Mayagüez; J. Cordero, University of Puerto Rico Medical Campus; J. Meeker, University of Michigan

Puerto Rico has the highest rate of preterm birth in United States, and one of the highest in the world. Preliminary investigations suggest that the increase in preterm birth rates in Puerto Rico cannot be explained by changes in known factors, and that there is evidence that exposure to some contaminants contributes to preterm birth. Of special concern are the Chlorinated Volatile Organic Compounds (CVOCs) and phthalates. These contaminants have been found in karst groundwater system of northern Puerto Rico. Complex hydrogeologic characteristics of this system impart an enormous capacity to mobilize, store, and release contaminants from sources to potential exposure zones. Combined with variations in hydrologic events and conditions, these characteristics lead to potential dynamic exposure to water contamination. This study assesses spatial and temporal contamination changes in the karst aquifer of northern Puerto Rico during different hydrologic conditions. The assessment applies temporal analysis and GIS technologies to CVOC and phthalate detections and concentrations data obtained from historical records and field samples. Historical records are collected from the literature and governmental entities, cleaned, categorized, and integrated into a database. Groundwater and tap water field samples are collected during different hydrologic conditions and analyzed for CVOCs, phthalates, and common ions. Historical data and field samples show spatial and temporal variations in the detection and concentrations of contaminants in the study area. The variability is associated with dynamic sources of contaminants and changes in the fate and transport processes that affect the storage, release, and mobility of these contaminants. CVOCs are detected more frequently in groundwater, as single contaminants and mixtures. Phthalates are detected more frequently in tap water, and are associated with discontinuous sources of contamination. Nitrates are frequently found in both, but tend to show lower concentrations in tap water. Detection and concentrations of CVOCs, phthalates, and nitrates vary as a function of hydrogeologic conditions, but a direct correlation has not yet been established, indicating a need for geospatial multi-variate analysis. The results suggest a potential exposure of contaminants through karst aquifers that is temporally and spatially variable. Future work will assess and model this variability.

RP085 Community-Focused Remediation and Risk Assessment at a Former Manufactured Gas Plant Site

E. Shay, J. Panko, Cardno ChemRisk

As part of a comprehensive program to evaluate conditions at historic manufactured gas plant (MGP) locations, we worked with a utility to help them assess and manage potential health risks to the surrounding community during the remediation of one of their properties the former MGP sites. Due to the potential for release and transport of MGP-related constituents from impacted soil and subsurface materials during remedial activities, the project was targeted toward the protection of the nearby community members. Specific efforts included conducting a site-specific remediation-based human health risk assessment for the purpose of determining fenceline concentration objectives (FCOs) for 14 potential fenceline and non-fenceline community receptors surrounding the site. Additionally, potential emissions during remediation were modeled using site investigation soil data and the detailed remediation plan, and the predicted concentrations were compared to the FCOs. Emissions from remediation vehicles were also considered. Community involvement was fostered by making information available through various channels, including establishing a public repository at the local library for site-related documents, press releases, meetings with the community and township officials, mailings, and a dedicated project website containing information such as project status, "what to expect during remediation," FAQs, links to additional information, and contact information. From the view of a "classic" site risk assessor, this total effort represents a rather uncommon case in which numerous community receptors were specifically considered in the risk assessment process, and in which community members also had the opportunity to be involved and informed throughout the entire remediation process.

RP086 Relative contribution of inhalation of lead soil particulate matter to gardener's lead exposure

J. Tabony, Boston University School of Public Health; W. Heiger-Bernays, Boston University School of Public / Department of Environmental Health

Gardening and farming in urban settings has risen in popularity in Boston, as the city has seen an increase in community gardens and urban farms in the past years. However, due to the historic use of lead-based paint and leaded gasoline, lead is ubiquitous in many urban soils, posing a potential risk to human health. Lead exposures in utero and to the young child can result in adverse effects on neurocognition, measured as learning disabilities and behavioral disorders. Evidence of older child and adult lead exposures can result in adverse effects on several organ systems, including the

central nervous, gastrointestinal, reproduction and renal systems. While the USEPA's Integrated Exposure Uptake Biokinetic (IEUBK) Model provides a useful tool for exposure and risk assessment from lead in children, it fails to account for inhalation of particulate lead from playing in the soil while gardening. The IEUBK model only assesses risk associated with incidental ingestion while working the soil. Inhalation is addressed only for lead vapor and not the particulate lead from the soil. Literature on lead bioavailability through the lungs based on lead species and particle size is reviewed in order to better understand the behavior of the lead in the human respiratory tract. Empirical data are collected during simulated gardening activity. To our knowledge, these data are the first to be reported in the context of defining exposure through inhalation of particles while gardening in lead-contaminated soils. The literature-based data on bioavailability and the fine particulate matter estimates of lead intake are combined to provide modeled estimates for a pathway not typically considered in assessment of lead risk. These data can be used to inform regulatory guidelines and soil Best Management Practices for gardeners working in lead-contaminated soils.

RP087 Sideritis Brevibracteata improve memory and learning (experimental study in mice)

K. Zerrouki, Univ Motaganem / Biology; N. Djebli, Department of Biology, Health & Sciences Faculty / Univ of Mostaganem

The neurotoxicity of heavy metals such as aluminium is largely spread recently, without any exception of a young and old peoples, Alzheimer's is the most common form of dementias result by this metals. The current therapy of AD that is used in patients on a daily basis is exclusively hypothesis-driven and not causal. Indeed, there are only a limited number of drugs currently available. All these compounds are ACh-esterase inhibitors, which have only a modest effect on the progression of AD. Although stabilization of the neurotransmitter ACh is an obvious approach and may help to some extent to keep up the ACh-driven neurotransmission necessary for complex processes such as cognition and memory, these drugs are not the long awaited breakthrough. The idea of recent approach is by decreasing oxidative stress by means of antioxidant plants. The objective of this study is to clarify the positive effect of sideritis brevibracteata used as a moderated treatment against neurodegenerative diseases includind Alzheimer's caused by aluminum chloride $AlCl_3$. The mice were randomly divided into four groups; each group containing seven mice (for each experience: neurotoxicity (AD): control group, neurotoxicity, treated intoxicated groups and the control treated groups. $AlCl_3$ was dissolved in distilled water administrated orally (100 mg/kg) for the intoxicated group, and treated intoxicated groups, given for chronically (8 weeks); in parallel of Sideritis Brevibracteata administration (60mg/kg oraly) respectively for the intoxicated treated group and the control treated groups received the same doses of Sideritis Brevibracteata (60mg/kg). The results of the neurologic studies showed that there are typical neuropathological changes in almost of treated intoxicated mice's behavior & memory. In this investigation the effect of Sideritis Brevibracteata with over load of aluminum chloride to mice lead to reduction of neurotoxicity and Alzheimer's disease appeared as improvement in neurologic apearances.

Analytical Considerations for Ecological Investigations

RP088 Biota Lessons Learned – Collection, Preparation, and Analysis Associated with the TVA Kingston Ash Recovery Project

E. Rodgers, Environmental Standards / Chemistry

On December 22, 2008, an ash storage cell dike failure at the Tennessee Valley Authority (TVA) Kingston Fossil Plant in Kingston, Tennessee, resulted in the release of an estimated 5.4 million cubic yards of fly ash into the surrounding environment. As part of the comprehensive monitoring program initiated following the release, TVA expanded its historical biological monitoring activities to include the sampling of a broad variety of biological specimens (e.g., tree swallows, blue heron, osprey, benthic macro invertebrates, amphibians, turtles, and raccoons) for analysis of a suite of 26 ash-related metals constituents. Personnel from several organizations collaborated during the sample collection, processing, analysis, quality control, and data evaluation processes. The complexity of such a robust biological monitoring program resulted in challenges for the TVA Kingston Ash Recovery Project team from its initial planning stages to the final reporting and assessment stages. Specifically, the project team faced challenges with regard to planning sampling events; effectively communicating among personnel across multiple organizations; conducting the sampling events; documenting sampling and analytical procedures and implementing these procedures; coordinating sample analysis; and performing data review and evaluation. Careful review and evaluation of each project phase was necessary to ensure the resulting data met the data quality objectives for the project. Effective identification of issues encountered and resolution of these challenges resulted in a high degree of confidence in the results obtained and provided mechanisms for continuous improvement to the TVA biological monitoring program. This paper presents a summary of the challenges encountered as part of the TVA Kingston Ash Recovery Project biological monitoring program and the approaches used to overcome those challenges as a way of sharing lessons learned to improve biological monitoring processes and, ultimately, data quality and usability.

RP089 Porewater collection and risk evaluation

J. Gruzalski, Environmental Standards; J. Markwiese, R. Vitale, Environmental Standards, Inc; W. Rogers, N. Carriker, Tennessee Valley Authority; D. Thal, Environmental Standards, Inc

Investigating the ecological impacts of contaminants released into the environment requires the integration of information from multiple lines of evidence. The collection and analysis of interstitial water is often used as one of the lines of evidence for assessing ecological risks of contaminated sediment that can produce a strong line of evidence for developing benthic exposure estimates in aquatic ecosystems. It is a well-established principle that chemical and toxicity data on interstitial water samples should represent in-situ conditions. This underscores the importance of maintaining sample integrity throughout the sample collection process to avoid alteration of the in-situ geochemical conditions. This presentation will focus on collection and processing of porewater under both aerobic and anaerobic conditions and on how these methods can influence analytical results. For example, several metals (notably iron and manganese) can be solubilized in sedimentary reducing environments. Conversely, exposure to oxygen can result in the precipitation of these metals and the release of other metals (e.g., cadmium, copper, lead, nickel and zinc) that are otherwise sulfide-bound in anaerobic sediments. Sampling-dependent geochemical transformations such as these highlight the importance of minimizing atmospheric exposure during sample collection and processing in preventing bias in the estimation of in-situ concentrations.

RP090 Reporting Results to Method Detection Limits: Quality Control Measures to Evaluate Analytical Accuracy at Low Concentrations

R.J. Vitale, Environmental Standards

State agencies often require data to be reported to the method detection limit (MDL) for various programs, typically for meeting health-based standards or for demonstrating compliance with a discharge permit. Commercial environmental laboratories typically perform MDL studies in accordance with 40 CFR Part 136, Appendix B to determine matrix-specific and analyte-specific MDLs for a given preparative technique and analytical method. This method has received significant technical criticism but remains the most wide-spread method for determining the MDL. Calculating the MDL at the 99% confidence interval (student's t times the standard deviation of a set of measurements of samples approximately at the MDL) allows for the probability that 1% of the samples analyzed with a true concentration slightly below the MDL will be reported as false positives. Additionally, the Part 136 definition assumes the instrument response curve passes through zero; that is, the Part 136 MDL does not allow for the effect of a non-zero signal in a blank sample. In order to generate detection limits with a high degree of defensibility and show that a particular analyte can be "seen" at the MDL, project-specific MDLs for metals analyzed by ICP and ICP/MS in aqueous, solid, and biological matrices were developed which accounted for the non-zero signal in blanks for a large multi-year environmental cleanup utilizing months of blank data. Additionally, a series of MDL standards was required to be analyzed with each analytical sequence to demonstrate the laboratory's continuing ability to detect the project-specific MDL. These "PjMDL" check standards were constructed to allow for non-zero blank signals and also for component aging since the last time the Part 136 MDL was determined. This paper discusses the development of the project-specific MDLs and the results from implementation of these MDLs with the MDL standards.

RP091 Quantifying accurate minimum detectable level performance for sensitive LC/MS/MS methods; application to pharmaceuticals in surface and wastewaters

K. Ruffatto, Illinois Institute of Technology; M. Murphy, J. Pritt, M. Burkhardt, EPA Region 8

The US Environmental Protection Agency Region 8 Laboratory performs ultra-trace analysis of surface water samples for pharmaceuticals using liquid chromatography tandem mass spectrometric (LC/MS/MS) methods. These sensitive instruments have the capability to identify and quantify concentrations in the sub parts-per-trillion to parts-per-quadrillion range. Toxicologists, risk assessors, and ecologists have described ecological and health impacts exist at these very low levels of detection. Our experience shows that the current published methods frequently used for determining minimum detectable levels provide poor estimates of the true method performance. Additionally, current detection level methods do not include the very important compound identification features of the LC/MS/MS measurement process. We have tested a logistic regression technique on five pharmaceutical compounds representing a range of analytical performance for determining minimum detectable levels that uses both identification and quantification data. The identification data are produced by parent/daughter ion transitions, relative ion abundances, and signal to noise ratios following the guidance provided by European Union Standards in the Official Journal of the European Union (2002). Selecting the most appropriate calibration model from a host of options available by the instrument calibration software provides the quantification data. We show that using logistic regression to model the analytical measurement sequence along with proper calibration selection can produce highly reliable quantification of compounds in the range of the minimum detection level reducing the need to qualify low-level data. Minimum detectable values determined by logistic regression are compared to the EPA MDL method in 40 CFR 136 Appendix B and Lowest Concentration Minimum Reporting Level (LCMRL) that is used for updated methods such as 524.3 and the ISO Standard 11843 for determining the critical level and minimum detectable level.

RP092 Percent Moisture in Biological Samples – Minimum Sample Mass Requirements and Storage Considerations

R.L. Forman, Environmental Standards

Data for solid samples, including biological matrices, may be reported on a dry-weight or wet-weight basis. Sample results that are reported on a dry-weight basis account for the moisture content in the sample by utilizing the results from the percent moisture determination for that sample to correct the wet-weight analytical results. The decision to report sample results on a dry-weight or wet-weight basis is often determined by project or regulatory requirements and whether the end users need to compare sample results to risk-based standards or action levels that are developed on a dry-weight or wet-weight basis. Most moisture determination procedures specify the use of several grams of biota sample to perform the test. Sample mass is typically not an issue when sampling and analyzing fish tissue, but would certainly become a question for the collection and analysis of smaller-sized biota samples or aliquots of samples such as a mayfly or fish organs. Just how much sample mass is needed to perform a percent moisture test to provide reproducible results? Will storage duration and conditions impact moisture determinations for very small sample masses? Why are precision and accuracy of the percent moisture determination important? This presentation will provide data from moisture determination studies involving various sample amounts and storage durations for which the test was performed.

RP093 Results of Mercury Analyses Significantly Beyond Recommended Holding Times – Potential Implications for Mercury Holding Times in Biological Samples

J.N. Gable, Environmental Standards, Inc.

The US Environmental Protection Agency (USEPA) recommends a maximum technical holding time of 28 days for the analysis of mercury in biological tissue samples (*QA/QC Guidance for Sampling and Analysis of Sediments, Water, and Tissues for Dredged Material Evaluations-Chemical Evaluations*. EPA 823-B-95-001). However, as noted in this document, this recommended holding time is based on guidance that is administrative, rather than technical in nature. The 28 day holding time is codified in 40 CFR Park 136 for aqueous samples; however, no promulgated holding times exist for biological samples. In fact, some studies appear to indicate that mercury is stable in biological matrices for significantly longer time periods, particularly when the tissue is frozen or freeze-dried. Additionally, many standard reference materials (SRMs) of biological origin are stable for several years. As part of the biological monitoring program for the Tennessee Valley Authority (TVA) Kingston Ash Recovery Project (KRP), mercury analyses were performed on a variety of biological matrices, including whole-body fish, benthic invertebrates, bird eggs and nestlings, and mammal blood. As part of a comparison study, aliquots of a subset of these samples were reanalyzed for mercury significantly beyond the USEPA-recommended technical holding time; the samples were reanalyzed for mercury 1 to 2 ½ years after collection. This presentation will compare mercury results for these samples generated within and beyond the USEPA-recommended maximum holding time. In addition, this presentation will discuss the implications of these comparisons on the currently-recommended USEPA holding times for mercury in biological samples.

RP094 Overcoming Analytical Challenges Associated with the Analysis of Cadmium Uptake in Chara australis

S.R. Wegst, University at Buffalo / Department of Chemistry; M. Bisson, University at Buffalo / Department of Biological Sciences; D.S. Aga, University at Buffalo / Department of Chemistry

Plant-based cleanup techniques are becoming increasingly common extraction practices for heavy metal contamination remediation. Initial laboratory tests are necessary to determine the viability of a particular plant for phytoremediation. The macrophytic alga *Chara australis* is being investigated for its ability to accumulate high levels of Cd. Since we found that the presence of Zn improves survival of *Chara* exposed to toxic levels of Cd, we hypothesized that Zn competes with Cd to prevent its uptake by the plant. However, measurements showed that Cd uptake was not reduced in the presence of Zn, leading us to propose that Zn increases Cd resistance by alleviating oxidative stress by increasing glutathione concentrations. Multiple analytical difficulties are associated with the study of the plant tissue including refractory materials, matrix effects, and detection limits. Experiments were performed to determine the refractory materials present following nitric acid digestion, and dissolution steps were taken. Using this sample preparation, recoveries were determined and method limits of detection and quantification were established in order to account for false positives and negatives resulting from the matrix. Standard addition was deemed necessary for accurate quantification due to matrix effects. Glutathione concentrations were measured to test its possible role in the ability of Zn to reduce stress induced by the presence of Cd. However, sample preparation and analysis required optimization due to naturally low concentrations found in *Chara*.

RP095 Lend an Ear: Overcoming Analytical Complexity in Analyzing Manganese in Rat Ear Tissue by Inductively Coupled Plasma-Mass Spectrometry

E.J. Mullin, S.R. Wegst, University at Buffalo / Department of Chemistry; R. Salvi, University at Buffalo / Center for Hearing and Deafness; J. Roth, University at Buffalo / Department of pharmacology and toxicology; D.S. Aga, University at Buffalo / Department of Chemistry

Trace levels of manganese (Mn), approximately 2 mg a day, is beneficial to humans, however, at high levels (above 10 mg daily) it can cause adverse effects, including neurological disorders exhibiting symptoms similar to Parkinson's disease. Rats, exposed to Mn via drinking water, were the experimental system utilized to determine the effects of Mn accumulation in the ear. Inductively Coupled Plasma-Mass Spectrometry was used to quantify Mn concentrations at low parts per billion levels. Since the tissue samples are sub-milligram in size, a micro-scale digestion technique was developed in conjunction with extensive method validation to verify accuracy in the data. Spike recovery experiments were conducted to ensure that the biological matrix was not suppressing the signal. Use of a collision cell indicated that isobaric interferences were not significant for Mn. A rhodium internal standard was used to account for sample dilution as well as instrumental drift. In order to further minimize the error due to the small tissue weights, a larger number of samples were analyzed. The optimized instrument conditions were then used to measure the accumulation of manganese in different portions (basilar membrane, modiolus, and stria vascularis) of the ear. Control samples exhibited detectable concentrations as low as 2.5 parts per billion. Other biologically relevant metals (Fe, Cu, Zn) were also monitored to determine if their levels were affected by increased manganese. Project funded by NIOSH grant 1R010H010311-01.

RP096 Biological validation of enzyme-linked immunosorbent assays for detection of Cry proteins in the environment

V.C. Albright, Iowa State University / Department of Entomology; R.L. Hellmich, USDA-ARS / Corn Insects and Crop Genetics Research Unit; J.R. Coats, Iowa State University / Department of Entomology

As the use of transgenic crops expressing genes for the production of insecticidal crystalline (Cry) proteins increases, concerns that these proteins may have adverse effects on non-target organisms also increase. Accurately detecting these proteins in various environmental matrices is essential for determining possible exposure of non-target organisms to these proteins. Enzyme-linked immunosorbent assays (ELISAs) have been widely used for detection of Cry proteins in the environment. However, their results have not been validated biologically to ensure that only bioactive Cry proteins are being detected; thus, accurate concentrations of the bioactive protein may not be properly represented in ELISA results. This could potentially lead to overly conservative risk assessments and unnecessary regulation. Thus, in order to properly study Cry proteins in environmental matrices, standardized methods of detection that can be biologically validated are needed. The overall objective of this research project is to develop a model system to predict whether Cry protein fragments can produce for false-positive ELISA results. Development of a model system will allow solutions containing fragments of Cry proteins to be generated and tested to determine whether any of the solutions are capable of repeatedly producing false-positive ELISA results. The model system will consist of pure Cry1Ab protein added to a buffer with a digestive enzyme or pure bacterial culture. Several concentrations and time points will be screened by SDS-PAGE to determine optimum conditions for degradation of the protein. Aliquots will be taken for analysis with ELISA. Solutions of Cry protein fragments capable of producing a positive ELISA result will then be saved and used in biological validation of the ELISA procedure. Many current ELISA procedures have been validated for buffer effects, matrix effects, etc., but few, if any, have been validated for biological activity. The use of bioassay validation will evaluate if the proteins or protein fragments detected by ELISA are still bioactive. The information obtained will be utilized to propose a framework of a bioassay validation protocol that can be adapted to quantify the Cry protein of interest from a variety of environmental matrices against representative susceptible species in order to validate ELISA results. Current progress on this project, including preliminary bioassays and model system development, will be presented.

Better Benthic Biomonitoring for Risk Assessments, Criteria Development, and Causal Analyses

RP097 A comparison of the sensitivity of mayfly taxa to a simulated high dissolved solids discharge

J. Kinney, Marshall University; M.Y. Armstead, Marshall University / Integrated Science & Technology; M. Wilson, Marshall University

The discrepancy between field derived benchmarks and toxicity values generated using traditional laboratory testing organisms exposed to mining discharges with elevated dissolved solids has prompted consideration as to whether laboratory generated endpoints are protective of aquatic communities. The question becomes whether the aquatic communities are more sensitive to dissolved solids than traditional test organisms or are additional variables affecting in-stream response. Exposure of field collected macroinvertebrates to a simulated mining discharge, representative of Appalachian streams with elevated specific conductance from sulfate dominated effluent, allows for evaluation of community level as well as individual taxa responses in a controlled environment. Rock baskets containing organisms from reference streams were exposed to dilutions of the elevated specific conductance water in stream mesocosms for 10-day and 30-day exposures. Collections of macroinvertebrates at multiple reference sites provided diverse assemblages and multiple sensitive taxa for comparison. Effects to the sensitive mayfly taxa (Order: Ephemeroptera) are considered herein with an evaluation of specific conductance and sulfate levels associated with loss of abundance of specific taxa and richness of this sensitive order.

RP098 Cryptic species diversity: a potentially confounding factor for risk assessment and biomonitoring?

A. Feckler, Institute for Environmental Sciences; J.P. Zubrod, A. Thielsch, K. Schwenk, R. Schulz, Institute for Environmental Sciences; University of Koblenz-Landau; M. Bundschuh, Department of Aquatic Sciences and Assessment; Swedish University of Agricultural Sciences

During ecological risk assessment it is assumed that the endpoints used to assess species sensitivity towards stress vary around one mean. This assumption, however, ignores the existence of cryptic lineages (i.e., genetically distinct but morphologically similar subunits within one nominal species), a widespread phenomenon in aquatic benthic invertebrates. Although the considerable genetic distance may lead to deviations in biological traits as e.g. sensitivity towards chemical stress among these groups of organisms, the relevance of cryptic diversity has mostly been ignored in an ecotoxicological context. Hence, we investigated the sensitivity of two lineages comprised within the cryptic complex of the benthic freshwater amphipod *Gammarus fossarum* (i.e., A and B) towards the model stressors ammonia and two currently applied pesticides (namely tebuconazole and thiacloprid) using up to five field populations per lineage. Meta-analyses of all test results uncovered that individuals of lineage A are up to a factor of six more sensitive relative to lineage B. This lineage-specific higher sensitivity seems most likely triggered by the meaningful genetic difference, since confounding factors possibly influencing organisms' sensitivity were either controlled for (life-stage, sex, season of collection, and parasitism) or displayed only minor deviations between lineages (energy reserves and land-use pattern of upstream catchments). In this context, it may be questionable if currently applied safety factors – used as a conservative tool to accommodate uncertainties related to the assessment of chemicals' risks – are still protective. Data for ecological risk assessment may underestimate impacts of pesticides if the species included in the evaluation harbor cryptic lineages. Also macroinvertebrate biomonitoring used for the assessment of water quality (e.g., taxa richness, density) should proactively account for cryptic speciation by molecular identification of sampled organisms. This seems adequate as occurrence of more tolerant cryptic lineages may mask low water quality, leading to deceptive interpretations of the ecological status of ecosystems.

RP099 Site-specific ammonia recommendations for the South Toe River's (North Carolina) endangered freshwater mussels

T. Augspurger, US Fish and Wildlife Service / Ecological Services

Benthic macroinvertebrate monitoring of the South Toe River (Yancey County, North Carolina, USA) has documented 110 species of insects, oligochaetes, mites, crayfish and snails. Monitoring which targets other taxa adds 42 species of fish, amphibians, clams, and mussels (including the endangered Appalachian elktoe, *Alasmidonta raveneliana*) to the known taxa of this high elevation river. A proposed municipal wastewater treatment plant discharge to the South Toe River necessitated the derivation of a site-specific limit on ammonia for protection of Appalachian elktoe. The 2009 draft national water quality criteria for ammonia was a valuable source of toxicity data including 12 freshwater mussel species mean acute values for seven genera, and three mussel species mean chronic values for two genera, but there were no toxicity data available for the Appalachian elktoe or the genus *Alasmidonta*. Data were not available to construct interspecies correlation models for the Appalachian elktoe, so a freshwater mussel species sensitivity distribution was used to derive estimates of protective concentrations for acute and chronic exposures. Long-term South Toe River pH data were used to tailor recommendations to local conditions. The final estimates were reasonable when compared to toxicity data for the most taxonomically-related species, the most ecologically-related species, the most sensitive species, and ambient ammonia concentrations in reference watersheds. Biomonitoring data help illustrate a common concern over the paucity of toxicity data in comparison to the stream's aquatic life. Even for a relatively well-studied compound like ammonia (for which the 2009 draft update to the water quality criteria includes 98 species mean acute values for 67 genera and 17 species mean chronic values for 12 genera), toxicity data were available for only 8% of the South Toe River's genera and 3% of its species (with no data for the endangered mussel). Regionally, data limitations are further highlighted when considered against biomonitoring compilations for southeastern rivers and streams which include over 4,000 species of insects, 490 species of fishes, 269 species of mussels, 313 species of snails, and 20 species of clams. With the vast majority of taxa untested, recalculations which add data or resolution (by focusing on species instead of genera, for example) are encouraged rather than recalculations which eliminate data for sensitive species.

RP100 What are benthic invertebrate surveys good for: types of assessments and types of studies?

G.W. Suter, S.M. Cormier, USEPA

Benthic invertebrate surveys have been used for various purposes with highly variable success. Success might be improved if benthic ecologists better understood the assessments that use their data, the needs of those assessments, and decisions that they inform. A framework for fully integrating environmental assessments provides a guide to those uses. The first of the four major types, condition assessments, is probably the most familiar. They answer the question, is the community impaired and in what way? Second, causal assessments determine the cause of any impairment and its source. Third, predictive assessments determine the likely outcomes of alternative decisions including risks to the environment, human health, and the economy. Finally, outcome assessments consider the results of chosen actions in terms of their success in meeting goals and in terms of the degree of validation of the predictive assessments. A condition assessment need only consider the properties of the community, but if it is to be followed by a causal assessment, measurements of habitat and water quality properties must accompany the biology. Similarly, if an assessment of risks is to be based on biological surveys, exposure-response models must be developed to predict the results of manipulating causal factors. Finally, if a cost-benefit analysis or other decision support assessment is conducted, the services provided by benthic invertebrates must be identified and, as far as possible, quantified. Collaboration between data generators and data users could benefit both. The views expressed in this abstract are those of the authors and do not necessarily represent the views or policies of the US Environmental Protection Agency.

New and Emerging Methods in Aquatic Toxicology

RP101 Case Study: Zebra Mussel (Dreissena polymorpha) Embryonic Testing as a Freshwater Biomonitoring Tool in the United States

M.K. Chanov, W.L. McCulloch, EA Engineering, Science, and Technology, Inc. / Ecotoxicology Laboratory; W.L. Goodfellow; R.A. Connelly, EA Engineering, Science, and Technology, Inc.

The presence of freshwater mussels (Unionidae) in the United States is unlike that of any other country, comprising 297 of the world's 1,000 species. Currently, of those 297 species, 35 are considered extinct, with another 70 federally listed as endangered or threatened, making this group one of the most at risk in the United States. Research involving early life stage testing with glochidia and juvenile mussels demonstrates that current water quality criteria may not be adequate for the protection of freshwater mussel populations. The preservation of native mussel populations necessitates a cost effective and efficient methodology for assessing toxicant effects. While current research has developed multiple assays with early life stage mussels, most studies conclude that further method refinement and development is needed. One methodology that has not been widely considered is the utilization of an invasive species for the protection of native mussels. Zebra mussels (*Dreissena polymorpha*) represent a unique opportunity in freshwater mussel toxicity testing as they have a waterborne embryo and can reside in the majority of habitats where native freshwater mussel populations are found, making them a viable surrogate. All other species of freshwater mussels found in the United States use internal fertilization and embryonic development as their reproductive strategy. The waterborne embryonic stage potentially allows for a sensitive stage of mussel development to be tested without intrusive procedures. While additional research may be necessary to determine the comparability of waterborne zebra mussel embryos to the internally developing embryos of native mussels, the ability to test a developing embryo stage presents a unique opportunity in freshwater environments. The results of this case study will determine if zebra mussel embryonic toxicity testing is a viable alternative to current early life stage testing with freshwater mussel glochidia or juveniles. Additionally, based on current data, an idealistic method for early embryonic life stage zebra mussel testing for the preservation of native freshwater mussels is possible as test temperatures and conditions could mimic a multitude of mussel habitats. It should be noted, that proper control of accidental release and distribution must be an essential component of the method and testing plans.

RP102 Fish and Invertebrate Behavior Analysis: A Review of the Current Literature and Recommendations for Future Research

K.I. Stoddard, University of North Texas / Department of Biological Sciences; M.J. Foster, University of North Texas; D.B. Huggett, S.N. Garcia, University of North Texas / Department of Biological Sciences

Animal behavior has been the subject of scientific study for scholars and philosophers since the time of ancient Greece. The first scholarly contribution to the field of animal behavior is largely believed to be made by Aristotle (384-322 BC), who wrote *Historia animalium* (*The History of Animals)*. Centuries later, Charles Darwin emerged as another figure whose ideas and writings would greatly influence the field of animal behavior. Today the study of animal behavior has permeated many fields of science including, but not limited to, genetics, physiology, neurobiology, ecology, and toxicology. Additionally, behavioral studies have expanded beyond using mammals to include fish and invertebrates test species. Due in part to advancing technology, behavioral observations of test species, are becoming increasingly more quantitative in addition to qualitative. This shift has the potential to significantly impact the many science disciplines which utilize behavioral studies. Such impacts could include improved strength of behavioral studies and increased reliability and reproducibility of results. One field of science which is beginning to see the benefits and potential real-world applications of improved and quantitative behavioral analyses is aquatic toxicology. A thorough review of the aquatic toxicology literature and regulatory guidance reveals that despite a wealth of knowledge and information that is now available regarding behavioral responses of aquatic test species to contaminates, the specialized field of behavioral toxicology generally lacks cohesion, direction, and meaningful regulatory guidance. The purpose of this poster is therefore to: Provide a brief review of the current state of knowledge of behavior studies in the field of aquatic toxicology; Discuss the prominent behavioral assays and endpoints used in aquatic toxicology; Discuss the challenges currently facing the use of aquatic toxicological behavioral endpoints; and Frame a discussion for future research needs and solutions to these challenges.

RP103 Studies to Support Implementation of Aquatic Life Criteria for Poorly Soluble Metals

N. Love, GEI Consultants, Inc.; R.W. Gensemer, GEI Consultants / Ecological Division; S.D. Baker, GEI Consultants, Inc.; S.P. Canton, GEI Consultants, Inc. / Ecological Division; S. Skigen, GEI Consultants; S. Sanderson, Colorado Mining Association; E. Fry, Peabody Energy

Recent efforts are being made in various states to update aquatic life water quality criteria for aluminum and iron. Both metals are poorly soluble under commonly encountered water quality conditions (i.e., circumneutral pH), presenting challenges both for derivation and implementation of these criteria. Water quality criteria are typically based on the dissolved or total recoverable fraction of an element. However, because the total recoverable fraction measures all forms of a given metal, metals associated with non-toxic particulates (i.e., clays and soils) are also captured, potentially overestimating the concentration of metal that would actually be toxic to aquatic life. This talk will use field data from Colorado and New Mexico to discuss how sampling and analysis techniques can be modified to obtain more reliable water quality data, which can then be used for more accurate attainment evaluations. Our results suggest that a coarse sample pre-filtration step would adequately remove significant portions of the non-toxic, particulate mineral phase metals in samples collected as part of water quality monitoring studies. A pre-filtration step using 5 µm or 10 µm filters effectively removes the aluminum and iron associated with the particulate form of TSS, while leaving the smaller-sized colloidal (i.e., potentially toxic) form in the sample. Aluminum and iron concentrations in unfiltered water were significantly higher (up to 43x in some cases) than acute and chronic criteria concentrations, yet none of the samples showed acute toxicity, and only two demonstrated low levels of chronic toxicity which were likely not associated with either aluminum or iron. We conclude that coarse sample pre-filtration, which is an approach recently adopted in New Mexico for aluminum criteria, is an appropriate method to consider to more accurately determine toxic concentrations of poorly soluble metals for regulatory purposes.

RP104 Are morphological effects on P. subcapitata relevant to assess the impact of effluents? Experiences on the evaluation of treated groundwater samples

F. Martinez-Jeronimo, Escuela Nacional de Ciencias Biológicas-I.P.N. / Zoología; S. Guerra-Martinez, Instituto Politécnico Nacional. Escuela Nacional de Ciencias Biológicas

Assessment of toxic effects of treated effluents in primary producers is fundamental as they constitute the first level of any food web in aquatic environments. *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) is a small-size, green microalgae frequently used as test organism in aquatic toxicity evaluation, and several guidelines using this species are available at present. The endpoint measured in these protocols is inhibition in population growth after 72 or 96 h exposure to selected concentrations. Some toxicants are able to produce morphological effects that cannot be detected when biomass, chlorophyll content, or absorbance are measured as endpoints; when cell density is measured through counts in the Neubauer chamber, is possible to discriminate between normal and atypical cells, and a better determination of toxic effects is achievable. In this study the 96-h effects of treated groundwater samples on *P. subcapitata* were monthly determined during several years, applying the method USEPA 1003.0 (Green alga, *Selenastrum capricornutum*, growth test). Samples were filtered (0.45 µm nitrocellulose membranes) to avoid interferences with particles or microorganisms present in the water, and different concentrations (6.25, 12.5, 25, 50, 75 and 100%) were tested in triplicate; samples of each replicate were daily obtained and counted in the Neubauer chamber. During all the studies morphological alterations were easily perceived, so normal and altered cells were counted; morphological modifications included deformed, aggregated, multinucleated, and giant cells, but also disruption in *cytoplasm* and loss of pigments were observed. In most of the studies, significant inhibition in total and normal cells were determined. The growth tendencies were graphed and statistical analysis evidenced differences with respect to the control. In order to determine if morphological effects and growth inhibition could be exhibited in other microalgae, *Ankistrodesmus falcatus* (Chlorophyceae) was exposed in the same conditions; this is a large species (≈40 µm in length), with acicular shape. Growth inhibition and percentage of deformed cells were similar to those determined for *P. subcapitata*, but *A. falcatus* has as advantages its larger size (that make easier the cell count), and its simple shape, that make more perceptible the effects. It is necessary to include the evaluation of atypical cell as an additional endpoint in toxicity tests with microalgae.

RP105 The effects of a simple pesticide mixture on population and interspecific dynamics

R.A. Erickson, USGS – UMESC (CNB) / Dept. of Environmental Toxicology; S.B. Cox, Texas Tech University / Dept. of Environmental Toxicolgy; K.R. Long, Texas Tech Univeristy / Mathematics and Statistics; T.A. Anderson, C.J. Salice, Texas Tech University / Environmental Toxicology

Chemical exposures in environmental settings rarely involve an individual chemical. Instead, most exposures occur as mixtures. Furthermore, most biological assays consider individual organisms and do not consider populations or interspecific dynamics. We studied the effects of pesticides on the population dynamics and interspecific competition between two species of *Daphnia*. A laboratory study was conducted where populations of *D. magna*, *D. pulex*, and both species together were exposed to different pesticide treatments and the populations were followed through time. Specifically, we used malathion, pendimethalin, and permethrin. A simple mixture of these three chemicals was used as a fourth treatment. The results from these studies were incorporated into a two species model. This model was parameterized using a Bayesian framework. We found that the pesticide exposure altered both the population and interspecific dynamics. However, the effects of the pesticide mixture on the population dynamics and competition dynamics were not expected a priori and illustrates the benefits of considering both population dynamics and interspecific competition as part of ecotoxicological studies.

RP106 In silico predicted adverse effects of progestins on steroidogenic function in female fathead minnows (Pimephales promelas)

D. Hala, University of North Texas; L.H. Petersen, University of North Texas / Institute of Applied Science; D. Carty, M. Cantu, University of North Texas; D. Martinovic, University of St. Thomas; D.B. Huggett, University of North Texas / Department of Biological Sciences

There is great interest in understanding how adverse (and diverse) environmental stressors impact organism fitness, such as survival, growth and reproduction. It is expected that the 'structural' underpinnings of biochemical networks contribute greatly to functional susceptibility or robustness under perturbation. In this poster we present computational analyses (using *constraints-based reconstruction and analysis*)to assess effects of exposure to a synthetic (norethindrone) and natural progestin (progesterone) on female fathead minnow steroidogenesis. In order to do this, a previously developed stoichiometric model of piscine steroidogenesis was constrained with production rates of various steroid hormones. Specifically ovary growth was 'anchored' with a biomass reaction constraining the contributions of various steroid metabolites to biomass production (fmol/mg ovary weight). We also quantified *ex vivo* steroid hormone productions (fmol/mg/hr) which also constrained performance of the *in silico* steroidogenesis model. Simulations involved calculating optimal flux distributions using flux balance analysis (FBA), under control and exposed conditions. This allowed identification of reaction flux distributions (or optimal pathways) that 'maximized' the biomass objective function (or ovary growth). The work presented is a step towards the inclusion of a regulatory-relevant apical endpoint (i.e., ovary growth) with *in silico* predictions. The presentation of this analysis will provide predictions of altered (or susceptible) metabolic pathways that are representative of compound-specific modes of actions.

RP107 Mixture Effects of Triclosan and Triclocarban on Daphnia magna

K. Albanese, The Ohio State University, Environmental Science Graduate Program; R. Lanno, The Ohio State University / Department of Evolution, Ecology and Organismal Biology; Y. Chin, The Ohio State University / School of Earth Sciences; C. Hadad, The Ohio State University / Department of Chemistry

Triclosan and triclocarban are two ubiquitous anti-microbial compounds commonly found together in surface waters. Both compounds have been shown to undergo photodegradation in surface waters, with triclosan forming a variety of products including 2,8-dichlorodibenzodioxin, whereas the degradation products of triclocarban are unknown. Both compounds have also been shown to exert toxic effects in aquatic organisms, although the toxicities of mixtures, as well as the toxicities of photodegradation products in tandem with any residual parent compound have not been studied. In this study we investigated possible synergistic/antagonistic effects of triclosan and triclocarban on their photodegradation and toxicities, as well as the effects of the presence of other naturally occurring compounds. Triclosan and triclocarban water samples were spiked with dissolved organic matter (DOM) and salts and placed in a solar simulator, with samples taken until a half-life could be estimated. 96-hour lethality tests were used to determine the relative toxicities of triclosan and triclocarban, as well as binary mixtures, to *Daphnia magna*, and water and tissue samples were analyzed by high-pressure liquid chromatography and mass spectrometry to determine the phototransformation products, the degree of phototransformation and the ratio of degradates to parent compound, as well as lethal body burdens of the degradates and parent compound.

RP108 Read Across of Sucralose Mammalian Toxicological Data For Environmental Decision Making

D.B. Huggett, University of North Texas / Department of Biological Sciences; K. Stoddard, University of North Texas

The intense artificial sweetener sucralose has been detected in municipal wastewater effluent and surface waters at concentrations ranging from ng/L to low ug/L. Mammalian toxicology data have been shown to be useful in prioritizing environmental safety issues in fish and other aquatic vertebrates. The premise of mammalian to environmental read-across is based on the conservation of pharmacological and toxicological pathways across species. A wide array of mammalian data (e.g., acute, sub-chronic and chronic rodent studies) suggest that sucralose is unlikely to produce acute or chronic toxicity in aquatic vertebrates. Specifically, no changes in growth or reproduction (e.g., fertility) have been reported in the mammalian literature, suggesting a low probability of growth or reproduction changes in aquatic vertebrates (e.g., fish). Further, sucralose does not accumulate in mammalian tissues following exposure. Acute and chronic fish studies with trout, bluegill and fathead minnow support the mammalian read-across. No lethality or growth changes were observed in fish up to 100 mg/L, which is well above concentrations detected in aqueous matrixes. In addition, bioconcentration factors in fish are < 3, indicating that sucralose in not bioaccumulative. Collectively, the mammalian and environmental data suggest that sucralose poses a negligible risk to aquatic vertebrates.

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SETAC Africa | SETAC Asia/Pacific | SETAC Europe | SETAC Latin America | SETAC North America

Administrative Offices:

Pensacola Office

229 S Baylen St., 2nd Floor
Pensacola, FL 32502 USA
T +1 850 469 1500
F +1 850 469 9778
E setac@setac.org

Brussels Office

Av. de la Toison d'Or 67
B-1060 Brussels, Belgium
T +32 2 772 72 81
F +32 2 770 53 86
E setaceu@setac.org

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